# *Session 13. Miscellanea*

## Posters

## P13.1

### Brassinosteroid-enhanced the level of primary metabolities in *Wolffia arrhiza*

Andrzej Bajguz

*Department of Plant Biochemistry, Institute of Biology, University of Bialystok, Bialystok, Poland*
*e-mail: abajguz@uwb.edu.pl*

Brassinosteroids (BRs) are natural plant steroidal compounds that promote growth and affect a broad spectrum of physiological responses at nanomolar to micromolar concentrations. The effect of 24-epibrassinolide (epiBL; $10^{-15}$–$10^{-6}$ M) on growth and levels of chlorophylls, carotenoids, sugars and protein in *Wolffia arrhiza* after 7 days of cultivation is reported. Application of epiBL ($10^{-12}$–$10^{-7}$ M) to *W. arrhiza* cultures stimulates the growth and increases the content of photosynthetic pigments, sugar and protein. The greatest effect of epiBL is observed at a concentration of $10^{-9}$ M. At concentrations below $10^{-13}$ M, epiBL had no influence on the growth of *W. arrhiza*. Thus, there are two types of response to epiBL in a dose-dependent fashion. One is the stimulation of growth, where stimulation is directly related to epiBL concentration ($10^{-12}$–$10^{-7}$ M). The second response to epiBL is toxicity of the cultures (≥$10^{-5}$ M). In general, the stimulatory effect of epiBL was characterized by little activity on the growth and biochemical changes in *W. arrhiza* cultures. However, knowledge of the role played by BRs in *W. arrhiza* is still fragmentary but the above results suggest that BRs are importantly involved in regulation of many processes in this plant. The further studies are required to explain unclear the mechanism of BR action in *W. arrhiza*.

## P13.2

### Role of acetylcholinesterase in the physiology of endothelial cells

Aneta Balcerczyk[1], Łukasz Pułaski[1, 2], Mirosław Soszyński[1], Grzegorz Bartosz[1]

[1]*Department of Molecular Biophysics, University of Lodz, Lodz, Poland;* [2]*Department of Transcriptional Regulation, Center of Medical Biology, PAS, Lodz, Poland*
*e-mail: abal@biol.uni.lodz.pl*

Acetylcholinesterase (AChE) is an essential enzyme of the cholinergic neurotransmission system, expressed in several types of cells, mainly in neuronal cells, but also in cardiovascular system cells like erythrocytes or lymphocytes. Recently it was detected also in endothelial cells (EC). The primary and the best-known function of enzyme is to hydrolyze acetylcholine, the endogenous agonist of nicotinic acetylcholine receptors (nAChRs), synthesized and stored in endothelium. Literature data show that nAChR agonists mediate proliferation and capillary network formation, but there are no results explaining the role of acetylcholinesterase in physiological processes of vascular endothelial system. To get insight into its role, we have examined the effect of inhibitors of acetylcholinesterase (BW285c51, eserine, paraoxon) alone and in co-treatment with AChR agonists (acetylcholine, nicotine, pilocarpine) on proliferation and angiogenetic potential of EC. We have also investigated the effect of aldosterone on expression of AChE, because of impaired response of cells to acetylcholine and dysfunction of endothelium under conditions of hormone excess. All experiments were performed on primary endothelial cells isolated from umbilical cord, HUVECs, and microvascular endothelial cells, HMEC-1. We have found 4-times lower activity of AChE in endothelial cells in comparison with erythrocytes. MTT proliferation assay showed decreased viability of cells after treatment with AChE inhibitors, but for concentrations higher than those sufficient to inhibit the enzyme activity. Inhibition of enzyme in agonist-treated nAChR cells increased proliferation and capillary formation of HUVECs. Analysis of expression of AChE, performed by real-time PCR technique, confirms the role of the enzyme in aldosterone-mediated endothelial dysfunction.

## P13.3

### Impaired balance between JNK and MAP kinase cascades activation in postsynaptic density after transient brain ischemia

Małgorzata Beręsewicz, Barbara Zabłocka

*Molecular Biology Unit, Medical Research Centre, Warsaw, Poland*
*e-mail: mberesewicz@cmdik.pan.pl*

Transient cerebral ischemia leads to a selective, delayed damage of neurons in vulnerable (CA1), but not in resistant (CA2, CA3, and *gyrus dentatus*) region of hippocampus. It is believed that neuronal injury is a result of the balance between death (JNK) and survival (MAPK) pathways. We have recently demonstrated in gerbils that transient ischemia activates both pathways, however amount of P-JNK dominates in CA1 and P-ERK dominates in the resistant region. The mechanism of that is not well un-

derstood however we have concentrated on the role of the postsynaptic density (PSD) in that phenomenon. We have studied the influence of ischemia/reperfusion on the proteins implicated in the regulation of MAPK pathway (PSD95, SynGAP, CaMKII) as well as engaged in the activation of JNK cascade (PSD95, kalirin, MLK3). In P2 fraction obtained from ischemia-vulnerable and resistant parts of hippocampus we have examined the level of SynGAP-PSD95 and kalirin-PSD95 complexes and solubility. Our data indicate that relationship among MAPK-associated proteins is modified in CA1, with an exception for CaMKII, which amount increases in ischemia-resistant area. We conclude that these changes lead to the impaired activation of MAPK in CA1 region. On the other hand, ischemia elevates the amount of kalirin which is implicated in the activation of JNK pathway, in both hippocampal regions. Further study are needed to clarify the postischemic relations between these two opposite cascades especially on the most upstream stages connected with PSD proteins.

Supported by MES 2P04A 024 28.

## P13.4

### Differential action of human metabolism regulators on the lymphocyte susceptibility to oxidative stress

K. Brzóska[1], T. Iwaneńko[1], M. Wojewódzka[1], J. Woliński[2], M. Kruszewski[1]

[1]*Department of Radiobiology and Health Protection, Institute of Nuclear Chemistry and Technology, Poland;* [2]*Department of Gastrointestinal Physiology, The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Poland*
*e-mail: marcinkr@orange.ichtj.waw.pl*

Leptin, an adipose tissue cytokine is known to reduce food intake and increase energy expenditure. On the contrary, ghrelin stimulates feeding and reduces energy expenditure *via* activation of growth hormone secretagogue receptor (GHSR). Both leptin receptor and GHSR are expressed on the surface of the peripheral blood mononuclear cells and the existence of a reciprocal regulatory network, by which ghrelin and leptin control immune cell activation and inflammation is anticipated. Ghrelin leads to a dose-dependent inhibition of leptin-induced cytokine expression, while leptin upregulates GHS-R expression in human T lymphocytes. Both modulate the activation of peripheral blood mononuclear cells, but leptin is known to have a proinflammatory action, whereas ghrelin inhibits the expression of proinflammatory cytokines. In this work, we compared the influence of leptin and ghrelin on the cellular susceptibility to oxidative stress. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by density gradient centrifugation on Histopaque 1137, resuspended in RPMI1640 medium supplemented with 20% of FCS, stimulated with PHA and pre-treated with leptin (20 μg/ml) or ghrelin (1–5 aa) (100 ng/ml) for 20 h. After pre-treatment cells were treated with different DNA damaging agents, such as X-radiation (dose 0–3 Gy, 200 kV, 5 mA, dose rate 1.2 Gy/min), $H_2O_2$ (0–250 μM) in PBS for 15 min at 4°C, SIN-1 (0–50 μM) in PBS for 15 min at 4°C. The extent of DNA damage was evaluated by alkaline comet assay. Neither leptin nor ghrelin had any effect on PBMCs proliferation after PHA stimulation. Pretreatment with leptin had no effect on the level of X-radiation- or $H_2O_2$-induced DNA damage. On the contrary, in PBMCs treated with ghrelin we observed a significant increase in the level of X-radiation-, $H_2O_2$- or SIN1-induced DNA damage as compared to untreated cells. However, no effect of ghrelin was observed on cells' capacity to repair DNA damage. Our results indicate that in addition to the opposite effects on food intake and energy expenditure, leptin and ghrelin also have different effects on cellular susceptibility to DNA damaging agents. Overproduction of ghrelin may lead to excessive DNA damage and consequent cytotoxicity, especially in inflammatory condition, where DNA damaging agents, such as ROS or NOS, are produced. Thus, this unexpected action of ghrelin may also have some relevance to the pathophysiology of obesity.

## P13.5

### Looking for the carbohydrates in Fab fragment from human IgG

Monika Buchaniewicz, Bożena Krotkiewska

*Department of Biochemistry, Medical University, Wrocław, Poland*
*e-mail: bozena@bioch.am.wroc.pl*

It was recognized many years ago that the carbohydrate moiety of certain glycoproteins, in some diseases, is altered. An example is human serum IgG, which in rheumatoid arthritis (RA) has agalactosylated conservative N-glycans, present in both heavy chains (Asn-297); this agalactosylation is proportional to the severity of the disease. IgG, which physiologically is poorly glycosylated, has also some oligosaccharide chains in Fab fragment and this amount is statistical. So far there is no precise data on the structure of those oligosaccharides in pathological cases. To explore the problem it is important to isolate, with a good purity, Fab fragment of IgG. We started this isolation with IgG samples from healthy subjects and from the patients with RA. The samples were digested with pepsin and the reaction mixture was fractionated, using Protein A-Sepharose affinity chromatography and Sephadex gel filtration; the purity of $F(ab)_2$ fragments was checked by SDS/PAGE. Further experiments will regard carbohydrate analysis of $F(ab)_2$ fragments by reaction with selected lectins using biosensor BIAcore with the surface plasmon resonance as a detection method.

# P13.6

## New trends in antifibrotic therapy

V. Buko

*Institute of Biochemistry, Grodno, Belarus*

Drug targeting to the activated hepatic stellate cells (HSC) is now possible by use of different agents which inhibit HSC proliferation, collagen synthesis and activate fibrolysis. However, very limited number of these agents is effective in suitable animal models *in vivo*. We studied the effects of losartan (LOS), sylimarin (SYL), interferon alpha2 (IFN), mycophenolate mofetil (MMF) and pioglytazone (PGZ), singly or in combination, in rat liver fibrosis reversal induced by thioacetamide (TAA). The TAA treatment during 3 months induced micronodular liver fibrosis with expressed deposition of collagen fibers. The treatment with LOS, IFN, MMF, LOS+IFN, LOS+MMF significantly decreased the square of liver connective tissue. The expression of MMP-13 was enhanced in rats treated with IFN, LOS+IFN and LOS+MMF. Transcripts of TGF-beta1 in animals treated with MMF, SYL and LOS+SYL and the PAI-1 expression in rats treated with LOS+PGZ were significantly down-regulated. Two combinations, LOS+MMF and LOS+IFN, were most effective. In other trial we evaluated the effects of NO synthase (NOS) inhibitors, L-nitroarginine methyl ester (L-NAME) as inhibitor of eNOS and aminoguanidine (AG) as inhibitor of iNOS on the TAA-induced liver fibrosis reversal. Both inhibitors significantly increased the square of liver connective tissue and the liver hydroxyproline content. The NOS inhibitors up-regulated collagen I, MMP-13, TIMP-1 and PAI-1 mRNA expression where the effect of AG was more pronounced. AG increased mRNA expression of TGF-beta1. Thus, NOS inhibitors developed a clear profibrotic effect, where the effect of aminoguanidine is more pronounced. Our data propose a significant antifibrotic role for NO producing by iNOS rather than eNOS.

# P13.7

## EPR studies of melanoma cells from human skin

Ewa Chodurek[1], Dariusz Kuśmierz[2], Małgorzata Latocha[2], Barbara Pilawa[3]

*[1]Department of Biopharmacy, [2]Department of Cell Biology, [3]Department of Biophysics, School of Pharmacy, Medical University of Silesia, Sosnowiec, Poland*
*e-mail: echodurek@slam.katowice.pl*

Electron paramagnetic resonance (EPR) spectroscopy at X-band (9.3 GHz) was used to the examination of human melanoma tumor cells isolated from skin. High melanin content characterizes the studied biological samples. EPR spectra were measured by RADIOPAN (Poznań) spectrometer with modulation of magnetic field 100 kHz and Rapid Scan Unit produced by firm JAGMAR (Kraków). The first derivative EPR lines were recorded at time of $10^{-4}$ s. To determine free radical concentration in the sample EPR spectra were measured at low microwave power 0.7 mW. The EPR spectra were analysed using Elf program of JAGMAR firm. For the EPR lines the following parameters were determined: amplitude (A), integral intensity (I), linewidth ($\Delta B_{pp}$) and g-factor. g-Value was calculated from resonance condition ($g = h\nu/\mu_B B_r$, where: h – Planck constant, $\nu$ – microwave frequency, $\mu_B$ – Bohr magneton, $B_r$ – magnetic resonance induction). The spin-lattice relaxation in the cells was studied by continuous microwave saturation method. The influence of microwave power in the range from 0.7 to 70 mW on parameters of the resonance lines was obtained. Changes in microwave saturation of EPR lines of the studied melanoma cells and model eumelanin – DOPA-melanin were compared. Lineshape of the measured EPR spectra indicate that mainly eumelanin exist in the skin tumor cells. Similarly to DOPA-melanin high concentration of o-semiquinone free radicals was found for the tested melanotic cells. Broad EPR lines and their saturation at low microwave powers were observed. Strong spin-spin and slow spin-lattice relaxation processes occur in the analysed cells.

# P13.8

## Single channel measurements of ATP regulated potassium channel from rat brain mitochondria

Katarzyna Choma[1,2], Piotr Bednarczyk[1,2], Adam Szewczyk[2], Krzysztof Dołowy[1]

*[1]Department of Biophysics, Agricultural University SGGW, Warsaw, Poland; [2]Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Warsaw, Poland*

Various types of ion channels are present in mitochondria membrane. During last ten years they have become object of many studies. It was proved that mitochondrial ATP-regulated potassium channel ($mitoK_{ATP}$) and mitochondrial Ca-activated large conductance potassium channel ($mitoBK_{Ca}$) are involved in cytoprotection but the mechanism of this event is still unclear. In our study, single channel activity was measured after reconstitution of inner mitochondrial membrane from rat brain into a planar lipid bilayer. The potassium channel with a mean conductance of 219 ± 15 pS in symmetrical 450/450 mM KCl (*cis*/*trans*) solution was recorded. The effect of different channel modulators on single channel activity was examined. The channel activity was inhibited by complex ATP/$Mg^{2+}$ and the effect was reversed by BMS 191095 and the channel activity was reduced. The reason of that amplitude decrease is the presence of magnesium ions. Magnesium ions change channel activity only after addition to the *trans* compartment in our experimental conditions. Apart from, inhibitor of $mitoBK_{Ca}$ channel – iberiotoxin IbTx and inhibitor of mitochondrial voltage gated potassium channel (mitoKv1.3) – margatoxin MrTx have no effect on channel activity. Additionally, inhibitor

of mitoK$_{ATP}$ channels – 5-hydroxydecanoid acid (5-HD) does not change channel activity. Thus, we conclude that the mitoK$_{ATP}$ is present in rat brain mitochondria but in opposite to mitoK$_{ATP}$ from other tissues it is insensitive to 5-HD.

This work was supported by the Ministry of Science and Information Society Technologies grant No. PBZ-MIN-001/P05/11 and by President of the Agricultural University SGGW.

# P13.9

## *In vitro* effect of integrilin, glycoprotein IIb/IIIa antagonist, on various responses of porcine blood platelets

Michał Ciborowski, Marian Tomasiak

*Department of Physical Chemistry, Medical University of Bialystok, Poland*
*e-mail: michalc@amb.edu.pl*

Integrilin (synthetic cyclic heptapeptide), a potent glycoprotein IIb/IIIa receptor antagonist, has been shown to be effective in reducing thrombotic episodes in subjects undergoing coronary angioplasty or in patients with acute coronary syndromes. However, its efficacy in patients with unstable and stable anginas without coronary intervention was found to be remarcably low. This study evaluates the *in vitro* effect of integrilin on various responses of porcine platelets. It was found that integrilin at the concentrations up to 40 μg/ml did not affect calcium signal produced by thrombin. Integrilin in concentration-dependent manner reduced platelet aggregation evoked by ADP ($IC_{50}$ = 24 μg/ml), collagen ($IC_{50}$ = 19.9 μg/ml), or thrombin ($IC_{50}$ = 11 μg/ml). The concentrations of integilin completely blocking aggregation evoked by ADP or collagen failed to eliminate totally microaggregate formation. Integrilin was rather poor inhibitor of the lysosome secretion induced by ADP or collagen ($IC_{50}$ = 40.5 μg/ml). Lumiaggregometry measurements revealed, that the integrilin concentartions totally reducing platelet aggregation (induced by thrombin) failed to block completely dense granule secretion. Integrilin (40 μg/ml) inhibited the adhesion of platelets to fibrinogen coated surfaces induced by thrombin, collagen or ADP by 30%, 22% and 35%, respectively. Integrilin was poor (less than 50% reduction) inhibitor of platelet procoagulant response induced by collagen. The obtained results show that significant differences exist in the sensitivity of various platelet responses to integrilin. It is concluded that integrilin effectively reduces platelet aggregation but is very poor inhibitor of platelet adhesion to fibrinogen and rather weak inhibitor of platelet dense granule and lysosome secretion. This may explain its variable clinical efficacy.

# P13.10

## Mutational analysis of the putative catalytic motif of the *Hin*dIII restriction endonuclease isospecific homologs

Magdalena Cichowicz[1], Iwona Mruk[1], Janusz M. Bujnicki[2], Tadeusz Kaczorowski[1]

*[1]University of Gdansk, Department of Microbiology, Gdańsk, Poland; [2]Bioinformatics Laboratory, International Institute of Molecular and Cell Biology, Warszawa, Poland*

Our efforts are focused on investigating the nature of the isospecifity phenomenon among type II restriction-modification (R-M) enzymes. We are especially interested in finding out what enzymes which recognize the same specific sequence have in common. As a model we have chosen a group of enzymes isospecific to *Hin*dIII R-M system of *Haemophilus influenzae*, which recognizes the sequence 5′-AAGCTT-3′. Here, we present results on the the structure of the catalytic center of the *Hin*dIII endonuclease (R.HindIII) and its isospecific homologs. Magnesium dependent nucleases possess in their structure a motif PD/EX$_{10\text{-}30}$D/EXK which is essential for DNA cleavage and Mg(II) binding. This motif is present in R.HindIII, PE$^{52}$X$_{56}$DXK$^{111}$ and its homologs: R.EcoVIII (*Escherichia coli* E1585–68), PE$^{66}$X$_{54}$DAK$^{123}$; R.LlaCI (*Lactococcus lactis*), PD$^{49}$X$_{54}$DXK$^{106}$; R.Csp231I (*Citrobacter* sp.), PE$^{106}$X$_{55}$DXK$^{164}$; R.BstZ1II (*Bacillus stearothermophilus*), PD$^{58}$X$_{55}$DXK$^{116}$; BbrI (*Bordetella bronchiseptica*), SE$^{80}$X$_{52}$DXK$^{135}$. In an effort to understand the interaction of these proteins with their DNA recognition sequence, we have mutagenized selected amino acids of R.EcoVIII and R.Csp231I genes and identified variants with reduced or null catalytic activity (R.EcoVIII: D108A, D121A, K123A, P65A, S72A, E75A, L77A, K80A, V119A, R126A, R129A, N133A, D136A, K138A, W145A, P157A, D108E, D121E, K123R; R.Csp231I: P105A, D149A, D162A, K164A, D149E, D162E and K164R). This data, compared with analysis of the R.HindIII molecular model prompted us to propose that the catalytic motif for each of these enzymes is slightly different from suggested above. In case of R.HindIII the motif is D$^{94}$X$_{14}$DXK$^{111}$. Thus by analogy suggested catalytic motifs for isospecific endonucleases are as follows: R.EcoVIII, D$^{108}$X$_{12}$DXK$^{123}$; R.LlaCI, D$^{91}$X$_{12}$DXK$^{106}$; R.Csp231I, D$^{149}$X$_{12}$DXK$^{164}$; R.BstZ1II, D$^{100}$X$_{12}$DXK$^{116}$; R.BbrI, D$^{119}$X$_{12}$DXK$^{135}$.

# P13.11

## Flow cytometry as a research tool for determination of fluorescent anion transport across the plasma membrane

Wojciech Ciszewski[1], Błażej Rychlik[1], Paulina Sicińska[1], Janusz Skolimowski[2], Grzegorz Bartosz[1]

*[1]Department of Molecular Biophysics, University of Łódź, Łódź, Poland; [1]Department of Organic Chemistry, University of Łódź, Łódź, Poland*
*e-mail: brychlik@biol.uni.lodz.pl*

Fluorescent compounds are frequently used as substrates

of organic anions transporters including multidrug resistance proteins – members of ABC superfamily. However most of the assays are based on monitoring the dye movement in or out the cell thus making the detailed kinetic analysis highly complex. We previously (Rychlik *et al*., 2003, *J Membr Biol* 193: 79) established a system for direct measurement of fluorescent dye accumulation into inside-out vesicles derived from human erythrocyte plasma membrane. Due to instrumental limitations we could not apply our system to other types of cells. To solve this problem we used flow cytometry which resulted in increase of method sensitivity and enabled us to minimize the material consumption. We were able to demonstrate that 5/6-carboxyfluorescein (CF) and 2',7'-bis(carboxyethyl)-5/6-carboxyfluorescein (BCECF) are actively accumulated in inside-out vesicles prepared from red blood cell plasma membrane. We also showed that the accumulation of BCECF is markedly increased in inside-out vesicles derived from HL60ADR cells overexpressing ABCC1 protein when compared to control and was completely inhibited by MK571 – a well known inhibitor of ABCC proteins. To further characterize the BCECF and CF transport we tested the modulatory properties of other organic anion transport inhibitors including: colchicine, diclofenac, KO-143, novobiocin, reserpine, sulphasalazine, TMB8 and verapamil. Surprisingly, some of these compounds acted as CF transport stimulators and not inhibitors. To identify the molecular determinants responsible for this stimulatory action, we tested a series of derivatives of veratrylaldehyde. The results of this survey may shed a new light on allosteric activation of organic anion transporters.

## P13.12

### Pyruvate- and 2-oxoglutarate dehydrogenases as the essential regulatory points of metabolism

Jan Czerniecki, Paweł Dobrzyń, Adam Tylicki, Jolanta Kondracikowska, Maria Czygier, Sławomir Strumiło

*Department of Animal Biochemistry, University of Bialystok, Bialystok, Poland*
*e-mail: jaczer@uwb.edu.pl*

Pyruvate dehydrogenase (PDH) and 2-oxoglutarate dehydrogenase (OGDH) are large enzyme complexes, very similar to each other in their structure. Both of them are regulated by allosteric mechanism and covalent modification, but in different manner. The main difference is presence of specific PDH-kinase phosphorylating and simultaneously inactivating PDH, and PDH-phosphatase which dephosphorylates and reactivates it. Acetyl-CoA and NADH are strong activators of the PDH-kinase, whereas $Mg^{2+}$, $Ca^{2+}$ and especially $Mn^{2+}$ are main activators of PDH-phosphatase. Moreover, thiamine pyrophosphate (TPP) inhibits PDH-kinase. We also established a direct influence of exogenous TPP and divalent ions on the kinetic parameters of PDH. The exogenous TPP and divalent ions lead to shortening in the lag-phase of the catalysed reaction and a strong decrease of the $K_m$ for pyruvate, CoA and $NAD^+$. This direct influence of the coenzyme on the PDH affinity for its substrates seems to be important regulatory mechanism. The second complex, OGDH is strongly activated by ADP, $P_i$, $Ca^{2+}$ and $Mn^{2+}$, which significantly decrease the $K_m$ for 2-oxoglutarate without any change in the maximum rate of the reaction. The sigmoid curve of the dependence of the initial OGDH reaction rate on ADP concentration indicates positive cooperativity of the binding sites. OGDH is inhibited by ATP *via* opposition to ADP activation, as distinct from PDH, where ATP regulates its activity by specific PDH-kinase. Moreover, both complexes are inhibited by their end-products by universal, negative feedback inhibition.

## P13.13

### Disturbance in vitamin B1 metabolism under stress

Iwan Czernikiewicz[1], Jerzy Tarasow[2]

*[1]Grodno State Medical University, Grodno, Belarus; [2]Institute of Biochemistry, NAS of Belarus, Grodno, Belarus*

## P13.14

### Biochemical activity of indomethacin in alga *Chlorella vulgaris* (*Chlorophyceae*)

Romuald Czerpak, Alicja Piotrowska, Anna Pietryczuk, Anna Olesiewicz, Marta Wędołowska

*Departament of Plant Biochemistry, University of Białystok, Poland*
*e-mail: alicjap@uwb.edu.pl*

Indomethacin is a synthetic derivative of indole-3-acetic acid an auxin natural occured in plants. This coumpound finds application as an anti-inflammatory and painkilling medication. Because indomethacin contains the indole ring in its structure, it may be supposed that possesses auxin-like activities in plants. For this reason the influence of indomethacin in the range of the optimal cocentration of $10^{-8}$–$10^{-4}$M on the growth and changes of dry weigh, photosynthetic pigments, proteins, monosaccharides and nucleic acids content in the unicellular green alga *C. vulgaris*, was examined. It was detected that $10^{-7}$M indomethacin possesses the highest stimulatory effect on alga onthe 5th day of cultivation. The greatest increase of cells' number to 181%, dry weigh to 192%, the content of chlorophyll *a* to 249% and *b* to 229%, total carotenoids to 182%, monosaccharides to 198%, DNA to 160%, RNA to 169% and water-soluble proteins to 179% with respect to the control (100%), was obtained. The SDS/PAGE analysis of the total cellular proteins isolated from the *C. vulgaris* on the 5th day of cultivation showed that under the influ-

ence of the $10^{-7}$M indomethacin are synthesized 14 new polypeptides with molecular weights range of 41–139 kDa. Moreover the $10^{-7}$M indomethacin caused the highest increase of the extracellular secretion of algal proteins to 168% when compared to the control (100%). It was determined that indomethacin is characterized by the auxin-like activity and stimulates the growth and increase of analysed biochemical parameters content in green alga *C. vulgaris*.

## P13.15

### Tryptophan hydroxylase pathway in the brain of rats under alcohol withdrawal: effects of ethanolamine and tryptophan

Darashenka Ya.[1], Zolotukhin M.[1], Razvodovsky Yu.[1], Smirnov V.[2]

*[1]Grodno State Medical University; [2]Yanka Kupala Grodno State University, Grodno, Belarus*

Amino acid imbalance plays an important role in the pathogenesis of neurochemical disorders which accompany chronic alcohol intoxication as well as alcohol withdrawal syndrome. The correction of the imbalance in the content of amino acids and their derivatives including precursors of biogenic amines can therefore be considered as the essential for the metabolic therapy of alcoholism. Ethanolamine is known as neuromodulator possessing both hepatoprotective and antioxidant properties and is therefore considered as possible agent preventing undesirable effects of excessive amounts of tryptophan when the latter is administered for the correction of central serotoninergic functions. Thus, the aim of our study was to compare the effects of thyptophan and ethanolamine administered to rats separately under conditions of alcohol withdrawal regarding the levels of the compounds related to tryptophan hydroxylase pathway to estimate the direction of their actions. We investigated the influence of ethanolamine (EA) and tryptophan on the formation of the pool of hydroxylase pathway products in the brain of rats under conditions of alcohol withdrawal (AW). Male Wistar rats (140–160 g) undergoing subchronic forced ethanol intoxication (8 days, 5 g/kg twice a day, intragastrically) were administered with EA or Trp (intragastrically, 100 mg/kg for both preparations) and AW (12 h). The levels of Trp, 5-hydroxytryptophan (5-HTP), serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), tryptamine (Trn), *N*-acetylserotonin (NAS) and melatonin were assayed in the brain regions of rats by ion-pair HPLC with the detection by natural fluorescence. We found a decrease of Trp content in the hypothalamus, striatum, and brain hemispheres after 12 h of ethanol withdrawal which was accompanied by a decrease of 5-HTP content. Trp administration led to normalization of all these changes. These facts may indicate the presence of depression of the central serotoninergic function during ethanol withdrawal which may be a consequence of the decrease of both the main precursor availability and the rate of serotonin synthesis. An increase in 5-HT and 5-HIAA content in hypothalamus *vs* ethanol withdrawal was observed after Trp injection; both were significant also in comparison with control. This fact indicates that Trp can accelerate both synthesis and degradation of transmitter thus allowing achieving an activation of serotoninergic system in AW. Changes in the parameters studied in the brain hemispheres were similar to those found in the hypothalamus. In the striatum only Trp level increased. Both 5-HT and Trp levels were elevated in the midbrain. We also found a decrease in the melatonin concentration in the pineal after tryptophan administration while 5-HT level was significantly increased. EA administration was found to have no effect on the levels of tryptophan derivatives assayed in the hypothalamus except for tryptamine (approx. 3.2-fold decrease was found). No effect was observed in the striatum. In the midbrain and brain hemispheres the level of NAS was slightly decreased. The only significant change in the parameters of interest induced by EA was the slight decrease in the hemispheres NAS level. We can conclude that Trp administration during ethanol withdrawal can correct the changes in the central serotoninergic functions during AW. The effects of Trp do not include an activation of decarboxylation of aromatic amino acids. Ethanolamine was found to have nearly zero effect on the levels of tryptophan and products of its hydroxylase branch of conversions. Our findings suggest the simultaneous administration of EA and Trp or including EA into tryptophan-containing amino acid compositions to be promising.

## P13.16

### Synthesis of trehalose in tissues of *Ascaris suum* (Nematoda)

Małgorzata Dmitryjuk, Elżbieta Łopieńska-Biernat, Marek Farjan

*Department of Biochemistry, University of Warmia and Mazury, Olsztyn, Poland*
*e-mail: m.dmit@uwm.edu..pl*

Trehalose (1-O-$\alpha$-D-glucopyranosyl-1→1-glucopyranoside) fulfills in nematodes many physiological functions. It acts as the energy reserve being the resource and transport from of glucose to tissues. Trehalose has a protective function under stress conditions and participates in the mechanism of hatching the larvae from eggs. As a consequence of the above roles the importance of trehalose metabolism for nematodes, including the parasitic nematode *Ascaris suum*, is very significant. In the majority of Eucaryota in the process of trehalose synthesis participate two enzymes: the first – trehalose-6-phosphate synthase (TPS; EC 2.4.1.15), which synthesis trehalose-6-phosphate from UDPG and G6P; the second – trehalose-6-phosphate phosphatase (TPP; EC3.1.3.12) decomposing T6P to trehalose and inorganic phosphate. There is much less infor-

mation available on synthesis of trehalose in nematodes that's why in the present research we decided to mark determinig the activity of both enzymes participating in trehalose synthesis in tissues of *A. suum*. Activity of TPS was determined using the method by Giaever *et al*. (1988), and that of TPP by Kaasen *et al.* (1992). The end product of the reaction – trehalose was determined using HPLC. The highest activity of TPS was found in reproductive system (289.84 ± 253.38 u/mg), lower in haemolymph (206.45 ± 17.41 u/mg) and the lowest in muscle of *A. suum*. (27.45 ± 13.45 u/mg). Totally different than TPS, T6P phosphatase demonstrated the highest activity in muscle (646.08 ± 222.82 u/mg), slightly lower in reproductive system (462. 97 ± 376.33 u/mg) and the lowest in haemolymph (200.87 ± 92.63 u/mg) of that parasite. We did not demonstrate the activity of both enzymes participating in trehalose synthesis in cuticle and intestine of *A. suum*.

References:

Giaever HM, Styrvold OB, Kaasen I (1988) Biochemical and genetic characterization of osmoregulatory trehalose synthesis in *Escherichia coli*. *Bacteriol J* 170: 2841-2849.

Kaasen I, Falcenberg P, Styrvold OB, Strøm AR (1992) Molecular cloning and physical mapping of the otsBA genes, which encode the osmoregulatory trehalose pathway of *Escherichia coli*: evidence that transcription is activated by KatF (AppR). *Bacterial J* 174: 889-898.

# P13.17

## Comparison of Ang II effect on tyrosine kinase activity in hormone-dependent and hormone-independent human prostate cancer line

Domińska Kamila[1], Rębas Elżbieta[2], Lachowicz-Ochędalska Agnieszka[1]

*[1]Department of Comparative Endocrinology, [2]Department of Molecular Neurochemistry, Medical University of Lodz, Lodz, Poland*
*e-mail: kamila-107107@wp.pl*

As our recent studies showed, angiotensin II (AngII), the main peptide of the renin-angiotensin system (RAS), can not only regulate the water-electrolytic balance and control the arterial blood pressure. Ang II is also involved in cell proliferation of different types of cells, in normal and pathological as well. The present study was to examine the influence Ang II on tyrosine kinase (PTKs) activity in two humane prostate cancer lines: androgen-dependent LNCaP and androgen-independent DU-145. The PTKs play important role in growth cell, differentiation and apoptosis. 7-days old cell cultures of LNCaP and DU-145 human prostate cancer were used as a source of protein tyrosine kinases. These cultures were exposed to different concentrations of Ang II ($10^{-11}$–$10^{-9}$ M). In phosphorylation reaction as a donor of phosphate was used $\gamma^{32}$P-ATP and as the substrate the synthetic peptide – poly Glu, Tyr (4:1), known as an artificial substrate for all types of protein tyrosine kinases. The specific activities of PTKs were defined as pmoles $^{32}$P incorporation into exogenous poly Glu, Tyr/mg of protein/min. Ang II in every studied concentration inhibited PKTs activity in androgen-independent human prostate cancer but not in androgen-dependent. We can assume Ang II influence on tyrosine kinase activity is modulated by hormonal status of the cell line and the presence or lack of androgen receptors in cells may play important role in this process. The obtained results suggest that AngII might be new therapeutic agent in therapy of prostate cancer.

Work was supported by Grant Nr 507 – 11 – 270.

# P13.18

## Copper induced non-enzymatic lipid peroxidation in *Arabidopsis thaliana* and its consequences for plant growth

Maria Drążkiewicz, Ewa Skórzyńska-Polit, Zbigniew Krupa

*Department of Plant Physiology, Maria Curie-Skłodowska University, Lublin, Poland*
*e-mail: mdrazki@biotop.umcs.lublin.pl*

One of symptoms of oxidative stress induced in organisms living in polluted environment is lipid peroxidation. This process can be carried out in enzymatic way by lipoxygenases and in non-enzymatic way. In the latter process reactive oxygen species are engaged, formed in different compartments, and transition metal ions. A subject of our interest was the question: can lipid peroxidation affect plant growth? In the present work non-enzymatic lipid peroxidation was measured as production of malondialdehyde (MDA) and hydroxyalkenals (HAEs). Moreover, membrane damage was determined basing on measurements of electrolyte leakage from leaf discs, and changes in some growth parameters were studied. The experiments were carried out in leaves of *Arabidopsis thaliana* exposed to elevated copper concentrations (5 and 50 μM) for 7 days. Level of MDA+ HAEs increased with increasing copper concentrations in the nutrient medium. This increase was accompanied by enhanced electrolyte leakage from leaf discs and by diminished leaf fresh weight as well as a size of *Arabidopsis thaliana* rosettes. Mechanism of copper excess action on plant growth is discussed.

# P13.19

## Structure of carboxypeptidase III from triticale

Adam Drzymała, Agnieszka Grabowska, Wiesław Bielawski

*Department of Biochemistry, Warsaw Agricultural University, Warsaw, Poland*
*e-mail: drzymala@delta.sggw.waw.pl*

Carboxypeptidase III from triticale grains belongs to the group of enzymes synthesized in aleurone layer in re-

sponse of gibberellic acid stimulation (Drzymała *et al.*, 2005). Sequence of the enzyme was determined by molecular biology method of cDNA clone sequencing and peptide sequence analysis by mass spectrometry method. Carboxypeptidase III is monomer of molecular weight 46.6 kDa and exhibit 99% homology to carboxypeptidase III from wheat and 98% homology to correspond enzyme from barley. Position of amino acids responsible for catalysis were assigned by comparison with results obtained by Chiba *et al.* (1995). Catalytic triad of carboxypeptidase III from triticale is composed of serine 143, aspartic acid 331 and histidine 388. Another important group in the active site of the enzyme is an "oxyanion hole", which contain backbone amides of glicine 50 and tyrosine 144. Comparative modelling of tertiary structure of the enzyme by Geno-3D (Combet *et al.*, 2002) demonstrated, that arrangement of these elements in active site of carboxypeptidase III from triticale resembles arrangement of corresponding groups in carboxypeptidase Y from yeast and carboxypeptidase II from wheat. Moreover there is an "acid bridge" in active site, which is observed in serine carboxypeptidases of known structure, and can be considered as stabilizing element of native conformation in acidic pH.

References:

Drzymała A, Prabucka B, Bielawski W (2005) *Acta Physiol Plant* 27: 539-547.

Chiba Y, Midorikawa T, Ichishima E (1995) *Biochem J* 308: 405-409.

Combet C, Jambon M, Deléage G, Geourjon C (2002) *Bioinformatics* 18: 213-214.

# P13.20

## The analysis of adherential abilities and hydrofobic interactions on a *E. coli* bacteria cells surface

Krystyna Dworniczak, Grażyna Ginalska

*Department of Biochemistry, Pharmaceutical Division, Medical University of Lublin, Lublin, Poland*
*e-mail: tetradion@lublin.mm.pl*

Latest studies have displayed that the distinct influence on adhesion, the formation of biofilm and the virulence of a lot of pathogenic bacterial strains (*Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus* sp., *Pseudomonas* sp.) may have extracellular biopolimers in the coat slime (exopolysaccharide –EPS) (1,2). Mucus bacterial, both of the Gram Positive and the Gram Negative bacteria, is usually a mixture of such organic structures as superficial proteins, polysaccharides polymers and teichoic acids. In addition to this the bacteria in a biofilm are more refractory to antibiotics than free descendant cells. In a previous study there were no precise analysis in a subject of molecular regulation of adhesion phenomenon in a virulent strains *E. coli*, in connection with it, valid is the inspection of the nature of those occurrences with the participation of the strains mentioned. In the individual studies were found, that rich culture medium has an influence on EPS biosynthesis. We showed that in presence of 1% of maltose as a source of carbon, the examined strain of *E. coli* (ATCC 3521) showed the highest content of hydrofobic structures and the increased activity of phosphoglucomutase (PGM), opposite to the experiments with 1% or 2% glucose in medium. Initially there were defined the hydrofobic occurences, which are probably responsible for the adhesion native *E. coli* cells to the contact surface. Studies such as evaluative of the aggregation of the cells ability in solution ammonium sulfate and the estimation of adherence to hydrofobic layer ability were taken. The results of those investigations show the hydrofobic nature of *E. coli* cells. In a SAT test strain did not deference with aggregation in water and in 0.4 M ammonium sulfate, however it aggregate in 0.8 M and 1.6 M solutions $(NH_4)_2SO_4$. In turn, the MATH test were used for investigate the native cells, after the sonication and treating it with 0.1% tripsin. We proved the 6% amount of cells proceeding from the buffer layer to the organic layer (p-xylene). However the HIC test showed the 30% amount of cells adsorption to the hydrofobic layer. It was appointed by the decrease of OD density in the eluat, against the initial density of the bacterial suspension measured with the wave 600 nm lenght. It was showed that the native cells of investigated strain evidented the 18% concentration of hydrofobic structures on the surface. Also it was observed that the hydrofobicity of cells on their surface may have a fundamental influence on biofilm formation, beside such elements as: biochemical contain of medium, concentration of cations such as: Mg (II), Mn (II), Ca (II), or Fe (II, III), inherence of EDTA and urea. We experimented also a initial identification of hydrocarbon components of coat slime (glycocalyx) by means of paper chromatography and reduction tests. The results received encourage to the further biochemical studies related to isolation, purification and the investigations of phosphoglucomutase properties – the enzyme directly involved in EPS of bacteria biosynthesis.

References:

1. Rodney M, Donlan J, Costerton (2002) Biofilms. *Clin Microbiol Rev* 15: 167-193.

2. Darryl R, Absolom F, Lamberti V (1983) Surface thermodynamics of bacterial adhesion. *Appl Environ Microbiol* 46: 90-97.

# P13.21

## Calcium signaling evoked by BzATP in glioma C6 cells

Anna Dygas, Jolanta Barańska

*Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology, Warszawa, Poland*
*e-mail: adygas@nencki.gov.pl*

In glioma C6 cells ATP acting on P2Y metabotropic receptors leads to the elevation of free cytosolic $Ca^{2+}$ level

in the process mediated by phosphoinositide turnover. ATP can also activate P2X ionotropic receptors which mRNA (P2X1-4 and P2X6-7) we have recently detected in glioma C6 cells. Western blot analysis confirmed a presence of P2X2, P2X4, P2X6 and P2X7 receptor proteins. P2X2 receptor is detected in two isoforms (a and b) both on the mRNA and protein levels. Unfortunately, it is impossible to measure $Ca^{2+}$ response evoked by activity of both, P2X2 and P2X4 receptors because of the lack of their specific agonists/antagonists. In contrast, BzATP, the relatively specific rat P2X7 agonist effectively stimulates $Ca^{2+}$ flux into glioma C6 cells. This effect was observed at high BzATP concentration (300 μM), however 100 μM is also effective. KN-62 is (CaMKII inhibitor) a noncompetitive, selective P2X7 antagonist was used at concentration between 5–20 μM, but has a little effect on the P2X7 rat glioma C6 cells. Thus, the action of P2X7 receptor in these cells appears to be not related to CaMKII. In contrast, the P2X7 specific antagonist, oATP (200 μM), irreversibly abrogates [$Ca^{2+}$] signal, however only that being evoked by BzATP. ATP added after BzATP when the calcium response reaches the basal level, usually increases intracellular calcium again. Thus we conclude that whereas the first [$Ca^{2+}$]i elevation is due to BzATP-evoked calcium influx through by P2X7 receptor, the second one is the result of $Ca^{2+}$ release from the internal stores mediated by ATP-stimulated P2Y receptors.

# P13.22

### Degradation of phenol by free and immobilized mixed cultures of *Pseudomonas* strains

Janusz Dzirba, Izabela Greń, Marta Rudzińska, Sylwia Łabużek

*Department of Biochemistry, Faculty of Biology and Environment Protection, University of Silesia, Katowice, Poland*
*e-mail: jdzirba@us.edu.pl*

Phenol is the environmental pollutant that is very toxic even at low concentrations. Therefore, the treatment of phenol effluent is very important. This aromatic substance is degradated by many bacterial strains, e.g. *Pseudomonas, Acinetobacter* or *Arthrobacter*. Biodegradation of organic compounds by pure bacterial cultures can produce toxic intermediates. More effective degradation of these chemicals can be achieved by use of mixed cultures, as they have a wider spectrum of metabolic properties. Using immobilized bacteria in wastewaters treatment is promising and has many advantages, e.g. it gives possibility of higher concentrations of chemicals' degradation and cells protection by matrices used for immobilization. The aim of this study was to compare phenol degradation's effectiveness by mixed: free and immobilizated bacterial strains. We have examined cells viability exposed to different phenol concentrations. The four *Pseudomonas* strains were used in this work. Bacteria were entrapment in calcium alginate of concentration 2%, 3% and 4%. Twelve different phenol concentrations (1–20 mM) were used. Phenol degradation by immobilized co-cultured bacterial strains was faster than by mixed free cells. The time needed to 1–10 mM phenol degradation was twice lower when immobilized cells were used, than when free cells were applicated to biodegradation of this compound. The 15 mM phenol concentration was degraded only by immobilized bacteria. We have not observed complied degradation of the highest phenol concentration either by free or immobilized cells. 3% calcium alginate gave the best protection of bacterial cells against high concentrations of phenol which was demonstrated by the viability tests.

# P13.23

### Glutathione peroxidase, superoxide dismutase, tumor necrosis factor alpha, C-reactive protein and vascular cell adhesion molecule 1 are markers of metabolic syndrome

Tomasz Francuz, Jan Gminski, Krzysztof Siemianowicz, Barbara Posielezna, Aneta Wojcik

*Department of Biochemistry, Silesian Medical Academy, Katowice, Poland*
*e-mail: tfrancuz@mp.pl*

Metabolic syndrome is defined as a constellation of dyslipidemia, elevated blood pressure, impaired glucose tolerance, and central obesity. The prevalence of metabolic syndrome varies by definition used and population studied; the etiology of the metabolic syndrome has not been established definitively, yet. The aim of the study was to compare the concentration of biochemical markers of oxidative stress and inflammatory status in patients with newly diagnosed metabolic syndrome and matched controls; the other aim was to found predictors of the syndrome with additional value to the present diagnostic criteria. Patients and methods: In the study we enrolled 40 non-smoking patients: 20 patients with newly recognized metabolic syndrome with fulfilled all NCEP ATP III diagnostic criteria, and 20 age, BMI and WHR-matched patients without the syndrome. In all patients glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), VCAM-1, hsCRP and TNF-$\alpha$ were determined using commercially available kits. Inter group comparisons were conducted using Kruskal-Wallis ANOVA. For choosing the best predictors of metabolic syndrome among checked parameters discriminant function analysis was performed. Results: Patients with the syndrome had statistically significant elevations of VCAM-1 (886.38 ± 237.2 *vs* 1029.89 ± 177.8; $P < 0.05$), CRP (0.20 ± 0.12 *vs* 0.44 ± 0.37; $P < 0.05$) and TNF-$\alpha$ (18.83 ± 7.24 *vs* 25.18 ± 11.0; $P < 0.05$), SOD (1072 ± 173 *vs* 1303 ± 326; $P < 0.01$) and decrease of GPx (44.04 ± 16.38 *vs* 31.19 ± 12.01; $P < 0.01$ ). CAT was not statistically different between groups (216.9 ± 48.3 *vs* 205.6 ± 26.4; P = n.s.). Using discriminant functions analysis we found that HDL glucose, diastolic pres-

sure, SOD, TNF-$\alpha$ and catalase were the strongest predictors of metabolic syndrome in tested groups and had the best distinguishing power. Conclusions: In conclusion, patients with newly diagnosed metabolic syndrome had disturbed antioxidant status and elevated levels of proinflammatory factors, such as TNF-$\alpha$, CRP and VCAM-1. Some of the measured parameters could be used as predictors of metabolic syndrome with additive value to present criteria such as HDL, triglycerides concentration, obesity and blood pressure.

# P13.24

## Immunogenic properties of pea seeds proteins Varietes"Ramrod" and its hydrolyzates

Regina Frączek, Elżbieta Kostyra, Beata Zimnoch, Agata Wojkian

[1]*Departament of Biochemistry, University of Warmia and Mazury, Olsztyn, Poland*
*e-mail: regina.fraczek@uwm.edu.pl*

Peas are cultivated nearly all over the world. The seeds of this plant, similarly to other legume crops, are an excellent source of proteins, having high nutritive value. Due to this feature, pea proteins are successfully used instead of animal proteins. However, it should be considered that proteins, which are glyco-nucleo-metalo-phenoloprotein complexes, co-occur in pea seeds with other antinutritional substances and this fact makes them potential allergens. The aim of this study was to describe immunogenic properties of the protein extracts from pea varieties "Ramrod" and its hydrolyzate. The McLeester (1973) method was used for protein extraction. The protein extracts were hydrolyzed with pepsin and tripsin in 37°C. Immunogenic potential was determined by indirect and competitive ELISA. Describing the molecular mass of antigens quantitatively by immunoblotting (Towbin, 1979) and affinity chromatography (Hudson, 1980). As a result of conducted analyses, it was found out that the two-stage enzymatic hydrolysis of pea proteins caused proteolysis of some protein fractions above 65–97 kDa and below 53–29 kDa. The protein extract from pea seed "Ramrod"characterized with higher growth of index of polyclonal antibodies 1:12800 than its hydrolyzate 1:3200. Level of cross-reactions between proteins from pea cv. "Ramrod", and its hydrolyzate determined by using antibodies against "Ramrod" was 26.7%. After immunoblotting and affinity chromatography 15 antigenic fractions from protein extracts were obtained as well as 8 fractions from its hydrolyzate.

References:

Hudson L, Hay FC (1980) Chromatografia powinowactwa na kolumnie Aktywacja sepharose 4b I immobilizacja białka. In *Practical Immunology*, Ed. II, Oxford, UK.

Mc Lester RC, Hall TC, Sun SM, Bliss FA (1973). Comparison of globulin proteins from Phaseolus vulgaris with those from *Vicia faba*. *Phytochemistry* 2: 85-93.

Towbin H, Staehelin T, Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheet: Procedure and some applicatons. *Biochemistry* 76: 4350-4354.

# P13.25

## The second methyltransferase of NcuI restriction modification system is a homolog of M2.MboII methylase

Beata Furmanek-Blaszk, Marian Sęktas

*Department of Microbiology, University of Gdansk, Gdansk, Poland*
*e-mail: furmanek@biotech.univ.gda.pl*

DNA methyltransferses (MTases) are sequence-specific enzymes which transfer a methyl group from *S*-adenosyl-L-methionine (AdoMet) to the amino group of either cytosine or adenine within a recognized DNA sequence. Methylation of a base in a specific DNA sequence protects DNA from nucleolytic cleavage by restriction enzymes recognizing the same sequence. Our research is focused on the nature of the isospecifity phenomenon among type IIS restriction-modification systems, where we are especially interested in finding out what these enzymes have in common. The enzymes encoded by the NcuI restriction-modification (R-M) system of *Neisseria cuniculi* ATCC 14688 recognize a pentanucleotide sequence 5′-GAAGA-3′,3′-CTTCT-5′. Restriction endonuclease cleaves the DNA 8 and 7 nucleotides downstream from the recognition site leaving a single 3′ protruding nucleotide. M1.NcuI modifies the last adenine in the recognition sequence 5′-GAAGA-3′/3′-CTTCT-5′ to $N^6$-methyladenine. We have cloned and purified M2.NcuI to electrophoretic homogeneity using a three-step chromatographic procedure. M2.NcuI is a protein with a $M_r$ = 30 000 ± 1 000 under denaturing conditions and exists in solution as a monomer. We have demonstrated that M2.NcuI modifies the internal cytosine in the recognition sequence 5′-GAAGA-3′,3′-CTTCT-5′ yielding $N^4$-methylcytosine. Divalent cations ($Ca^{2+}$, $Mg^{2+}$, $Mn^{2+}$ and $Zn^{2+}$) inhibited the methylation activity of M2.NcuI. The biochemical characteristics (molecular mass, activity dependency on divalent cations) were very similar to those determined for M2.MboII. We also observed the close relationship of M2.MboII and M2.NcuI from the nucleotide sequence coding regions of these proteins showing 83% identity. The amino acid similarity between two methyltransferases is even higher and revealed 88% identity indicating that these proteins have emerged from a common ancestor.

# P13.26

## Oscillations of glucose concentration in medium upregulate angiotensin II type 1 receptor gene expression in cultured human umbilical artery endothelial and smooth muscle cells

Tomasz Gawlik, Tomasz Francuz, Wojciech

Garczorz, Elżbieta Kotrys-Puchalska, Teresa Jurczak, Jan Gmiński

*Department of Biochemistry, Silesian Medical Academy, Katowice, Poland*
*e-mail: gawlik@mp.pl*

Atherosclerosis is the leading cause of excess in mortality in diabetics. Hyperglycemia is important contributor in the pathogenesis of diabetes' complications. There is increasing evidence that postprandial glycemia may have essential, independent influence on atherosclerosis complications. Angiotensin II plays an important role in atheroma formation acting mainly through its type 1 receptor ($AT_1$). The aim of this study was to assess the influence of oscillations in glucose concentration in the medium on the $AT_1$ receptor gene expression in human umbilical artery endothelial and smooth muscle cells in culture. Methods: The cells were incubated for 48 h in the medium with glucose concentration oscillating between 5 and 20 mM every 6, 8 or 12 h. The gene expression was assayed using QRT-PCR. The relative protein content was assessed using Western-blotting technique. Results: In both cell types oscillating glucose concentrations caused significant overexpression of $AT_1$ receptor, greater than in cells incubated in medium with constantly high glucose concentration. Conclusions: The presented data demonstrate that $AT_1$ receptor overexpression can play major role in the detrimental effect of postprandial hyperglycemia.

# P13.27

## The effect of NaF on the $Na^+/H^+$ exchanger in unstimulated human platelets

Magdalena Gąssowska, Barbara Dołęgowska, Dariusz Chlubek

*Department of Biochemistry and Medical Chemistry, Pomeranian Medical University, Szczecin, Poland*
*e-mail: basiad@sci.pam.szczecin.pl*

Introduction: Fluorides permeate into the cells and induce unspecific metabolic changes. Probably NaF influences $Na^+/H^+$ exchanger in human platelets. The membrane sodium – hydrogen exchanger (NHE) is the major regulator of intracellular pH ($pH_i$). It mediates the electroneutral exchange of intracellular $H^+$ for extracellular $Na^+$. The aim: The aim of the study was to investigate the effect of NaF on the $Na^+/H^+$ exchanger in human platelets. Materials and methods: The platelets were obtained from blood-donation centre from healthy human volunteers. The platelets rinsed with Tyrode's buffer were incubated at 37°C for 0, 10, 15, 20 min with NaF concentration ranging from 2.5 to 10 mM. The effect of fluoride was investigated by monitoring changes in $pH_i$ with fluorescent $pH_i$ indicator BCECF. Statistical analysis was performed by the Student's *t*-test, taking the level of significance as $P < 0.05$. Results: Unstimulated platelets had a $pH_i$ of $7.33 \pm 0.01$ ($n = 108$). Addition of 2.5 mM NaF slightly decreased $pH_i$ during first 10 minutes ($7.32 \pm 0.01$), then increased it to the initial values in the next 10 min. An increase in $pH_i$ occurred within 5 min in response to stimulation with 5 mM, 7.5 mM and 10 mM NaF. Subsequently acidification was observed until $pH_i$ of $7.31 \pm 0.01$ was achieved at 20 min. Conclusion: Higher concentrations of sodium fluoride (7.5 mM, 10 mM) significantly inhibited the $Na^+/H^+$ exchanger in unstimulated human platelets.

# P13.28

## Effects of steroids and salicylic acid derivatives on the activity of cholesterol sulphate sulphohydrolase of lysosomal membrane

Jadwiga Gniot-Szulżycka, Katarzyna Roszek, Magdalena Fret

*M. Kopernik University, Institute of General and Molecular Biology, Biochemistry Department, Toruń, Poland*
*e-mail: kroszek@biol.uni.torun.pl*

Cholesterol sulphate sulphohydrolase (CHS-ase) activity is present in human placenta lysosomes at a particularly high rate. Our investigations on the sublysosomal localization of the CHS-ase showed that total activity of the enzyme in the lysosomal membrane compartment was about 92% of the whole lysosomes' activity. Chromatographic analysis of lysosomal membranes with HPLC method revealed especially high content of cholesterol sulphate that serves as a natural substrate for the cholesterol sulphate sulphohydrolase of lysosomal membrane. The purified lysosomal CHS-ase exhibited optimal activity at pH 3.4. The $K_m$ value was established to be $3.6 \pm 0.95 \times 10^{-5}$ mol/l. The molecular weight was $36.5 \pm 2.5$ kDa and the isoelectric point (pI) had the value 5.7. The effect of different steroids and salicylic acid derivatives on the activity of the cholesterol sulphate sulphohydrolase was determined. Anti-inflammatory compounds like hydrocortisone and acetylsalicylic acid are known to stabilize the lysosomal membrane. Both of them effected inhibition of the CHS-ase activity to $66\% \pm 7\%$ and $36\% \pm 17\%$ of the control activity, respectively. On the other hand, agents with labilizing influence (membrane labilizers) like oestrogens, effected only slight inhibition of the CHS-ase activity. These data suggest that lysosomal membrane CHS-ase may be responsible for the regulation of cholesterol and cholesterol sulphate content in the lysosome limiting membrane. The activity of the CHS-ase is influenced by the stability (fluidity) of lysosomal membrane. The relatively low activity of CHS-ase in the presence of anti-inflammatory compounds may be due to an impeded access of a substrate to the active site of enzyme. Nevertheless, the exact mechanism of this influence awaits elucidation.

## P13.29

### Development of phage T4 *rI* and *rIII* mutants in slowly growing host cells

Piotr Golec[1], Aleksandra Wiczk[1], Grzegorz Węgrzyn[1,2], Marcin Łoś[1]

*[1]Department of Molecular Biology, University of Gdansk, Gdansk, Poland; [2]Institute of Oceanology, Polish Academy of Sciences, Gdynia, Poland*

Bacteriophage T4 is a very well studied organism. However, its development was investigated mainly in standard laboratory conditions which are very different from those occurring in the natural habitat. In the natural environment it had to develop strategies allowing it to sustain its presence. Phage T4 as a representative of virulent phages, belonging to the T-even group, is not able to lysogenise the host cell, but employs a strategy which could be termed "controlled virulency". Bacteriophage T4 development depends on host cell physiology. It is able to adapt its development to the growth rate of the host cells and, in some conditions, it can stop it's development by establishing pseudolysogens. Phage T4 is also able to prolong its development after superinfection by another T-even phage. These abilities are dependent on the action of at least two genes, namely *rI* and *rIII* which functional products were previously assumed to be involved only in the lysis inhibition (LIN) phenomenon. We demonstrate the results of culture lysis time experiments in which proteins RI and RIII were overproduced from plasmids with tetracycline promoter. In one-step growth experiments, when slowly growing *E. coli* was infected by T4*rI* mutant, cell lysis is caused significantly quicker relative to the T4wt (wild type) phage. We noticed also an increase in latent period length and decrease in phage yield (from 40 to 5) when generation time of bacteria was reduced. Both mutants, T4*rI* and T4*rIII*, developed more rapidly in comparison with wild type phage. Latent period and lysis times were different in T4wt comparing to studied mutant phages.

## P13.30

### Identification of histone H1.c isoforms in the chicken populations

Ewa Górnicka-Michalska, Jan Pałyga, Andrzej Kowalski

*Department of Biochemistry and Genetics, Świętokrzyska Academy, Kielce, Poland*
*e-mail: egorn@pu.kielce.pl*

A histone H1 family in higher eukaryotes is represented by several primary-sequence subtypes (non-allelic variants) encoded by different genes. The six H1 genes (11L, 11R, 03, 0.10, 01 and 02) identified in the chicken genome encode for the following H1 subtypes: H1.a, H1.a', H1.b, H1.c, H1.c' and H1.d. Several non-allelic avian H1 variants may be represented by two or more allelic isoforms. In this work we identified a variability of chicken erythrocyte histone H1.c. Two isoforms of erythrocyte subtype H1.c, H1.c1 and H1.c2, were identified in the acetic acid-urea gel within Leghorn G99 chickens. The differences in the electrophoretic mobility between H1.c1 and H1.c2 in the acetic acid-urea gel and only a slight shift in their migration during electrophoresis in second dimension in the polyacrylamide gel containing SDS seem to indicate that both proteins can mainly differ in a net charge. The H1.c1 and H1.c2 allelic isoforms may form three phenotypes: c1, c2 and c1c2. In the two-dimensional gel, the proteins H1.c1 and H1.c2 in the phenotype c1c2 have not been fully separated as they migrated very close to each other. Therefore, we determined the phenotype c1c2 in the chicken population using the acetic acid-urea gel. Heterozygous individuals c1c2 and homozygous individuals c2 were most abundant in Leghorn population and occurred at frequency 0.527 and 0.369, respectively, while the homozygous birds c1 appeared relatively rarely (0.104). The inheritance date were consistent with hypothesis that the observed phenotypes were controlled by a gene with two codominant allels (*c1* and *c2*) at the *locus*.

## P13.31

### Oxalic acid as a potential important factor in hydrogen peroxide generation in *Abirtiporus biennis* cultures

Marcin Grąz, Anna Jarosz-Wilkołazka, Elżbieta Malarczyk

*Department of Biochemistry, Maria Curie-Sklodowska University, Lublin, Poland*
*e-mail: mgraz@biotop.umcs.lublin.pl*

Fungi classified to *Basidiomycetes* are the most effective decomposers of lignocellulose complex. Organic acids are important low molecular weight compounds involved in initiation of wood decay process. It has been reported that oxalic acid is a predominant organic acid secreted in wood-rotting fungi cultures. Oxalic acid can serve as a proton and electron source, strong metal chelator, factor which stabilize osmotic potential and pH of fungal growth environment. White rot fungi secrete an array of oxidases and peroxidases for lignin degradation. The three of them, laccase (Lac), manganese peroxidase (MnP) and lignin peroxidase (LiP) are considered as the main enzymes involved in this process. In enzymatic action of MnP, oxalic acid can facilitate the catalitic cycle by chelating $Mn^{3+}$ ions. Both LiP and MnP require extracellular $H_2O_2$ as a substrate. Potential pathways for hydrogen peroxide generation involve enzymatic action of e. g. glucose oxidase, pyranose oxidase, aryl oxidase, methanol oxidase. In the present work novel role for oxalic acid as a factor providing initial concentration of $H_2O_2$ by enzymatic degradation *via* OXO in *Abortiporus biennis* cultures

is proposed. Correlation between MnP, Lac activity, $H_2O_2$ concentration and secretion and enzymatic degradation of oxalic acid in *Abortiporus biennis* liquid cultures are investigated in this study.

## P13.32

### Influence of monochlorophenols and dichlorophenols on 1,2-catechol dioxygenase activity from *Pseudomonas putida* N6

Izabela Greń, Danuta Wojcieszyńska, Anna Wiechetek, Urszula Guzik, Sylwia Łabużek

*Department of Biochemistry, Faculty of Biology and Environment Protection, University of Silesia, Katowice, Poland*
*e-mail: igren@us.edu.pl*

Structure of catechol as the key intermediate in aromatic compounds degradation can be cleaved by dioxygenase of different types. Division of these enzymes is usually connected with the presence of the specific substituents attached to carbons creating the aromatic ring. 1,2-Dioxygenase cleaves carbon–carbon bond only when each of carbon atoms is substituted with hydroxyl groups. 1,6- and 2,3-dioxygenases are so-called extradiol enzymes and they prefer cleaving aromatic structure between two carbon atoms, from which only one is substituted with hydroxyl groups. Presence of chlorine atom in *metha* position of aromatic ring strongly inhibited activity of 2,3-dioxygenase. The aim of this work was to determine the influence of monochlorophenols and dichlorophenols on 1,2-catechol dioxygenase activity in the crude cell extract from the cells of *Pseudomonas putida* N6 strain after 3 mM phenol induction. The optimum temperature was 35°C and the optimum pH was 7.4 for 1,2-catechol dioxygenase activity. The enzyme was strongly inhibited in the presence of $Cu^{2+}$. The best substrate for 1,2-dioxygenase was catechol without any additional substituents in the aromatic ring. After 2 days of induction with 0.5 mM monochlorophenols and dichlorophenols activity of 1,2-catechol dioxygenase was heavy reduced (more than 75%) in the presence of monochlorophenols and 2,4- and 2,5-dichlorophenol. Dichlorophenols with two chlorine substituents in the *ortho* or *metha* position and without any chlorine in the *ortho* position reduced cleavage enzyme activity in 50% or less.

## P13.33

### Theoretical modeling of PrkCc, serine-threonine protein kinase catalytic domain, with ATP and K; Shah's ATP derivatives

Paweł Gruszczyński[1], Rajmund Kaźmierkiewicz[1], Michał Obuchowski[2]

[1]*Faculty of Chemistry, University of Gdańsk, Gdańsk, Poland;* [2]*Department of Medical Biotechnology, Intercollegiate Faculty of Biotechnology, Medical University of Gdańsk, Gdańsk, Poland*
*e-mail: blakrose@hebe.chem.univ.gda.pl,*

PrkC is a member of a super-family of the eukaryotic-like receptor protein kinases. It forms dimers and is anchored in the membrane, with a cytoplasmic kinase domain and an external domain, presumably acts as a sensor. PrkC enables biofilms formation of *Bacillus subtilis* which show a high degree of spatial organization. They colonize various surfaces and produce complex antibiotic resistant communities. PrkC also displays kinase activity with myelin basic protein and some other activities. These include auto-trans-phosphorylation of two phospho-threonine clusters and Ser214 identified by mass spectrometry in the catalytic domain of the PrkC (PrkCc), affecting catalytic activity and perhaps partner recruitment respectively, as well as trans-membrane signaling. Structure of the PrkCc includes an activation loop, which conformation may regulate the kinase activation state by controlling the access of the ATP and the substrate molecules. PrkC acts as a Ser/Thr kinase with features of the receptor kinase family of eukaryotic Hanks-type kinases. Our current study involved the theoretical modeling of the protein kinase PrkC complexes with ATP and modified ATP molecules. There were no attempts before to use molecular modeling methods to make PrkC-ATP/ATP-analogs complexes. We report our results of docking ATP derivatives into PrkC substrate pocket and the analysis of 5ns molecular dynamics simulations in AMBER force field.

This work was supported by grant BW-8000-5-0293-6 form the Polish State Commitee for Scientific Research (KBN). The calculations were carried out at the Academic Computer Center in Gdańsk (TASK).

## P13.34

### Catabolism of nucleoside phosphoramidates in higher plants can be controlled by nucleoside phosphoramidase (Hint protein) and dinucleoside triphosphatase (Fhit protein)

Andrzej Guranowski[1], Paweł Bieganowski[2], Janina Baraniak[3], Anna Rydzik[4], Janusz Stępiński[4], Jacek Jemielity[4]

[1]*Department of Biochemistry and Biotechnology, University of Agriculture, Poznań, Poland;* [2]*Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland;* [3]*Center of Molecular and Macromolecular Studies, Polish Academy of Sciences, Łódź, Poland;* [4]*Department of Biophysics, Institute of Experimental Physics, Warsaw University, Poland*
*e-mail: guranow@au.poznan.pl*

Adenosine 5′-phosphoramidate (AMP-$NH_2$), the simplest representative of nucleoside phosoramidates, is a naturally occurring compound formed by enzymatic displacement of sulfate from adenosine 5′-phosphosulfate

by ammonia. Supposition that nucleoside phosphoramidates play a biological role is supported by the fact that the cells contain enzymes which can degrade these compounds. We have demonstrated in the extracts of yellow lupin seeds two activities that hydrolyze AMP-$NH_2$ to AMP and ammonia. One of them is a specific nucleoside phosphoramidase (32 kDa protein) and the other – nucleoside triphosphate hydrolase (41 kDa protein) for which AMP-$NH_2$ appeared to be just one of different nucleoside derivatives from which this enzyme releases NMP. This study evidences the existence of nucleoside phosphoramidase activities in plants for the first time. *Arabidopsis* FHIT and HINT genes have been isolated and used for the production of corresponding proteins in *Escherichia coli*. Their molecular properties and substrate specificities confirmed identity of these recombinant proteins with proteins isolated from the yellow lupin seeds. We have also tested different chemically synthesized nucleoside phosphoramidates such as AMP-lysine and various GMP-phosphoramidates. Most of these compounds were substrates for the both enzymes.

# P13.35

## The effect of hormonal changes on formation of the lactational function

Ludmila Gutikova[1], Ruslana Anisim[2], Oleg Kuznetzov[2]

*[1]Chair of Obstetrics and Gynaecology, Grodno State Medical University, Belarus; [2]Department of Clinical Laboratory Diagnostics, Grodno Regional Clinical Hospital, Belarus*
*e-mail: klam@tut.by*

There was made an estimation of the level of hormones taking part in regulation of lactogenesis and lactopoesis and comparison of the lactational function of puerperas. In blood-serum of women (249 having preeclampsia of different degree and 96 healthy) in their 36–40 weeks of pregnancy on the 6 day of puerperium there was determined the content of estradiol, estriol, progesterone, prolactin and placental lactogen using immune-enzyme analysis applying automatic immune-enzyme analyser "AxSYM" (2003, USA), by means of standard set of reagents Abbott AxSYM system. Results of the research work were processed statistically using the programme "ANOVA". The level of estradiol, estriol, progesterone and prolactin in blood-serum of the pregnant having preeclampsia was reliably lower in comparison with the screening group. The content of placental lactogen in the screening group made up 20.1 ± 0.4 mkg/ml. In main groups its level was reliably lower in comparison with the screening group (by 9.43 and 62% correspondingly). Puerperas had the content of estradiol, estriol, progesterone decreased in comparison with similar groups of the pregnant. On the 6 day the level of prolactin of puerperas of the screening group and main groups reliably increased 2.5, 1.7, 1.5 and 1.2 times correspondingly. The lowest indexes of average daily quantity of milk had puerperas with medium and severe degree of preeclampsia. On the 6 day puerperas having severe preeclampsia had the volume of milk decreased 1.7 times in comparison with the screening group. Puerperas suffered from preeclampsia had no full formation of the lactational function as the result of changed hormonal status during pregnancy and in early puerperium that leads to the development of hypogalactia and agalactia.

# P13.36

## Amplification of 2,3-(chloro)catechol dioxygenase's gene of *Stenotrophomonas maltophilia* KB2

Urszula Guzik, Izabela Greń, Sylwia Łabużek, Ilona Kula, Katarzyna Hupert-Kocurek

*Department of Biochemistry, Faculty of Biology and Environmental Protection, University of Silesia, Katowice, Poland*
*e-mail: uguzik@us.edu.pl*

Phenol and its chlorinated derivatives are very difficult to degrade. One of the most efficient way to its removal from the environment is bioremediation. Under aerobic conditions microbial degradation of these coumpounds lead to formation of catechol or its derivatives. Cleavage of the monocyclic aromatic structure of catechol with catechol dioxygenases causes substrate more susceptible to biodegradation thus dioxygenases play a central role in catabolism of aromatic substrates. The aim of the study was to optimize the conditions of the amplification of (chloro)catechol 2,3-dioxygenase gene (*C23O*) from *Stenotrophomonas maltophilia* KB2. *C23O* gene was amplified with *Pfu* and *Taq* polymerases. In both cases nonspecific products beside specific 738 ± 8 bp fragment for *Pfu* polymerase and 427 bp fragment for *Taq* polymerase were obtained. Amplification with 51–65°C annealing temperature as well as temperature gradient was conducted. The best results for *C23O* gene amplification were obtained for both polymerases with temperature gradient 59°C to 55°C. Different magnesium and potassium ion concentration were tested individually for each polymerase. Highest amount of PCR product was obtained with 2.5 mM magnesium ion for *Pfu* polymerase and with 3 mM potassium ion for *Taq* polymerase. Optimal product synthesis was also observed with 10 mM and 25 mM potassium ion concentration for *Pfu* and *Taq* polymerase respectively. Results of our studies have shown that PCR reaction with *Pfu* polymerase at 3 mM magnesium ion and 10 mM potassium ion concentration and temperature gradient 59°C to 55°C was optimal for (chloro)catechol 2.3-dioxygenase gene amplification.

# P13.37

## Antibacterial and antifungal properties of

### 3-(2-benzoxazol-5-yl)alanine derivatives

Katarzyna Guzow[1], Michał Obuchowski[2], Wiesław Wiczk[1]

[1]*Faculty of Chemistry, University of Gdańsk, Gdańsk, Poland;* [2]*Department of Medical Biotechnology, Medical University of Gdańsk, Gdańsk, Poland*
*e-mail: kasiag@chem.univ.gda.pl*

3-(2-benzoxazol-5-yl)alanine derivatives are a group of unnatural amino acid which can be used as fluorescent probes [1,2]. Because benzoxazole moiety is found in some biologically active compounds [3] as well as some synthetic benzoxazole derivatives possess antibacterial and antifungal activity [4,5], the antimicrobial properties of about 50 amino acid derivatives of this heterocyclic compounds were studied. An antibacterial activity of all studied compounds was screened by testing their activity against model Gram-positive (*Bacillus subtilis*) and Gram-negative (*Escherichia coli*) bacteria whereas antifungal activity was tested against yeast *Pichia pastoris* as well as *Candida albicans*. The active compounds were also tested against such pathogens as *Staphylococcus aureus*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*. All these tests were performed using antibiogram method whereas the minimal inhibitory concentrations were determined for each compound using two-fold serial dilution technique. It was found that compounds studied are more potent antifungal than antibacterial agents. Moreover, to some extent structure-antifungal activity relationship was observed.

This work was financially supported by the Ministry of Education and Science (Poland) under grants BW 8000-5-0264-5 and BW 8000-5-0287-6.

Katarzyna Guzow is the recipient of a fellowship from the Foundation for Polish Science.

References:

1. Guzow K, Szabelski M, Karolczak J, Wiczk W (2005) *J Photochem Photobiol A: Chem* 170: 215.

2. Szabelski M, Rogiewicz M, Wiczk W (2005) *Anal Biochem* 342: 20.

3. Rodríguez AD, Ramírez C, Rodríguez II, González E (1999) *Org Lett* 1: 527.

4. Yildiz-Oren I, Yalcin I, Aki-Sener E, Ucarturk N (2004) *Eur J Med Chem* 39: 291.

5. Rida SM, Ashour FA, El-Hawash SAM, ElSemary MM, M.H. Badr MH, Shalaby MA (2005) *Eur J Med Chem* 40: 949.

## P13.38

### The role of protein phosphatase PrpE in the UV resistance of *Bacillus subtilis* spores

Krzysztof Hinc[1], Michał Obuchowski[2]

[1]*Department of Molacular Biology, Univeristy of Gdansk, Gdansk, Polnad;* [2]*Department of Medical Biotechnology, Medical University of Gdansk, Gdansk, Poland*
*e-mail: khinc@biotech.univ.gda.pl*

Spores of *Bacillus subtilis* contain a number of small, acid-soluble spore proteins (SASP), which are synthesized midway during sporulation in the forespore and rapidly degraded after spore germination. The SASP's are divided into three groups: $\alpha$, $\beta$, and $\gamma$. Proteins belonging to $\alpha$ or $\beta$ types are coded by at least seven genes which showed high level of similarity. *SspA* and *sspB* genes code for major SASP (from $\alpha$ and $\beta$ group respectively) present in *B. subtilis* spores. Dormant spores produced by strains which carry deletion in *sspA* gene showed significantly decreased UV resistance. Deletion of *sspB* has relatively little effect, however, double deletion strain (Δ*sspA*, Δ*sspB*) produces spores much more sensitive to UV irradiation in comparison to wild-type spores or Δ*sspA*. We found that spores produced by strains with double deletion in the *sspA* and *prpE* genes or triple deletion *sspA*, *sspB* and *prpE* produced spores which showed resistance for UV irradiation similar to wild type. Surprisingly, qRT-PCR analysis of transcription of *sspB* gene revealed that PrpE phosphatase positively regulates its expression. Observed increase in UV resistance of spores produced by strains lacking SspA and SspB proteins in the background of Δ*prpE* supports hypothesis that other genes coding for SAPS may be also controlled by PrpE phosphatase. On this basis, we postulate that PrpE is an important element in a new signal transduction pathway in *Bacillus subtilis* which controls the expression of genes responsible for UV radiation resistance of spores.

## P13.39

### Cloning, expression, and purification of serprocidins in *Pichia pastoris*

Katarzyna Indyk, Paulina Wężyk, Wiesław Wątorek, Mariusz Olczak

*Institute of Biochemistry and Molecular Biology, University of Wroclaw, Wroclaw, Poland*
*e-mail: indyk@bf.uni.wroc.pl*

Azurophil granules and secretory vesicles of polymorphonuclear leukocytes (neutrophils) contain structurally related proteins–serprocidins. The serprocidin family includes serine proteases: elastase, cathepsin G, proteinase-3 and also azurocidin/CAP37 which is proteolytically inactive because of amino acid substitutions within the catalytic triad. Serprocidins are cationic glycoproteins (25–37 kDa), which play a crucial role in neutrophil-mediated inflammatory response (innate immune response). They induce activation of endothelial and epithelial cells, macrophages and lymphocytes, and increase vascular permeability during neutrophil extravasation. Furthermore, they possess antimicrobial properties against Gram-negative and Gram-positive bacteria, fungi, and protozoa. This group of proteins also displays proteolytic activity against a variety of extracellular matrix components, such as elastin, fibronectin, laminin, and type IV collagen. The aim of this project was to clone and express the serprocidins in *Pichia pastoris* expression system. The

cDNA for the serprocidins was amplified from HL-60 cell line total RNA by RT-PCR and cloned in the pPICZαB vector. Several constructs with or without propeptides were designed with the hexahistidine tag and *c-myc* epitope attached at the C-terminus. After induction with 2% methanol recombinant proteins were secreted into the medium. The purification procedure included concentration of medium on Amicon ultrafiltration device (YM = 1), dialysis and gel filtration on Superdex G-75 in 0.05 M sodium acetate buffer, pH 5.5, containing 0.15 M sodium chloride. The presence of recombinant serprocidins was monitored by SDS/PAGE and Western blotting using anti-6His and anti-*c-myc* polyclonal antibodies. Using this procedure we obtained electrophoretically pure recombinant proteins.

# P13.40

## The effectiveness of ultraviolet light irradiation in porcine collagen dye and sorbitol-mediated photooxidation

[1]Agnieszka Jura-Półtorak, Grzegorz Wisowski, Ewa M. Koźma, Paweł Olczyk, Krystyna Olczyk

[1]*Department of Clinical Chemistry and Laboratory Diagnostics, Medical University of Silesia, Sosnowiec, Poland*
*e-mail: ajura@slam.katowice.pl*

Dye-mediated photooxidation is a physical method of animal collagen crosslink formation, as potential for use in bioprosthetic heart valve material. The collagenous tissues treated by the dye-mediated photooxidation are resistant to chemical and enzymatic degradation, simultaneously maintaining the physical properties of a natural tissues. The implanted bioprostheses are nonimmunogenic, biostable and resistant to calcification. The effectiveness of tissue collagen crosslinking depends on the conditions of photooxidation. Our previous study showed, that the application of sorbitol at 0.5% concentration in photooxidation process of pericardial collagen with visible light and methylene blue, may increase the new crosslink formation. The aim of the present study was the evaluation of the ultraviolet light influence on the extent of porcine pericardial collagen crosslinking, in the presence of methylene blue and 0.5% sorbitol concentration. The effectiveness of porcine pericardial collagen fixation was evaluated on the basis of photooxidized tissue sensitivity to pepsin digestion. The hydrolysates of collagen components were characterized by polyacrylamide gel electrophoresis followed by densitometric analysis. The extent of photooxidation-derived crosslinking of pericardial collagen was estimated on the basis of the amount of collagen compounds released from the fixed pericardial samples, i.e. aggregates of collagen $\alpha$ chains, single collagen $\alpha$(I) and $\alpha$(V) chains and collagen degradation products. In conclusion, the pericardial collagen dye-mediated photooxidation with ultraviolet light and 0.5% sorbitol resulted in the significant decline of the tissue sensitivity to enzymatic degradation in contrary to untreated pericardium. However, the collagen modification stated in samples irradiated with ultraviolet light was less intensified, as compared with the collagen photooxidized with dye, sorbitol and visible light.

# P13.41

## The effect of Neuromedin U on endocrine function of pancreas

Przemysław Kaczmarek, Tomasz Szkudelski, Dawid Szczepankiewicz, Tatiana Wojciechowicz, Maciej Sassek, Ewa Pruszyńska-Oszmałek, Krzysztof W. Nowak

*Departament of Animal Physiology and Biochemistry, August Cieszkowski University of Agriculture, Poznan, Poland*

Neuromedin U (NmU) is a regulatory peptide found in significant concentrations in both the brain and gut of the rat and is named according to its ability to powerfully contract the uterus. The role of NmU in feeding behavior and identification of two types of NmU receptors were recently described. In the present study, we examined the presence of NmU receptors in pancreatic islets using RT-PCR and Western blotting analysis. We also investigated NmU role in regulation of insulin secretion *in vitro* using isolated pancreatic islets. We have confirmed that NmUR1 but not NmUR2 is specifically expressed in isolated pancreatic islets. In all tested doses (1, 10, 100 nmol/l) NmU we observed dose dependently decreased insulin output by isolated pancreatic islets. These inhibitory effects of NmU on insulin secretion may suggest the involvement of NmU in regulating the pancreatic branch of adipoinsular axis function. Thus, NmU can be included in that group of anorectic peptides, which are also involved in regulation of insulin secretion.

# P13.42

## The influence of polysiloxanes on the stability and conformation of proteins

Leszek Kadziński[1], Wojciech Sawuła[1], Magdalena Prokopowicz[2], Jerzy Łukasiak[2], Bogdan Banecki[1]

[1]*Department of Molecular and Cellular Biology, Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk, Gdańsk, Poland;* [2]*Division of Physical Chemistry with Instrumental Analysis Laboratory, Medical Academy of Gdańsk, Gdańsk, Poland*

Polysiloxanes (commonly known as silicones) are compounds containing silicon and oxygen atoms with various organic sidechains. Thanks to their physicochemical properties, silicone polymers have been widely used in consumer applications like food industry and many others. However, the most controversial is their application in medical and pharmaceutical industry. Because of the conviction of low reactivity of polysiloxanes, they have

been widely used in medical devices like hydrocephalus shunts, cardiac pacers and many others. Silicones were also rapidly taken up by surgeons as a compatible material for implanting, especially as breast augmentations or reconstruction implants. However, in the last three decades it has been reported that silicones can exhibit some adverse clinical effects, like autoimmune and connective tissue disease. In our studies we have determined the influence of linear and cyclic polysiloxanes on the stability and structure of proteins like fibrinogen, hemoglobin and mioglobin, using fluorescence spectroscopy. The tertiary structure of these proteins has been found to be altered by both low and high molecular weight silicones. Furthermore, we have developed a method of detection of the conformational changes in collagen (the most abundant protein in mammals) exposed to polysiloxanes. We have demonstrated that these changes decrease the ability of collagen molecules to self-assemble into fibrils. The extent of fibrillogenesis can be ascertained by measuring the increase in turbidity of the solubilized collagen solution by absorption spectroscopy. We have also demonstrated the influence of incubation of soluble collagen with polysiloxanes on the thermodynamic properties of this protein using scanning microcalorimetry.

# P13.43

### Does *Saccharomyces boulardii* treatment of experimental colitis influence colonic mucin structure? – preliminary studies

Dariusz G. Kisiel, Iwona Radziejewska, Andrzej Gindzieński

*Department of Medical Chemistry, Medical University of Białystok, Poland*
*e-mail: kisieldg@amb.edu.pl*

*Saccharomyces boulardii* is non-pathogenic yeast, resistant to gastric acidity and proteolysis, isolated from lychee fruit and used first time for diarrhea treatment in France in 1950. The yeast is an example of probiotic, living organism, which has beneficial effect on the maintenance of intestinal micro flora balance. *Saccharomyces boulardii* is presently used for prevention and treatment of infectious diseases in alimentary tract, especially in antibiotic associated diarrhea. Pilot studies have shown *S. boulardi* beneficial in the maintenance treatment of inflammatory bowel disease. The exact mechanism remains to be determined. After oral administration, *Saccharomyces boulardii* reaches in the gastrointestinal tract high concentration level in a very short time. As far as we know, the yeast does not permanently colonize colonic mucosa and does not live except in the intestinal channel, covered with protecting layer of carbohydrate-rich glycoproteins – mucins. The aim of our study were preliminary investigations of the possibility of changes in monosaccharide composition of mucins, isolated from mice colon with TNBS (2,4,6-trinitrobenzene sulfonic acid) induced colitis, before and after treatement with *Saccharomyces boulardii*.

# P13.44

### Evaluation of perfluorinated organic acids ($C_6$-$C_{10}$) toxicity *in vitro* with use of human cell lines

Konrad Kleszczyński, Paweł Gardzielewski, Andrzej C. Składanowski

*Department of Molecular Enzymology, Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk, Gdańsk, Poland*
*e-mail: acskla@amg.gda.pl*

Perfluorinated organic acids (PFOA) are widely used in various industrial fields, however they are known to be environmentally bioaccumulative carcinogens and peroxisome proliferators in rodents. Up to now, only few studies have been carried out providing preliminary reports on their toxicological properties. In this study we present *in vitro* toxicity data for PFOA with increasing chain length. The evaluation has been made with cell viability assays using MTT, neutral red uptake and lactate dehydrogenase leakage. Due to the potentially hazardous impact of contact with PFOA, we have tested cells of human origin: colon carcinoma (HCT116), osteosarcoma (143 B) and normal dermal fibroblasts (N-HDF). During 24 h treatment we have observed a structure-effect relationship for group of tested compounds. $EC_{50}$, the concentration at which 50% decrease of viable cells is observed, decreased with elongation of fluorocarbon chain length ($C_6$>$C_7$>$C_8$>$C_9$>$C_{10}$). Estimated $EC_{50}$ values were different for three tested cell types and HCT116 line was found as the most sensitive. The effect of increasing time exposure (4, 12, 24, 48, 72 h) was also evaluated with the special focus on 400 μM PFDA (perfluorinated decanoic acid) as the most effective compound. Cells treated through 48 and 72 h showed a dramatic decrease of viability, up to 85 and 95% respectively. A visible decrease of viability has been found then also at low 50–100 micromolar concentrations. Comparison of the estimated $EC_{50}$ values for PFDA and normal decanoic acid revealed a great increase of toxicity after introducing fluorine atoms to the natural metabolite. We hypothesize now that though PFOA have not revealed an acute-type of cytotoxicity at low micromolar concentration, the results may indicate on their chronic effect.

# P13.45

### Modifications of hemolytic activity of polyene antibiotics in the presence of mono- and disaccharides

Agnieszka Knopik-Skrocka, Józef Bielawski, Anna Bochanysz, Karolina Nowak, Katarzyna Wrzeszcz

*Department of Cell Biology, Adam Mickiewicz University of Poznań, Poland*
*e-mail: askro@amu.edu.pl*

The effect of some mono- and disaccharides on the kinet-

ics of hemolysis induced by filipin (Fil) and amphotericin B (AmB) in pig, horse and human erythrocytes was investigated by absorption spectrophotometric method. Differences between mono- and disaccharides in their influence on the mechanism of hemolysis and on the susceptibility of erythrocytes to the two hemolytic agents are analyzed. The mechanism of Fil-induced hemolysis in the isoosmotic mono- and disaccharide media is of the damage type. The hemolytic activity of Fil is slightly stimulated or inhibited. The effect of mono- and disaccharides on the mammalian erythrocyte resistance does not differ significantly. Mono- and disaccharides in much higher extent modify the hemolysis induced by AmB. Evidently higher inhibition of AmB hemolytic activity has been found in the disaccharide, compared to the monosaccharide media. Some difference in the extent of inhibitory effect of disaccharides with similar molecular weight suggests that molecular weight is not the only factor modifying AmB hemolytic activity. Large differences were observed in the kinetics of hemolysis induced by AmB in the media of the two groups of saccharides. In the presence of monosaccharides, the kinetics has revealed deflexions from the typical colloid osmotic hemolysis obtained in KCl and in the disaccharide media.

## P13.46

### Effect of food restriction on glycogen level in rat heart

Ewa Kossowska, Tomasz Letmański, Elżbieta Goyke

*Department of Biochemistry, Medical University of Gdańsk, Gdańsk, Poland*
*e-mail: ewakos@amg.gda.pl*

Although glycogen occupies about 2% of the cell volume in the adult cardiac myocyte, its physiologic role in the heart is not completely understood. Endogenous glycogen is believed to be an important energy substrate for the heart during low oxygen states, providing glucose phosphate for glycolytic ATP production. Therefore, cardioprotective effect of glycogen in the stressed ischemic heart is possible. It is known that myocardial glycogen levels are increased with fasting, whereas in the liver undergo reduction. We investigated the effect of refeeding after caloric restriction diet on the glycogen level in heart of rat. The rats obtained every morning for 30 days 50% of total amount of food consumed by control group. After one month of such treatment animals was allowed free access to food for 48 h. The rats feeding 50% restriction diet and then refeeding ad libitum for 48 h attained significantly less body weight as compared to the control animals. Glycogen concentration do not change significantly in group rats given 50% restricted diet, but increased about 30% in group of rats given 50% restricted diet and then refed ad libitum for 48 h, as compared to control. This diet did not affect significantly serum glucose concentration. Therefore, cardiac myocyte possesses mechanism by which the cell senses exogenous fuel deficiency and stores up endogenous fuel to guard vital processes of cell.

## P13.47

### Studies of leptin receptors expression in the hypothalamus of fasted and refed young and old rats

G. Kotlarz[1], M. Młotkowska, Z. Kmieć, K. Hinc, J. Kubasik-Juraniec[2], A. Myśliwski

*[1]Department of Histology and Immunology, Medical University of Gdańsk, Poland; [2]Laboratory of Electron Microscopy, Medical University of Gdańsk, Poland*
*e-mail: zgkotl@amg.gda.pl*

Introduction: Leptin – a pleiotropic hormon playing a key role in energetic balance of the organism. During ageing there is a tendency to increase the amount of body weight, especially the white adipose tissue both in humans and animals. Molecular mechanism, responsible for expression of leptin receptors in the hypothalamus in response to fasting/refeeding, is not known. Our studies aimed to estimate the role of protein receptors (OB-Rb) for leptin in the hypothalamus using Western blott technique, to comparise the distribution of OB-Rb receptors for leptin in the brain and to correlate distribution of Ob-Rb receptors for leptin and serum leptin level. Methods: Studies were carried out on Wistar male rats 5 and 22 months old. Rats were fasted for 48 h and 96 h and then refed for 24 h. Protein receptors for leptin was detected using Western blott technique and immunocytochemically using DAB (avidin-biotin, Santa Cruz) test. Leptin concentration was determined by test of RIA (Linco-Research). Conclusion: In young control rats immunopositive reaction for leptin receptors was stronger than in old ones. In old rats fasted for 96 h immunopositive reaction (ICH test) was marked more intensively in some nuclei of the hypothalamus: ARN, VMN, LH. Lower level of leptin in serum of old rats is caused by a lower mRNA expression in the adipose tissue. Fasting for 96 h is strong enough to decrease leptin level. This phenomenon was observed in old rats submitted to IHC test. Probably fasting for 96 h excites leptin receptors especially in the hypothalamus of the old rats.

## P13.48

### Influence of the dietary factors on the rate of modification of oxidative DNA damage repair in newborn pigs

Paweł Kowalczyk[1], Aleksandra Bieleń[1], Daniel Laubitz[2], Romuald Zabielski[3], Barbara Tudek[1]

*[1]Institute of Biochemistry and Biophysics PAS, Warsaw, Poland; [2]The Kielanowski Institute of Animal Physiology and Nutrition, Warsaw, Poland; [3]Department of Physiological*

*Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, Warsaw, Poland*

The diet exerts an important effect on the development and functioning of the digestive tract. We were investigating the effect of supplementation of the diet of lactescent sows with grains rich in polyunsaturated fatty acids (PUFA: flax-seed, rape-seed 4 kg/100 kg of feed each) as well as L-carnitine (15 g/100 kg), taurine (100 g/100 kg) and vitamin E (15 g/100 kg) on the rate of oxidative DNA damage excision in colons of their offspring. Diet supplementation was performed from the 80th day of pregnancy till 28th day of lactation. Repair activities in colons of the offspring from sows maintained on supplemented diet were higher than in unsupplemented group already on the first day after delivery and stayed constant until the end of experiment (28th day of life). The repair activity for 8-oxoG and εA (measured by the nicking assay) increased about twice in pigs, whose mothers were fed with supplemented diet rich in PUFA. However, diet supplementation had no effect on the repair activity of ethenocytosine (εC). We also studied the effect of supplementation of pigs with different doses of iron. Iron was administered in single injection at the dose 75 mg or 200 mg after the 3rd day of birth. The iron dose 75 mg increased significantly the repair activity for all three damages, but higher dose, 200 mg increased only the activity of 8-oxoG and εA glycosylases but not εC glycosylases. Thus dietary factors can modulate repair capacity for 8-oxoG, and εA, but have no effect on excision rate of εC from pigs intestines.

## P13.49

### Two erythrocyte isoforms of histone H1.b in a Grey partridge (*Perdix perdix*) population

Andrzej Kowalski[1], Jan Pałyga[1], Ewa Górnicka-Michalska[1], Joanna Wawszczyk[1], Marek Adamski[2], Zenon Bernacki[2]

*[1]Department of Biochemistry and Genetics, Świętokrzyska Academy, Kielce, Poland; [2]Department of Poultry Breeding, University of Technology and Agriculture, Bydgoszcz, Poland*
*e-mail: a.kowalski@pu.kielce.pl*

H1 histones extracted from erythrocyte nuclei of Grey partridge (Perdix perdix) individuals were resolved into six nonallelic subtypes in an acetic acid-urea polyacrylamide gel. Using a two-dimensional polyacrylamide gel electrophoresis for a population screening we detected two allelic isoforms of histone H1.b (b1 and b2). As the protein spot H1.b2 migrated faster than H1.b1 in the two dimensional gel and with a similar mobility as the H1. b1 in the acetic acid-urea gel, it seems that histone H1.b isoforms may differ with apparent molecular weights. These allelic variants form two homozygous (b1 and b2) and one heterozygous (b1b2) phenotype. The phenotype b1 with a frequency of 0.475 prevailed in the tested population while the other one occurred at a frequency of 0.135. In order to find H1.b domain in which a difference between H1.b1 and H1.b2 is located, we performed a preliminary experiment in which electrophoretically concentrated and purified H1.b isoforms were cleaved with N-bromosuccimide (NBS). This agent cleaves histone H1 molecule at a single $Tyr^{71}$ creating a short N-peptide (from the N-terminus to the $Tyr^{71}$) and a longer C-peptide (from the 72 residue to the C-terminus). The NBS-generated C-peptides derived from histone H1.b1 and H1.b2 migrated with a similar electrophoretic mobility in one dimension SDS polyacrylamide gel. The NBS-cleaved H1.b N-peptides were invisible in the gel after staining with Coomassie-Blue due to their low-molecular weights. Thus, we assume that the expected structural differences between histone H1.b allelic isoforms from Grey partridge erythrocytes could be located between the N-terminus and the 70th residue of their molecules, that is in the region spanning the N-terminal domain and/or beginning part of the adjacent globular domain.

## P13.50

### The role of PMCA isoforms in maintenance of calcium homeostasis in PC12 cells

Anna Kozaczuk[1], Janusz Szemraj[2], Christos Kargas[1], Ludmila Zylinska[1]

*[1]Department of Molecular Neurochemistry, Medical University, Lodz, Poland; [2]Department of Medical Biochemistry, Medical University, Lodz, Poland*
*e-mail: kozaczuk_a@yahoo.com*

Low free intracellular $Ca^{2+}$ concentration is essential for a proper functioning of the cell. Increased $Ca^{2+}$ inside cell is frequently related with apoptosis. One of the most important mechanisms for maintenance of calcium homeostasis is the active removing of calcium ions by plasma membrane $Ca^{2+}$-ATPases (PMCAs). The enzyme is coded by four independent genes, and four main isoforms exist. PMCA1 and 4 are ubiquitous, whereas PMCA2 and 3 are present in excitable cells. The aim of our study was to determine the concentration of cytosolic $Ca^{2+}$ in PC12 cells with full set of PMCA isoforms, and in PC12 cell lines with suppressed isoform PMCA2, PMCA3 and both. Spectrofluorimetric analysis using Fura-2/AM -loaded cells revealed the difference in basal $Ca^{2+}$ concentration in examined cell lines. Suppression of PMCA2 and 3 also altered the profile of apoptotic and necrotic cells, as was determined by flow cytometry. These results indicate that changes in intracellular $Ca^{2+}$ concentration in undifferentiated PC12 cells related to altered PMCA composition could have profound consequences for development and survive of PC12 cells.

Supported by the grants: 2P05A 03529 and PBZ-MIN-012KBN/02/2005 from the Ministry of Education and Science, Poland; 503-686-02 and 502-16-197 from the Medical University of Lodz.

## P13.51

### The enzyme responsible for digestion of chicken ovoinhibitor between domains

Agnieszka Kubiak, Antoni Polanowski

*Department of Biochemistry and Molecular Biology, University of Wrocław, Wrocław, Poland*
*e-mail: Agnieszka.Kubiak@mars.uni.wroc.pl*

Chicken ovoinhibitor is a seven domain Kazal-type serine proteinase inhibitor, which is present in egg white along with ovomucoid and it is also a major proteinase inhibitor in chicken plasma. In our laboratory we purified owoinhibitor and its active fragments from chicken liver, therefore, an attempt was made to isolate an enzyme responsible for processing of ovoinhibitor. It was found that 50% ethanole extraction from chicken liver anabled the separation of proteins possessing proteolitic activity. The active proteins degraded both casein and ovoihibitor. Degradation did not occur in the presence of leupeptin, E-64, chymostatin and cystatin indicated that sulfhydryl proteinases were involved in the digestion proces. The enzyme responsible for this activity was purified through $(NH_4)_2SO_4$ fractionation, tertbuthanole precipitation, gel filtration on Sephadex G-75, ion exchange chromatography on SP-Sephadex C-25 and affinity chromatography on cystatin-Sepharose 4B. Isolated enzyme degradates ovoinhibitor onto products of molecular weight close to previously obtained fragments of ovoinhibitor (Western blot analysis) possessing antyproteolytic activity. Sequence analysis of N-terminal fragment of enzyme showed its homology to chicken cathepsin S.

## P13.52

### Application of diazonium salt in the quantitative determination of alkylresorcinols in biological samples – comparison of standard and modified colorimetric micromethod

Mariola Kulawinek, Agata Gajda, Arkadiusz Kozubek

*Institute of Biochemistry and Molecular Biology, University of Wrocław, Poland*

Alkylresorcinols (ARs) are 1,3-dihydroxybenzene derivatives with an odd-numbered alkyl chain at position 5 of the benzene ring, a structure that gives them amphiphilic properties. This is important aspect with regard to their analysis, absorption, metabolism and potential bioactivity [Kozubek A. & Tyman J.H.P. (1999) *Chem Rev* 99: 1]. They occur in many plants, and in some bacteria and fungi. To investigate the content of ARs in cereal grains, their main source, and the effects of processing on their content in food, a rapid analytical method has been needed. Most general methods used for the determination of phenolic compounds such as ARs are based on their colourgenic reaction with diazonium reagents e.g. an orange colour with diazotized-*p*-nitroaniline [Briggs DE (1974) *Pchytochemistry* 13: 987]. ARs can be also visualized by iodine vapor and they turn a bright-red colour with anisaldehyde-sulphuric acid reagent [Reusch RN, Sadoff HL (1979) *Bacteriol* 139: 448]. Fast Blue B (methoxy-4-nitroaniline), a dye that stains phenolic compounds with resorcinolic ring to deep crimson depending on chain-length, has been commonly used for visualisation of AR on TLC plates. Nowday two Fast Blue B salts are available: $BF_4$ and Zn. Fast Blue $BxBF_4$ has been used for semi-sensitive estamination of alkylresorcinols [Tłuścik F *et al.* (1981) *Acta Soc Bot Polon* 50: 645]. This method is highly specific for 5-n-alkyl derivatives of resorcinols but has several disadvantageous properties e.g. carcinogenic and mutagenic effects of Fast Blue $BxBF_4$. Another diazonium salt: Fast Blue BxZn has been concluded to be used instead of Fast Blue $BxBF_4$ for finding more effective method of determination of ARs. This modified, fast and cheap procedure is more sensitive and about two fold more effective than standard method of determination of ARs with Fast Blue $BxBF_4$.

## P13.53

### Molecular and cellular effects of tacalcitol on human adenocarcinoma cell line Caco-2 in culture

Dariusz Kuśmierz, Alkesandra Zielińska, Dominika Nawrocka, Elektra Sliupkas-Dyrda, Małgorzata Latocha

*Department of Cell Biology, School of Pharmacy, Medical University of Silesia, Sosnowiec, Poland*
*e-mail: dkusmierz@slam.katowice.pl*

The last years brought new findings of the vitamin $D_3$ and its derivatives' role in the processes which control the cells' differentiation and proliferation. This properties have been already used in treatment of some disorders, which are caused by disruption balance between proliferation, differentiation and cell death, for example: psoriasis. Moreover, clinical trials are undertaken using some of vitamin $D_3$ derivatives as a therapeutic substances interfering with cell cycle regulation in autoimmune diseases and cancer. The aim of this work was to estimate the molecular and cellular effects of tacalcitol on human adenocarcinoma cell line Caco-2 in culture We analyzed survival and proliferation ability of Caco-2 cell line treated with tacalcitol in concentration of $10^{-3}$ to $10^{-9}$ M for 24, 48 and 72 h. Quantitative determination was made by the fluorescent marking technique. There were also used the trypan blue and DAPI tests which allow to state cells vitality and detect the presence of apoptotic cells. The RNA was isolated only from the chosen cell cultures. With the QRT-PCR technique we analyzed the expression of the proliferation markers' genes (histon H3 gene), pro-apoptotic genes (*BAX*, *FAS*) and anti-apoptotic genes (*BCL-2*). Our findings shown the tacalcitol's negative influence on the proliferation of the examined cells and we proved its

destruction function which is caused by the increased activity of chosen pro-apoptotic cells.

## P13.54

### Nitrosative stress on yeast: role(s) for flavohemoglobin and its impact on ferrireductase(s) activity

Anna Lewińska[1], Grzegorz Bartosz[1,2]

[1]*Department of Biochemistry and Cell Biology, University of Rzeszów, Rzeszów, Poland;* [2]*Department of Molecular Biophysics, University of Łódź, Łódź, Poland*
*e-mail: lewinska@univ.rzeszow.pl*

It has been postulated that flavohemoglobins from unicellular organisms may have similar functions but their exact roles must be elucidated. The proposed functions of these proteins include: oxygen transport, sensing and delivery; nitrite reduction; alkylhydroperoxide activity; reduction of siderophore-bound iron; protection from oxidative and/or nitrosative stress. Despite many studies, the physiological role of *Saccharomyces cerevisiae* flavohemoglobin is still a matter of controversy. We studied the effect of lack of *YHB1* gene on the phenotype of disruptant strain. Our results confirmed previous suggestions, that Yhb1 protein may act in nitric oxide detoxification since we showed concentration-dependent delay and inhibition of growth of the mutant lacking the Yhb1 protein after exposure to reactive nitrogen species e.g. peroxinitrite or GSNO. Also, intracellular level of NO after incubation of *Δyhb1* cells with NO-releasing agents was elevated with respect to parental strain. The largest difference in the NO level was noticed after addition such NO donors like GSNO, DETA NONOate, SIN-1, SNP and S-NAP. Additionally, no growth impairment of disruptant was seen after exposure to sources of reactive oxygen species (hydrogen peroxide, *t*-butyl hydroperoxide, cumene hydroperoxide, menadione, paraquat, juglone, chloramine and sodium hypochlorite) or to atmosphere of pure oxygen. These results suggest an indirect effect of Yhb1 protein in the protection against oxidative stress only by consuming NO and thus preventing inhibition mitochondrial respiration and enhanced ROS generation. We observed also a link between the presence/lack of functional *YHB1* gene and changes in activities of transmembrane electron transfer system (ferrireductase activity).

## P13.55

### Changes in intercellular adhesion molecule 1 expression and calcium homeostasis modulation by potassium channel openers in EAhy 926 cells

Agnieszka Łojek[1,2], Antoni Wrzosek[2], Adam Szewczyk[2], Krzysztof Dołowy[1]

[1]*Department of Biophisics, Agricultural University SGGW, Warsaw, Poland;* [2]*Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland*

EAhy 926 cells are a cell line derived by fusing human umbilical vein endothelial cells with the cell line A549. Treatment of the cell with tumor necrosis factor $\alpha$ (TNF-$\alpha$) caused an expression of intercellular adhesion molecule 1 (ICAM-1) an indicator of inflammatory responses of endothelial cells. Large conductance calcium-activated potassium channels ($BK_{Ca}$ channels) opener CGS7184 caused appearance of lower molecular mass ICAM-1 upon TNF-$\alpha$ stimulation of EAhy 926. The similar result was obtained when EAhy 926 cells were treated with thapsigargin an endoplasmic reticulum calcium pump inhibitor. The possible involvement of $Ca^{2+}$ pathway in this process was considered and the influence of mitoB-$K_{Ca}$ channel opener, CGS 7184, on calcium homeostasis in EAhy 926 cells was tested. Addition of CGS7184 to EAhy 926 cells at micromolar concentration caused an increase in $Ca^{2+}$ level as indicated with FURA-2 measurements. When calcium ions were absent in the external medium CGS7184 caused release of $Ca^{2+}$ from internal stores. Supplementation of external incubation medium with $Ca^{2+}$ resulted in increase of FURA 2 fluorescence ratio. The process was very similar to that observed upon addition of thapsigargin in process of capacitive calcium entry. However, some discrepancies were observed after addition of $Mn^{2+}$ (0.1 mM) to the incubation medium containing $Ca^{2+}$. The quenching of FURA-2 fluorescence by $Mn^{2+}$ was much faster for EAhy 926 cells treated with CGS7184 than with thapsigargin. It seems that CGS7184 mitoBK$_{Ca}$ channels opener influence not only endoplasmic reticulum but also involves the mitochondria and plasma membrane channels in calcium homeostasis in EAhy 926 cells.
This work was supported by President of Agricultural University SGGW.

## P13.56

### Gene encoding a new replication protein (RepRK) from plasmid pIGRK: expression characterization and optimalization

Anna Mazurkiewicz-Pisarek, Piotr Zaleski, Andrzej Płucienniczak[1]

[1]*Biotechnology and Antibiotics Institute, Bioengineering Department, Warsaw, Poland*
*e-mail: mazurkiewicza@iba.waw.pl*

Plasmids are (typically) circular double-stranded DNA molecules separate from the chromosomal DNA and capable of autonomous replication. They usually occur in bacteria, sometimes in eukaryotic organisms. Our department is attempting to isolate new plasmids that have the potential to be applied in biotechnology. Two examples of such plasmids are pIGMS31 (AY543072) and pIGRK (AY543071) isolated from the *Klebsiella pneumoniae* strain. In order to identify potential applications

it was necessary to determine their molecular biology and mechanism of replication. Two proteins RepMS and RepRK were identified as being responsible for replication of the plasmids. Analysis of amino acid sequences of these proteins showed that they belong to a *rolling circle replication* model group. Therefore it is important to obtain purified proteins RepMS and RepRK. The gene encoding RepRK protein sequence was cloned into pT7RS expression vector (AY923866). *E coli* BL21(DE3) strain was transformated with recombinant vector and Rep RK protein expression was obtained. After IPTG induction two bands were revealed by SDS electrophoresis in 12% polyacrylamide gel. The first one matched the molecular mass of protein RepRK (~30 kDa), the second had molecular mass of 20 kDa. Gene *repRK* sequence analysis showed that this sequence contains six ATG in open reading frame (ATG1-ATG6). Two of them have strong Shine-Dalgarno sequences (SD1-SD2). In order to increase protein RepRK expression, mutagenesis ATG1→CTG was performed. Comparison of the proteins by SDS electrophoresis in 12% polyacrylamide gel before and after mutagenesis showed that place ATG1 is not responsible for presence of additional band after IPTG induction therefore during next stage mutagenesis ATG2→CTG will be performed.

## P13.57

### The role of lysosomal iron in dinitrosyl iron complexes formation

Sylwia Męczyńska, Hanna Lewandowska, Marcin Kruszewski

*Institute of Nuclear Chemistry and Technology, Warszawa, Poland*
*e-mail: mesyl@ichtj.waw.pl*

Recently, it has been proved that the labile iron pool (LIP) remains in the form of complexes with low molecular mass ligands, labile enough to enter the Fenton reaction. This pool participates in control of the activity of iron containing proteins, cellular iron transport. It was also showed that labile iron pool takes part in the regulation of nitric oxide-dependent biochemical pathways through the forming dinitrosyl iron complexes (DNIC). These complexes are physiologically important transducers of nitric oxide. It has been shown that low molecular-weight DNIC possesses endothelium derived relaxing factor (EDRF) activity and modulate redox properties of the cellular interior through the inhibition of glutathione-dependent enzymes, such as transferase, reductase or peroxidase of glutathione. It is also known to inhibit the activity iron-sulphur cluster proteins, such as aconitase and DNA glycosylases. Formation of nitrosyl iron complexes, known as 2.03 complexes due to the G value of their characteristic EPR spectra, has been found in many kinds of plants, animals and bacteria. The sources of iron forming DNIC *in vivo* are still not precisely defined, one of the putative sources being iron proteins and another the labile iron pool. Neither are defined the cellular compartments, in which DNIC are formed. It was proposed that the most important part of the cellular LIP is located within lysosomes. In the present report we discuss the contribution of lysosomal iron to the formation of dinitrosyl iron complexes in living cells. To access two different pools of cellular LIP we used a permeable iron chelator, SIH, and lysosomotropic iron chelator, DFO. K-562, human myelogenous leukemia cells, (25 ml culture of density approximately $1 \times 10^6$ cells/ml) were treated with 1 mM DFO or 100 μM SIH in complete medium at 37°C for 1, 3 or 6 h in humidified 5% $CO_2$-incubator. In another series of experiments lysosomal proteolysis was inhibited by treatment with an inhibitor of lysosomal acidification, $NH_4Cl$. Cells were treated with 10 mM $NH_4Cl$ in complete medium at 37°C for 2, 4 or 6 h. After pretreatment cells were subjected to 70 μM DEANO (nitric oxide donor) to generate DNIC. Final concentration of NO was 100 μM. We showed that depletion of lysosomal labile iron pool by either chelation with deferoxamine or lysis inhibition leads to a considerable decrease (down to 50%, depending on the incubation time) of DNIC forming in the cells under the influence of nitric oxide. Depletion of total cellular LIP with permeable iron chelator, SIH, resulted in the similar decrease of DNIC formation. This would indicate a vital role of lysosomal labile iron in DNIC formation. Taken together, our present and previous results confirm the thesis on the considerable contribution of lysosomal iron to the total labile iron pool in the cell.

## P13.58

### The participation of reactive oxygen species in oxidation and reduction of iron in human placental microsomes

Ryszard Milczarek, Ewa Sokołowska, Anna Hallmann, Jerzy Klimek

*Department of Pharmaceutical Biochemistry, Medical University of Gdańsk, Gdańsk, Poland*
*e-mail: klimek@amg.gda.pl*

Free radicals and reactive oxygen species (ROS) play an important role in normal physiology but their overproduction may be the cause of some disease states. We proposed that endoplasmic reticulum of syncytiotrophoblast may be an important source of lipid peroxides in pregnancy. During NADPH- and iron-dependent lipid peroxidation placental microsomes produce similar amount of TBARS to placental mitochondria. The key role in mechanism of NADPH- and iron-dependent lipid peroxidation is played by interconversion of $Fe^{2+} \leftrightarrow Fe^{3+}$ and probably $Fe^{2+}/Fe^{3+}$ ratio. Mechanism of this interconversion is not clear. Thus the goal of this study was to examine the participation of ROS in $Fe^{2+} \leftrightarrow Fe^{3+}$ interconversion. Presence of NADPH-generating system (NADPH-GS) with $Fe^{3+}$ or $Fe^{2+}$ together cause strong rise in TBARS synthesis in human placental microsomes. When $Fe^{3+}$ was added TBARS

production was linear during incubation. If $Fe^{2+}$ was added lag phase lasting 10 min was observed and after this time TBARS quantity rose up at the rate similar to that observed when $Fe^{3+}$ was present in the incubation mixture. It can be explained that $Fe^{2+}$ have to be oxidized to achieved the proper $Fe^{3+}/Fe^{2+}$ ratio. Thus we measured iron oxidation and reduction in the presence of microsomes and in the presence of air. $Fe^{2+}$ was oxidized during incubation with microsomes with or without NADPH-GS. In the presence of NADPH-GS $Fe^{2+}$ was oxidized slower. SOD inhibited $Fe^{2+}$ oxidation. Catalase strongly accelerated $Fe^{2+}$ oxidation. NADPH-GS strongly accelerate ferric ion reduction. Heat inactivated microsomes could not reduce $Fe^{3+}$ to $Fe^{2+}$. It indicates that iron reduction in human placental microsomes is an enzymatic process. On the basis of this results we conclude that during NADPH- and iron lipid peroxidation in human placental microsomes reactive oxygen species take part in iron oxidation.

# P13.59

## Effect of ultrasound and phthalocyanines on nucleated erythrocytes membrane

Katarzyna Miłowska, Teresa Gabryelak

*Department of General Biophysics, University of Lodz, Lodz, Poland*
*e-mail: kasmilo@interia.pl*

A wide spectrum of ultrasound (US) application makes risk for human health, because high doses of ultrasound could induce numerous undesirable effects. Risk of threat for human health induces to investigation of effects and mechanisms their biological activity. Especially interesing process is a cavitation and its consequences as well as possibility of and used this phenomenon in tumor theraphy. The ultrasound-dependent enhancement of cytotoxic activities of chemicals (sonosensitizers) on tumor cells, is a promising modality for cancer treatment and it is known as sonodynamic therapy (SDT). The US can be focused in a small region and can penetrate deep in tissue and locally activate a preloaded sonosensitizer. However, the mechanism of SDT has not been fully elucidated. In this work, carp erythrocytes were used as nucleated cell model to test the hypothesis that ultrasound exposure can cause the change in plasma membranes. The erythrocytes were exposed to 1 MHz continuous wave at the intensities of 0.61–2.44 W/cm$^2$ for 5 min. These results showed that ultrasound sonication at the intensities of 1.90 and 2.44 W/cm$^2$ led to an increase in the degree of hemolysis. Ultrasound exposure caused increease in the fluidity of the surface of plasma membrane. Furthermore two phthalocyanines (zinc and chloroaluminum) have been tested as potential sonosensitizers for sonodynamic therapy. It was noticed that common US and phthalocyanines exposure led to an increase in the level of hemolysis and membrane fluidity in the surface region of lipid bilayer.

# P13.60

## Effect of creatine supplementation on antioxidant status in patients with chronic liver disease

Katarzyna Morawska-Staszak[1], Grzegorz Mielcarz[2], Krzysztof Linke[1], Aleksander Barinow-Wojewódzki[3]

*[1]Department of Gastroenterology and [2]Department of Chemistry and Clinical Biochemistry University of Medical Sciences in Poznan; [3]Provincial Specialize Hospital of Pulmunology in Ludwikowo/Poznan, Poland*

Introduction: Liver is the main organ for creatine biosynthesis and copper, zinc and selenium metabolism. Loss of liver function with chronic liver disease and ability to synthesize creatine in adequate amounts may underpin muscle loss and decrease total antioxidant body status. Objective: We investigated the *in vivo* effect of creatine supplementation on plasma total antioxidant status and plasma selenium, copper and zinc concentration in patients with chronic liver disease. We have suspect that creatine supplementation can improve liver function and increase not only physical efficiency, but also antioxidant status in these patients. Methods: All subjects were fasted overnight. 27 subjects were given 4 × 5 g/day starch as a placebo for one week. The other 27 patients with chronic liver disease were oral creatine supplemented 4 × 5 g/day for one week. Plasma selenium, copper and zinc were determined by atomic absorption spectroscopy (Perkin-Elmer Zeeman 3030). The "antioxidant power" of plasma was measured as FRAP (ferric reducing ability of plasma) assay, in which ferric to ferrous ion reduction at low pH causes a coloured ferrous-tripyridyltriazine complex form. It was done using Multiscan MS microplate reader. Results: After one week of creatine supplementation the group of patients with chronic liver diseases has a value of FRAP significantly higher ($P < 0.05$), then before supplementation. Concentration of plasma selenium was also higher ($P < 0.05$), but concentration of copper and zinc was not significant different ($P = 0.082$ and $P = 0.054$, respectively). We did not find significant differences in the placebo group. Conclusion: We concluded that creatine supplementation was effective in patients with chronic liver disease, indicating an indirect effect of creatine on plasma antioxidant status *in vivo*.

# P13.61

## Pig erythrocytes membrane damage induced by sodium glycodeoxycholate – effect of solution composition

Lucyna Mrówczyńska, Edyta Bonawenturska, Józef Bielawski

*Department of Cell Biology, Adam Mickiewicz University, Poznań, Poland*
*e-mail: lumro@amu.edu.pl*

Bile salts are known to induce cell membrane damage be-

cause of their detergent potency. Red blood cells are used as a simple model system for study disruptive interaction of amphiphiles with plasma membrane. The aim of present study was to investigate the kinetics and mechanism of hemolysis induced by hydrophobic bile salt, sodium glycodeoxycholate (GDC), in different combinations of solution composition. Washed pig erythrocytes were incubated in buffered media (pH 7.4, 37°C) containing increasing concentration of GDC. The hemolysis was quantified spectrophotometrically. The cytotoxicity of GDC was found to be both dose and time dependent and the mechanism of GDC-induced hemolysis was permeability type (colloid-osmotic). The effect of solution composition on GDC hemolytic potency was studied by replacing of standard buffer with divalent cations solutions. It was found that both calcium and magnesium cations stimulated GDC-induced hemolysis in the similar manner. To explain these effects, it was assumed that the changes in ionic strength affect the adsorption of GDC monomers to the erythrocyte membrane and/or their capacity to form more cytotoxic dimers or polymers co-aggregated in the rigid cholesterol-rich lipid bilayer region. Red blood cells were also incubated in standard buffer with GDC and other hydrophobic cholates (deoxycholate, lithocholate) simultaneously and first preincubated with sublytic concentration of other cholates followed by GDC. In both systems, the addition of other cholates to solution led to stimulation of GDC-induced hemolysis. These results suggest interesting questions concerning the capacity of cholates monomers to interact with other molecules.

## P13.62

### Cytogenetic study of pro-oxidative and antioxidative effects of 2,2′-azobis[2 amidinopropane]dihydrochloride (AAPH), hydrogen peroxide ($H_2O_2$), tryptophan and Phenol Red in human lymphocytes *in vitro*

Aleksander Myszka[1], Maciej Wnuk[1], Agata Piestrzyńska-Kajtoch[2], Monika Bugno[2], Zbigniew Kotylak[1], Ewa Słota[2]

*[1]Department of Genetics, University of Rzeszow, Rzeszow, Poland; [2]Department of Immuno and Cytogenetics, National Research Institute of Animal Production, Balice, Poland*
*e-mail: amyszka@univ.rzeszow.pl*

The aim of this study was to characterize the genotoxic as well as cytotoxic effects of 2,2′-azobis[2-amidinopropane] dihydrochloride (AAPH) and hydrogen peroxide ($H_2O_2$) on human lymphocytes *in vitro* in the presence or absence of tryptophan or Phenol Red. The exposure of cell cultures to AAPH and $H_2O_2$ was long-term. The cytokinesis-block micronucleus assay (CBMN) was applied to score micronuclei (MN), nucleoplasmic bridges (NPB), apoptotic and necrotic cells, nuclear division index (NDI) and cytotoxicity nuclear division index (CNDI). *In vitro* results showed that AAPH has a powerful cytotoxic effect whereas $H_2O_2$ is mainly responsible for chromosome damage and generation of micronuclei. There was also set an experiment *in vitro* with 0.05 mg/ml tryptophan and 0.01 mg/ml or 0.02 mg/ml Phenol Red added to cell culture in comparison to control. Analysis did not show any genotoxic effects of tryptophan and Phenol Red, but both compounds narrowly reduced the number of dividing cells and slightly demonstrated cytotoxic properties. Generally, tryptophan and Phenol Red, as antioxidants, decreased the number of micronuclei, nucleoplasmic bridges, necrotic and apoptotic cells. Tryptophan had stronger protective effect against genotoxicity of $H_2O_2$ than Phenol Red. Tryptophan or Phenol Red which were simultaneously added to cell cultures with hydrogen peroxide caused also cytostatic effect, probably by changing the cell redox status. These results suggested that some compounds can exhibit antioxidative properties as well as pro-oxidative. These low molecular weight compounds may be useful to enhance cytostatic and cytotoxic effects of many drugs and xenobiotics in medicine.

## P13.63

### YxaB participates in biofilm formation by *Bacillus subtilis*

Krzysztofa Nagórska[1], Arletta Nowodworska[2], Michał Obuchowski[2]

*[1]Uniwersytet Gdański, Katedra Biologii Molekularnej, Gdańsk, Poland; [2]Akademia Medyczna w Gdańsku, Międzyuczelniany Wydział Biotechnologii UG-AMG, Gdańsk, Poland*
*e-mail: quinia2@poczta.onet.pl*

Wild strains of spore-forming bacterium *Bacillus subtilis* are capable of forming architecturally complex communities of cells called biofilms. Critical to biofilm formation are the *eps* and *yqxM-sipW-tasA* operons, which are believed to be responsible for biosynthesis of an exopolysaccharide that binds chains of cells together in bundles. Unexpectedly, we have found *yxaB* gene, which appears to be very important to biofilm formation in spite of the fact that does not belong to any of those transcriptional units. Aminoacid sequence analysis of YxaB protein suggests that this enzyme is localized outside the cell and participates in the last stage of EPS synthesis understood as polimerization. However, direct evidences for a role in biofilm formation derive from the microscopic observations. They have showed differences in three-dimensional structure of those highly complex communities in case of either deletion or overexpression of *yxaB* gene in the variety of genetic backgrounds. Further insights on the role of *yxaB* in EPS biosynthesis were obtained from *yxaB* gene expression analysis, which proved a high level of *yxaB* expression in the mature biofilm. The further studies shed some light on regulatory pathway of *yxaB* as we believed there is a direct relationship between stress regulon which is under control of $\sigma^B$ and expression of *yxaB*

gene. It is also worth to point out that expression of *yxaB* increases in the presence of glucose, which supports our hypothesis that *yxaB* gene participates in EPS biosynthesis in *Bacillus subtilis*.

## P13.64

### Plasma homocysteine levels in pregnant women with birth defects' foetuses

Navumau A.V., Zolotukhin M.M., Plotski A.R.

*Grodno State Medical University, Grodno, Belarus*

Homocysteine (Hcy), a sulfur-containing amino acid, is involved in many metabolic pathways including *trans*-sulfuration in cysteine synthesis, *re*-methylation in methionine synthesis, *trans*-methylation of DNA, proteins, and lipids, and biosynthesis of small hormonal and neuronal signaling molecules. The normal range for total plasma homocysteine in adults is 5 to 15 μM with an average of 10 μM. Abnormal elevation of total plasma homocysteine is classified as moderate (16–30 μM), intermediate (31–100 μM), or severe (> 100 μM) hyperhomocysteinemia (Kang *et al.*, 1992). Numerous epidemiological studies have reported that as a risk factor, moderate hyperhomocysteinemia is associated with: 1) cardiovascular diseases; 2) neuropsychiatric disorders; 3) developmental disorders such as neural tube defects; 4) complications of pregnancy. The concentration of plasma Hcy is regulated by several factors including genetically determined alterations of relevant enzymes, nutritional status, underlying diseases, certain medication, age and pregnancy. However, pregnancy is known to be the only factor that specificaly decreases the concentration of plasma homocysteine. Elevated homocystein concentrations during pregnancy are associated with an increased incidence of spontaneus abortion, intrauterine growth restriction, placental infarction and complications of pregnancy, but the data concerning these problems is controversial. The aim of the studies was to measure the concentration of plasma homocysteine in both healthy pregnant women and those with diagnosed birth defects which were confirmed later after either delivery or artifitial abortion. Terms of sampling varied from 2 to 3 trimester. Blood samples were collected into tubes containing heparine, immediately chilled in ice and centrifuged. Samples prepared at the day of analyses. Total homocysteine was determined by direct HPLC after borohydride reduction with coulometric detection. Control group were women (34) who had live births that were unaffected by any birth defects. Another group comprised pregnant women with various birth complications. Women from control group have a moderate hyperhomocysteinemia. This may be a result of inappropriate periconceptional consumption of vitamins (more than 80% of pregnant women have a marked folate deficiency). Pregnant women with birth complications have significantly more severe hyperhomocysteinemia ($P < 0.01$). We found no difference in homocysteine plasma concentration compared to controls group in the cases of skeletal dysplasia, kindey and lung polycystosis and pathology of abdominal walls (omphalocele, gastroschysis and diaphragmal herinae). Our findings suggest that hyperhomocysteinemia could associated with certain birth defects and determination of Hcy level should be considered as essential for appropriate control of vitaminisation of both pregnant women and women before pregnancy.

## P13.65

### Mechanisms of defense against pathogen in plant *Chelidonium majus*

Nawrot R.[1], Mazur H[1], J. Łupicka[2], A. Goździcka-Józefiak[1]

*[1]Adam Mickiewicz University, Poznań, Poland; [2]Poznań University of Medical Sciences, Poznań, Poland*

Plants are constantly exposed to a great variety of potentially pathogenic organisms, such as viruses, fungi, bacteria, protozoa, mycoplasma and nematodes, and can be affected by adverse environmental conditions. Plants respond to invasion by pathogens with an array of biochemical and genetic changes, including the production of reactive oxygen species, antimicrobial proteins, antioxidants and signal molecules such as salicylic acid and jasmonic acid. Our study indicated that yellow sap from *Chelidonium majus* which has bacteriostatic and antiviral activity is also a very complex mixture of different proteins such as: nuclease, lectin and peroxidase. In this paper we report on the isolation and characterization of a 40 kDa NADH dehydrogenase and superoxide dismutase from this sap. 2DE and mass spectrometry (LC-MS/MS) were used to analyze total proteins from the sap. MS was conducted in the Polish Academy of Sciences in Warsaw. The activity of NADH dehydrogenase and superoxide dismutase was analyzed by spectrophotometry method and stained in gel. The role of two proteins in the defense mechanisms of *Ch. majus* will be discussed.

## P13.66

### Synergistic effects of *CYBA* and *PON1* genes polymorphisms and traditional risk factors on coronary artery disease

Niemiec Paweł[1], Balcerzyk Anna[1], Sarecka Beata[1], Żak Iwona[1], Wita Krystian[2], Wróbel Wojciech[2]

*[1]Department of Biochemistry and Medical Genetics, Medical University of Silesia, Katowice; [2]The First Department and Clinic of Cardiology, Medical University of Silesia, Katowice, Poland*

Background: Products of *CYBA* and *PON1* genes are involved in redox homeostasis of vascular cells. *CYBA* gene encodes p22phox protein, a component of NAD(P)H

oxidase, which is the main source of superoxide anion. *PON1* gene encodes an enzyme metabolizing lipids peroxides and protects its accumulation on LDL. The PON-1 activity is partially determined by common *PON-1* gene polymorphism Gln192Arg. 242T variant of *CYBA* gene causes increased LDL oxidation. As LDL oxidation plays an important role in the pathogenesis of atherosclerosis these two polymorphisms may affect coronary artery disease (CAD). Aim: Assessing a possible relationship between *PON1* or *CYBA* genes polymorphisms and CAD and estimating whether these polymorphisms modify the risk of CAD associated with traditional risk factors. Materials and Methods: We analyzed 329 individuals including: 165 patients with angiographically documented premature CAD, and 164 healthy blood donors without history of CAD. Both polymorphisms were genotyped using PCR-RFLP method. The results were analyzed using STATISTICA software. Results: We found the association between Q allele carriers of *PON1* gene and increased risk of CAD (OR = 3.68, P = 0.007). There was also observed strong synergistic effect of *PON1* gene polymorphism and smoking on risk of CAD (synergy index S = 16.25). Although there was no association between *CYBA* gene polymorphism and CAD, synergistic effect of *CYBA* polymorphism, smoking and elevated level of total cholesterol (≥ 200 mg/dl) was shown (S = 5.28). Conclusions: The molecular basis of atherosclerosis and CAD may be determined by polymorphic variants of *CYBA* and *PON1* genes, especially in the presence of some traditional risk factors.

## P13.67

### Gene-gene and gene-environment interactions between *ACE* and *PAI-1* polymorphisms and traditional risk factors of coronary artery disease

Niemiec Paweł[1], Sarecka Beata[1], Balcerzyk Anna[1], Żak Iwona[1], Krauze Jolanta[2], Trusz-Gluza Maria[2]

*[1]Department of Biochemistry and Medical Genetics, Medical University of Silesia, Katowice; [2]The First Department and Clinic of Cardiology, Medical University of Silesia, Katowice, Poland*

Background: The susceptibility to coronary artery disease (CAD) may be determined by environmental factors interacting with variants of polymorphic genes, encoding isoforms involved in atherogenesis. Angiotensin-1 converting enzyme (ACE) plays a major role in synthesis of potentially proatherosclerotic angiotensin II. Insertion-deletion polymorphism of *ACE* gene is related to increased plasma ACE activity. Plasminogen activator inhibitor-1 (PAI-1) is responsible for fibrinolysis inhibition. The –675_–674insG polymorphism in *PAI-1* gene is associated with differences in transcription activity. Aim: Estimating relationships between *ACE* or *PAI-1* genes polymorphisms and CAD and analysis of gene-gene and gene-environment interactions. Materials and methods: We analyzed 137 angiographically documented CAD patients and 158 healthy blood donors without history of CAD. *ACE* and *PAI-1* polymorphisms were evaluated by PCR and PCR-RFLP methods, respectively. Data were analyzed using STATISTICA software. Results: Homozygotes DD of *ACE* and *PAI-1* 5G allele carriers were more frequent in CAD patients than in controls (OR = 2.08, P = 0.024 and OR = 2.52, P = 0.0038, respectively). Combined analysis revealed that *ACE*(DD)+*PAI-1*(5G5G+4G5G) variant strongly differentiated CAD patients from controls (OR = 3.14, P = 0.005). We found synergistic effect between *ACE* DD genotype as well as *PAI-1* 5G allele carrier-state and smoking (synergy index S = 2.09 and S = 2.28, respectively). The strongest synergy with smoking was observed for *ACE*(DD)+*PAI-1*(5G5G+4G5G) combination (S = 3.03). Conclusion: Our results indicate association between *ACE* DD genotype or *PAI-1* 5G allele carrier-state and CAD, especially if both are present. Synergistic effects of ACE and PAI-1 isoforms with smoking may confirm presumptions that its proatherosclerotic variants may increase system susceptibility to disruptions caused by environmental factors.

## P13.68

### Simultaneous ontogenetic lesion of noradrenergic and dopaminergic neurons and 5-hydroxytryptamine level in the brain of adult rats

Przemysław Nowak, Ryszard Szkilnik, Aleksandra Bortel, Joanna Dąbrowska, Grażyna Szczerbak, Janusz Niwiński, Adam Kwieciński, Ryszard Brus

*Department of Pharmacology, Medical University of Silesia, Zabrze, Poland*
*e-mail: pharbrus@slam.katowice.pl*

*N*-(2-chlorethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4), an alkylating neurotoxin selective for noradrenergic neurons, crosses the blood-brain barrier and produces long-lasting noradrenergic denervation. Another common neurotoxin, 6-hydroxydopamine (6-OHDA), unable to cross the blood-brain barrier but often administered into the lateral ventricles (*icv*) of brain, is frequently used to destroy dopaminergic neurons, while simultaneously inducing sprouting and hyperinnervation of brain by serotoninergic fibers. In this study the reactivity of the central dopamine receptors was examined in adult rats lesioned as neonates, simultaneously with DSP-4 and 6-OHDA. Newborn male Wistar rats were injected with DSP-4 (50 mg/kg *sc* twice: day of birth and 3rd day of life) and on 3rd day of life with 6-OHDA (135 g *icv*, half in each lateral ventricle). Control newborn rats were injected with saline (*sc, icv*). At 8–10 weeks the analysis of biogenic amines and their metabolites (NA, MOPEG, DA, DOPAC, HVA, 3-MT, 5-HT, 5-HIAA) in the striatum, frontal cortex, hipocampus and cerebellum was performed by HPLC/ED technique. We found that NA levels in the hip-

pocampus and frontal cortex of adult rats injected with DSP-4, *sc* twice, as neonates were significantly reduced, but increased in the cerebellum. No changes in DA and other amine levels were observed. 6-OHDA applied to newborn rats *icv* on the 3rd day significantly decreased DA and DOPAC levels in the striatum and frontal cortex, and increased 5-HT and 5-HIAA in the striatum of adult rats, as compared to the control. Simultaneous injection of DSP-4 and 6-OHDA to newborn rats decreased NA, DA, DOPAC in the brain and increased 5-HT and 5-HIAA in the striatum in higher extent as compared to the control and 6-OHDA alone. From the above we conclude that simultaneous lesion of the central noradrenergic and dopaminergic system in neonatal rats increased central serotoninergic system activity, as estimated by biochemical method.

This study was supported by Medical University of Silesia: NN-1-001/06.

# P13.69

## Nitric oxide (NO) and [$^3$H]glucose uptake in the heart muscle of rats prenatally exposured to lead

Przemysław Nowak[1], Ryszard Szkilnik[1], Janusz Konecki[2], Grażyna Szczerbak[1], Marzena Drosik[1], Adam Kwieciński[1], Marek Swoboda[2], Ryszard Brus[1]

*[1]Department of Pharmacology, [2]Department of Histology and Embriology, Medical University of Silesia, Zabrze, Poland*
*e-mail: pharbrus@slam.katowice.pl*

Lead (Pb) is a highly neurotoxic agent, in both mammals and human. The developing brain of mammalians is more susceptible to the toxic effects of lead, as compare to mature brain tissue, due to a greater uptake of lead by fetal brain, which is a result of the slow development of the blood-brain barrier at this stage. In this study the effect of lead exposure during pregnancy of rats was examined on [$^3$H]glucose uptake in the peripheral tissues of adult offsprings, and the effect of nitric syntase (NOS) inhibitor, 7-nitroindazole (7-NI) on it was investigated. Wistar pregnant rats consumed in their drinking water 250 ppm of lead [$Pb(CH_3COO)_2 \times 3H_2O$] throughout their entire pregnancies. On the day parturition the lead containing water was replaced by tap water, and the offspring remained with their mothers for 21 days. Control pregnant rats consumed water without metal. Adult male offsprings from both groups (lead exposured and control) were pretreated with 7-NI 10.0 mg/kg *ip*. Other animals were injected with saline (control). Then 30 min later all rats were injected with 6-$^3$H-D-glucose (Amersham) 500 μCi/kg *ip*. Fifteen minutes later animals were sacrificed, and samples of left and right ventricle and atrial muscle of the heart were excised. In the samples radioactivity was measured in liquid scintillation counter and the results were expressed in DPM (Desintegration Per Minute) per 100 mg of wet tissue. It was shown that 7-NI decreased radioactivity in four examined parts of the heart muscle in similar extent both in control and lead exposured rats. From above we conclude that NO plays a role in the exogenous [$^3$H]glucose uptake and the lead exposure during prenatal development does not modify it.

This study was supported by the grant from the State Committee for Scientific Research, Warsaw, No. PO5D 066 27.

# P13.70

## Genistein induced metabolic and hormonal changes in male rats

Ewa Nowicka, Katarzyna Szkudelska, Tomasz Szkudelski, Leszek Nogowski

*Department of Animal Physiology and Biochemistry, August Cieszkowski University of Agriculture, Poznań, Poland*

Genistein belongs to the phytoestrogens and is present in clover, legumes, toothed medic and bluegrass. However the richest sources of genistein are soybean and soy products. Phytoestrogens demonstrate beneficial effect in the organism. Many studies indicate that genistein exhibits anticancerogenic activity. This isoflavone posses also antioxidant activity and can ameliorate parameters of lipoprotein oxidation. Genistein may exert favourable changes in some metabolic diseases. The aim of the experiment was to determine whether genistein changes some hormonal and metabolic parameters in rats. In experiment were used male Wistar rats weighing about 310 g divided into three groups (n = 9) – control group, group treated with 1 mg of genistein per kg body weight and group receiving 5 mg of genistein per kg body weight. Genistein was administered intragastrically once a day. After seven days rats were killed and their blood serum, livers and muscles were collected and stored until analysis. Serum insulin level was determined radioimmunologically, glucose content was assayed using the enzymatic method and free fatty acid concentration was determined by the method of Duncombe. Serum triglycerides were assayed according to Foster and Dunn. Results were statistically evaluated using one-way analysis of variance and Duncan's multiple range test. Results demonstrated that genistein (in the dose of 5 mg/kg b.w.) administrated for seven days reduced serum insulin level. There were no difference in serum glucose, triglycerides and free fatty acids level between groups. Results obtained in our experiment demonstrate that genistein may cause some hormonal and metabolic changes in male rats but only in animals receiving it at dose 5 mg/kg b.w.

References:

Bhathena SJ, Velasquez MT (2002) Beneficial role of dietary phyoestrogens in obesity and diabetes. *Am J Clin Nutr* 76: 1191-1201.

Murkies AL, Wilcox G, Davis SR (1998) Phytoestrogens. *J Clin Endocrinol Metab* 83: 297-303.

Setchell KD (1998) Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones. *Am J Clin*

*Nutr* 68: 1333-1346.
Kurzer MS, Xu X (1997) Dietary phytoestrogens. *Annu Rev Nutr* 17: 353-81.
Duncombe D (1964) The colorimetric micro-determination of nonesteryfied fatty acids in plasma. *Clin Chim Acta* 9: 122-125.
Foster LB, Dunn RT (1973) Stable reagents for determination of serum triglycerides by colorimetric Hatzsh condensation method. *Clin Chem* 19: 338-340.

# P13.71

## Phytochelatin based system of plant response to lead accumulation

Aneta Piechalak, Katarzyna Janczur, Dorota Mierzwa, Przemysław Nuc, Arleta Małecka, Barbara Tomaszewska

*Department of Biochemistry, University of Adam Mickiewicz, Fredry Poznan, Poland*
*e-mail: btomas@amu.edu.pl*

Worldwide heavy metals contamination causes severe hazard to ecological balance of ecosystems and to human health. Lead is well known for its toxicity an biomass production inhibition in plants. Phytochelatins-short metal binding thiol peptides act as one of the heavy metal detoxification mechanisms in plant cells. Their biosynthesis is underlain by translocation of dipeptide group from glutathione to donor molecule. The process is intensely limited by glutathione availability. Therefore γ-glutamylcysteine synthetase (γ-ECS) – an enzyme synthesizing glutathione precursor plays a key role in overall regulation of phytochelatins biosynthesis. Once transgenic plants over expressing γ-ECS could be possibly use for phytoremediation, its activity and expression level have been studied in lead stressed plants. *Pisum sativum* γ-ECS activity is higher in leaves than in roots in opposite to its expression level. Metal accumulation is followed by γ-ECS activity changes with lower Pb concentrations causing enzyme activation and higher Pb contents resulting in enzyme activity drop. For a more detailed analysis of the expression regulation of phytochelatins biosynthesis enzymes, several clones, containing genes of the three last steps in the pathway were selected from *Brassica napus* genomic BAC library: γ-ECS, glutathione synthetase and phytochelatin synthetase. Based on γ-ECS gene sequence it has been shown that at least two alleles of the gene are present in *B. napus* genome and that both are transcribed.

# P13.72

## A characteristic of satRNAs associated with Polish strains of Peanut Stunt Virus

Aleksandra Obrepalska-Steplowska[1], Marta Pelczyk[1], Henryk Pospieszny[2]

[1]*Interdepartmental Laboratory of Molecular Biology, Plant Protection Institute, Poznan, Poland;* [2]*Department of Virology and Bacteriology, Plant Protection Institute, Poland*
*e-mail: ao.steplowska@ior.poznan.pl*

The satellite RNAs are the small RNA particles associated mainly with some plant viruses having (+)ssRNA genome. They do not have infectious potential and their presence is not necessary for the virus biological activity. However, the presence of satRNAs in virus can modulate significantly the infection's symptoms, either intensifying or attenuating them. The nucleotide difference between both variants are usually very small, what suggests that one of the possible explanations for such a state can be their secondary structure. The nucleotide sequence of satRNAs shares no homology with their helper virus with the exception of 5′ and 3′ terminal fragments of the particle that are recognized by the same replicase system that replicates their helper virus. The origin and mode of action of that subgenomic particles are still very controversial and unclear. Some strains of Peanut Stunt Virus and other viruses belonging to *Cucumoviridae* family are known to posses a variety of satRNAs. In case of PSV, they usually have 393 nt. In our study we have analyzed three Polish isolates of PSV from cellery (Ag), pea (G) and yellow lupin (P). The presence of satRNA in PSV-P strain was reported before. Now, we have found that also the PSV-Ag strain can be associated with satellite particle. Both of them have 393 nt and differ only in one nucleotide. We also have ascertained on the basis of coat protein sequences that PSV-Ag and PSV-G shares very high level of the homology in that gene. However, the symptoms of infection are more intense in case of PSV-G. It can be due to the differences in other parts of their genomes or presence of satRNA in PSV-Ag that can attenuate the symptom's progression. In our study, we have tried to predict the possible secondary structure of the analyzed satellite RNAs, as well.

# P13.73

## The study on pyrethroid resistance in pollen beetle *Meligethes aeneus*

Aleksandra Obrepalska-Steplowska[1], Pawel Wegorek[2], Katarzyna Nowaczyk[1], Joanna Zamojska[2]

[1]*Interdepartmental Laboratory of Molecular Biology, Plant Protection Institute, Poznan, Poland;* [2]*Department of Zoology, Plant Protection Institute, Poznan, Poland*
*e-mail: ao.steplowska@ior.poznan.pl*

Pollen beetle (*Meligethes aeneus*) is one of the major pests parasiting on rape and causing significant losses in rape production. To eliminate it in Poland strong neurotoxines are used, among them those from pyrethroid group. However, there is very high resistance of that insect on aforementioned insecticides, resulting from common actions of many factors that can have influence on its sus-

ceptibility. There are many mechanisms that can be responsible for resistance in different organisms. Among them are reduction of toxin penetration through skin, sensitivity reduction of neural system and many other. In our study we have tried to determine susceptibility level to some pyrethroid insecticides and resistance mechanisms. Resistance mechanisms were detected using piperonyl butoxide, tributylinacetate and carbaryl as synergists. High synergism of pyrethroid insecticides with piperonyl butoxid (oxidative metabolism) and low with carbaryl (hydrolytic mechanism) and tributylin acetate (penetration factor) showed that the main mechanism of Pollen beetle resistance can be oxidative metabolism. We have also analyzed the mitochondrial cytochrome oxidase (I and II subunits) nucleotide sequence by using PCR and sequencing, as an example of an oxidative enzyme. Preliminary data indicates occurrence of many substitution in mtCOI and mtCOII sequences when compared with sequences already deposited in GenBank. The existing substitutions in both subunits DNA sequences entail amino acid substitution that can change secondary structure of those peptides. However, those substitutions can result from variability in populations geographically isolated. Therefore our study requires further analyzes on bigger number of population of *M. aeneus* from different parts of Poland and Europe.

## P13.74

### Application of immobilized fungal enzymes in synthesis and degradation of textile dyes

Anna Olszewska, Jolanta Polak, Anna Jarosz-Wilkołazka, Janina Kochmańska-Rdest

*Department of Biochemistry, Maria Curie-Sklodowska University, Lublin, Poland*
*e-mail: aolszewska@wp.pl*

Enzyme immobilization provides easy recovery and reuse of the enzyme and many other advantages, including easy in product separation and continuous operation. For successful development and application of immobilized biocatalysts, the enzyme support is generally considered as the most important component contributing to the performance of the reactor system. There is a variety of methods by which enzymes can be localized on/into support, ranging from covalent chemical bonding to physical entrapment but no single method and support is the best for all enzymes and their different applications. This is because of the widely different chemical characteristics and composition of enzymes, the different properties of substrates and products, and the different uses to which the product can be applied. The ideal support is cheap, inert, physically strong and stable. However, in many cases, immobilization affects the diffusion of the substrate towards the active site of the enzyme. For example the immobilized enzymes can be inactivated by the interactions with products formed in the reactions. Versatile peroxidase (VP) from *Bjerkandera* sp. and laccase (Lac) from *Cerrena unicolor* were immobilized using different carriers. Different strategies were considered concerning the type of supports and their activation. Different carriers were tested during experiments: alginate beads, polyacrylamide hydrogel, gelatin, Sipernat, controlled porosity glass (CPG), grit, and alumina. Among physical methods the best were alginate beads, among covalent chemical bonding method – Sipernat and CPG. Immobilized Lac was used in both decolourization processes and coupling reaction using different phenolic precursors. Immobilized VP was used in decolourization of simple model dyes and colour wastewaters.

This work was partially supported by EC FP6 Project SOPHIED (NMP2-CT-2004-505899) and the State Committee for Scientific Research (139/E-339/SPB/6. PR UE/DIE 450/2004-2007).

## P13.75

### A distinct pattern of Rab7 isoforms expression in single-celled eukaryote *Paramecium*

Magdalena Osińska, Liliana Surmacz, Jolanta Wiejak, Elżbieta Wyroba

*Department of Cell Biology, Nencki Institute of Experimental Biology, Warsaw, Poland*
*e-mail: e.wyroba@nencki.gov.pl*

We identified two *Rab7* genes, *Rab7a* and *Rab7b*, in single-celled eukaryote *Paramecium*. Two transcripts of 1.8 and 2.2 kb were detected by Northern blot analysis. The cloned cDNAs encode polypeptides of 206 amino acids that are 97.6% identical and differ in their C-termini. Up to now, Rab7 isoforms were identified only in human and plants. The predicted protein sequences of Rab7a/Rab7b contain all characteristic domains essential for Rab function and display 62.3–63.3% identity to human counterparts and 48.1–52.4% to Ypt7 of *S. cerevisiae* (Surmacz *et al., Acta Biochim Polon* 53: 149). Previously, Paramecium Rab7 was mapped to the late phagosomal compartment (Surmacz *et al., Biol Cell* 95: 69) and therefore distribution/expression of newly identified isoforms was of interest. A distinct pattern of Rab7 isoforms was detected upon onset of phagocytosis by immunolocalization studies with anti-peptide antibodies (Abs) specific for *Paramecium* Rab7a and Rab7b. Laser confocal multiple optical sections revealed that Rab7b prevailed in the region where dynamic membrane events take place and new phagosomes are formed. The reverse transcriptase-polymerase chain reaction (RT-PCR) analysis performed on the cells internalizing latex beads for 5 min revealed the increase in the level of mRNA of both Rab7 isoforms as compared to the non-phagocyting cells. However, expression of Rab7b was elevated to a higher degree than that of Rab7a. Such results may suggest a correlation between a distinct pattern of distribution and expression levels of both Rab7 isoforms of *Paramecium*.

This work was supported by grant 2 P04A 020 29 of the Ministry of Science and Higher Education and the statutory funds to the Nencki Institute of Experimental Biology.

## P13.76

### Proteasome inhibitor prevents LMP7 induction in the ischemic kidney of rat with renovascular hypertension

Halina Ostrowska[1], Irena Kasacka[2], Kamil Kruszewski[1]

*[1]Department of Biology and [2]Department of Histology, Medical University of Bialystok, Białystok, Poland*
*e-mail: osthal@amb.edu.pl*

Immuno-proteasome is responsible for the processing of intracellular antigens and is induced when cells are treated with the inflammatory cytokines promoting cellular immunity. We recently reported that LMP7 immuno-subunit is up-regulated in renal epithelial and endothelial cells in the ischemic kidney of rat with renal hypertension, and that there is a direct link between LMP7 induction, inflammation and renal atrophy. In this study we tested the hypothesis that LMP7 up-regulation can be prevented by *in vivo* administration of a proteasome inhibitor, PSI that is known to exert anti-inflammatory and cytoprotective effects by inhibiting NF-κB activation pathway. We found that administration of PSI (3 mg/kg/day, s.c.) for five days, 5 weeks after the induction of renal ischemia markedly reduced the levels of LMP7 subunit in epithelial and endothelial cells in the ischemic kidney. Moreover, the inhibition of LMP7 induction was accompanied by accumulation of IκBα protein, a known substrate of the 26S proteasome as well as by a slight attenuation of renal fibrosis and atrophy. Thus, these results indicate that ischemia-inducible LMP7 immuno-subunit is up-regulated in renal cells in response to proinflammatory cytokines produced locally or released from infiltrated macrophages, and further suggest an important role for LMP7 – containing proteasome in renal atrophy. Moreover, the inhibition of the 26S proteasome-dependent NF-κB activation may be also beneficial in reducing the development of renal fibrosis in rats with renovascular hypertension.

## P13.77

### Proteasome inhibition prevents tissue factor expression by human endothelial cells exposed to diverse agonists

Justyna K. Ostrowska-Roszczenko[1], Halina Ostrowska[2], Marek Z. Wojtukiewicz[1], Piotr Radziwon[3] , Marek Szczepanski[4], Sławomir Wolczynski[5]

*[1]Department of Oncology, [2]Department of Biology, [3]Regional Centre from Transfusion Medicine, [4]Department of Neonatology and [5]Department of Gynecological Endocrynology, Medical University of Bialystok, Poland*
*e-mail: halost@amb.edu.pl*

Proteasome inhibitors block the expression of many NF-κB-dependent genes whose products contribute to the pathogenesis of tumors and cardiovascular disorders. Because of this they are novel therapeutics for the treatment of certain human tumors and the stroke and myocardial infarction. In the present study we investigated whether proteasome inhibitors could prevent agonist-induced expression and activity of endothelial tissue factor (TF), which contributes to thrombotic episodes in a number of clinical disorders. To inhibit proteasomes, human umbilical vein endothelial cells (HUVEC) were pre-treated for 1 h with epoxomicin or Z-Ile-Leu(OtBu-)Ala-leucinal (PSI). Then the cells were stimulated with tumor necrosis factor-α (TNF-α) (20 ng/mL), bacterial lipopolisaccharide (LPS) (1 μg/mL) or angiotensin II (1 μmol/L) for 1–6 h to induce maximal NF-κB activation and TF expression and activity. We found that epoxomicin or PSI used in the concentrations inhibiting the proteasome dependent NF-κB activation prevented TNF-α, LPS or angiotensin II-induced TF expression on mRNA levels measured with RT-PCR. This was accompanied by the reduction in TF expression on protein levels determined by ELISA and TF amidolytic activity assessed by chromogenic assay. Thus, these data indicate that proteasome inhibitors may be promising antithrombotic agents in disease states associated with abnormal TF expression by endothelial cells.

## P13.78

### Mutagenic characterization of Hint, the phosphoramidates hydrolase

Magdalena Ozga, Agata Żebrowska, Dariusz Korczyński, Barbara Mikołajczyk, Agnieszka Krakowiak

*Centre of Molecular and Macromolecular Studies of the Polish Academy of Sciences, Department of Bioorganic Chemistry, Lodz, Poland*

Hint, *histidine triad nucleotide-binding protein*, is a universally conserved enzyme that hydrolyzes P-N bond in AMP-lysine, AMP-alanine, AMP-morpholidate and AMP-$NH_2$ with the rate ca. ~1 nmol $min^{-1}$ $\mu g^{-1}$. AMP-$SO_4$ and AMPS are also the substrates for this enzyme but the rate of the hydrolysis is below 0.2 nmol $min^{-1}$ $\mu g^{-1}$. The activity of Hint is due to the presence of conserved middle histidine of the histidine triad motif (His-112 for *rabbit* Hint), which is required for catalytic activity of HIT protein family. In contrast to the earlier suggestion of His-114 role as the acid-base catalyst in the process of P-N bond cleavage, the crystal structure of Hint-inhibitor complex provided information that the carbonyl oxygen of Gly-105 may assist the side chain hydroxyl of Ser-107 acting as the acid-base catalyst. The biochemical characterization of the S107A mutant indicated that the Ser hydroxyl plays a facilitative role in catalysis, although in its absence, the Gly-105 carbonyl may assist a water molecule bound by the S107A enzyme to provide residual activity for protonation of the leaving group and activation of the hydro-

lytic water. The aim of this study is to establish the role of conserved, third histidine of the HIT motif. Different mutants of Hint: H114G, H114D, H114N were engineered and the activity of these proteins towards different substrates (AMP-Lys, AMPS) is investigated. Experiments on the efficiency of H114G mutant in the presence and absence of imidazole are in progress.

## P13.79

### Intein-based fluorescence labeling of the DNA binding domain of ultraspiracle protein

Szymon Pakuła, Marek Orłowski, Andrzej Ożyhar, Piotr Dobryszycki

*Wrocław University of Technology, Faculty of Chemistry, Division of Biochemistry, Wrocław, Poland*
*e-mail: szymon.pakuła@pwr.wroc.pl*
*e-mail: marek.orlowski@pwr.wroc.pl*
*e-mail: andrzej.ozyhar@pwr.wroc.pl*
*e-mail: piotr.dobryszycki@pwr.wroc.pl*

Ecdysteroids are arthropod-specific hormones that function as the major inducing signals responsible for the regulation of development in insects. The functional receptor for ecdysteroids is a transcription factor comprised of two nuclear receptors, the ecdysone receptor (EcR) and a homologue of the mammalian retinoid X receptor, the ultraspiracle protein (Usp). The Usp/EcR heterodimer binds ecdysone response elements in the presence of the steroid hormone 20-hydroxyecdysone and stimulates transcription of targeted gens. DNA binding domain of the Usp protein (UspDBD) operates as an anchor dictating the polarity of the heterocomplex 5′-UspDBD–EcRDBD-3′ on the response element from the promoter sequence of gene encoding hsp27 protein *(hsp27*). In this communication, C-terminus of the UspDBD protein was labeled with fluorescent Cys-Lys-fluorescein dipeptide using an intein method. The obtained active protein was characterized. The degree of labeling was to be 47% while an excitation and emission maximum – 494 nm and 521 nm, respectively. Basing on Foerster theory, the labeled UspDBD was used for the measurements of the distance between UspDBD C-terminus and 5′-end of the *hsp27* (labeled with TAMRA fluorofore) in the absence and presence of EcRDBD. The distance between fluorescence donor (fluorescein) and acceptor (rhodamine) was to be 67 ± 3.4 Å and 70.1 ± 4.4 Å in the absence and presence of EcRDBD, respectively.

## P13.80

### Changes in fatty acids composition of the phospholipids of *Legionella lytica*

Marta Palusińska-Szysz[1], Wincenty Drożański[1], Rafał Kalityński[2], Andrzej L. Dawidowicz[2], Ryszard Russa[1]

*[1]Department of General Microbiology, [2]Department of Chromatographic Methods, M. Curie-Skłodowska University, Lublin, Poland*
*e-mail: martasz@biotop.umcs.lublin.pl*

*Legionella lytica*, formerly named *Sarcobium lyticum*, is the oldest known of *Legionellaceae* isolated from Polish soil as early as in 1954 year. The bacterium is an intracellular parasite of free-living amoebae and human derived phagocytes. The pathogenicity of *L. lytica* for humans has been established by the indirect immunofluorescent antibody technique. The aim of this study was to investigate of chemical composition of lipids and fatty acids composition from the bacterium grown intracellulary and on artificial medium. The lipids and phospholipids were extracted with chloroform/methanol and separated into nonpolar and polar fraction silicic acid column chromatography. Phospholipids were analyzed using high performance liquid chromatography coupled on-line with mass spectrometry (HPLC/ESI-MS) under-positive ionization conditions. Identifications of the individual phospolipid molecular species was based on the m/z ratio of their pseudomolecular ions, sodium adducts and head group-specific up-front fragmentation products. Cardiolipin (CL), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylcholine (PC) and two methylated derivatives of PE, i.e. phospatidyl-*N,N*-dimethylethanolamine (DMPE) and phospatidyl-*N*-monomethylethanolamine (MMPE), were found to make up the phospholipids of the analysed bacteria. In addition, the polar lipid, derived from the bacterium grown on BCYE medium showed the presence, short length, 3-hydroxy fatty acids. Characteristic feature of *L. lytica* phospholipids was the presence of branched iso and anteiso fatty acids and a clear difference in the fatty acids composition between the bacteria grown intracellulary and artificial medium. The possible taxonomic implication of these data are discussed.

## P13.81

### Influence of ultrasounds on human erythrocytes

Danuta Pałecz, Agata Stasiołek, Teresa Gabryelak

*Department of General Biophysics, University of Lodz, Lodz, Poland*
*e-mail: palecz@biol.uni.lodz.pl*

Influence of ultrasounds on biological material is variable depending on the acoustic and biological characteristics of the tissues, their localisation and the role they play. The aim of our work was to establish the response of human erythrocytes to ultrasound exposure. We avoid samples heating keeping controlled water bath so, the biological effect comes from cavitational origin. It is known that when cavitation occurs the H· and ·OH radicals are formed as well as other free-radicals are generated. In this study we measured the ultrasonically induced NO· formation. We

examined the level of NO· in human erythrocytes exposed to ultrasound (US) and we found that the content of NO· depended on the hematocrit, thus for the further study we choose 5% hematocrit erythrocytes. The sonication of 5% erythrocytes with 1 MHz continuous wave and the intensities of 0.61–2.44 W/cm$^2$ generated increased NO· level according to rising ultrasound intensities but the smallest ultrasound intensity stimulated the biggest increase. The estimations of NO· level in human erythrocyte membranes revealed higher level of this radical in the exposed human erythrocyte membranes than in the control. US treatment hemoglobin also showed a rise of NO· content according to applied intensities. On the basis of our work we can state that ultrasounds stimulate NO· generation in whole erythrocytes as well as in the hemoglobin and erythrocyte membranes. The level of NO· formatted under the cavitation action was evidently higher in the samples of lower hematocrit or protein concentration. We can conclude that cavitation bubbles are more active in lower concentration samples and provoke increased NO· formation.

# P13.82

### Structural and thermodynamic aspects of plant-hormone binding by CSBP

Oliwia Pasternak[1], Grzegorz D. Bujacz[1,2], Yasuyuki Fujimoto[3], Yuichi Hashimoto[4], Filip Jeleń[5], Jacek Otlewski[5], Michał M. Sikorski[1], Mariusz Jaskólski[1,6]

*[1]Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland; [2]Institute of Technical Biochemistry, Technical University of Lodz, Lodz, Poland; [3]Faculty of Pharmaceutical Sciences, Teikyo University, Kanagawa, Japan; [4]Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo, Japan; [5]Laboratory of Protein Engineering, Institute of Biochemistry and Molecular Biology, University of Wroclaw, Wroclaw, Poland; [6]Department of Crystallography, Faculty of Chemistry, A. Mickiewicz University, Poznan, Poland*
*e-mail: yenefer@ibch.poznan.pld*

Cytokinins are plant hormones that influence numerous physiological processes. They stimulate cell division, initiate shoot growth and retard senescence. The nataurally occuring cytokinins are adenine analogues with different substituents at the N6 atom of the purine ring. Also, some synthetic urea derivatives were shown to possess high cytokinin activity. The cytosolic fraction of *Vigna radiata* contains a 17 kDa protein which binds plant hormones from the cytokinin group, such as zeatin. Using recombinant protein and isothermal titration calorimetry as well as fluorescence measurements coupled with ligand displacement, we have reexamined the $K_d$ values and show them to range from about $10^{-6}$ M (for 4PU30) to $10^{-4}$ M (for zeatin) for 1:1 stoichiometry complexes. In addition, we have crystallized this cytokinin-specific binding protein (VrCSBP) in complex with zeatin and refined the structure to 1.2 Å resolution. Structurally, VrCSBP is similar to plant pathogenesis-related class 10 (PR-10) proteins, (sequence identity < 20%). This unusual fold conservation reinforces the notion that classic PR-10 proteins have evolved to bind small-molecule ligands. The fold consists of an antiparallel β-sheet wrapped around a C-terminal α-helix, with two short α-helices closing a cavity formed within the protein core. In each of the four independent VrCSBP molecules, there is a zeatin ligand located deep in the cavity with conserved conformation and protein-ligand interactions. However, in three cases, an additional zeatin molecule is found, in variable orientation but with excellent definition in electron density, which plugs the entrance to the binding pocket, sealing the inner molecule from contact with solvent.

# P13.83

### Transport and cellular localization of fluorescent analogs of diadenosine triphosphate

Róża Pęcherzewska, Maria Maszewska, Julia Kaźmierczak, Agnieszka Krakowiak

*Centre of Molecular and Macromolecular Studies of the Polish Academy of Sciences, Department of Bioorganic Chemistry, Lodz, Poland*

Despite the fact that diadenosine polyphosphates ($Ap_nA$) were discovered in the mid-sixties, their biochemical role has not been fully elucidated. $Ap_nA$ (n = 2–6) were recognized as intra- and extracellular signaling molecules and are considered as the class of new second messengers. Many physiological and pathophysiological processes are correlated with the alteration of their cellular level. Cell differentiation and apoptosis have substantial and opposite effects on $Ap_3A$ to $Ap_4A$ ratio in cultured cells. Although $Ap_nA$ can be non-specifically degraded by variety of phosphodiesterases and nucleotidases, the major role in their degradation belongs to the specific enzymes. One of them is Fhit (fragile histidine triad), a human $Ap_3A$ ($Ap_4A$) hydrolase. Fhit appeared to be involved in protection of cells against tumourigenesis. Tumour suppressor activity of Fhit is assumed to be associated with involvement of Fhit•$Ap_3A$ complex as a signaling molecule. To investigate this hypothesis fluorescent analogs of $Ap_3A$, which can be considered as a tools to study the transport and cellular uptake of $Ap_nA$ derivatives, are required. Appp-BODIPY [Appp-S-(4-4-difluoro-5,7-dimethyl-4-bora-3a,4a-diazasindacine-3-yl) methylaminoacetyl] and GpppBODIPY are diadenosine triphosphate analogs, which contain the fluorophore residue (BODIPY) instead of one moiety of nucleoside, fulfill those requirements. Since $Ap_nA$ are polar molecules with negative charge, what makes their passive diffusion across cellular membrane very difficult or not possible, different conditions (with and without carrier, like lipofectamin) to study the transport of these compounds across membrane in HeLa cells have been applied. Results of those experiments will be presented.

This project is financially assisted by the grant 2PO4A07929 (to A.K.).

# P13.84

## Laccase-catalyzed synthesis of coloured products from phenolic substrates

Jolanta Polak, Anna Jarosz-Wilkołazka, Marcin Grąz, Janina Kochmańska-Rdest, Elżbieta Malarczyk

*Department of Biochemistry, University of Maria Curie-Sklodowska, Lublin, Poland*
*e-mail: malar@hermes.umcs.lublin.pl*

Carotenoids, melanins, flavonoids, quinones and more specifically monascins, violacein or indigo there are coloured compounds synthesize by fungi in natural environment [1, 2]. However, there is a long way from Petri dishes to the industrial scale. Isolation of natural pigments and/or bioconversions of precursors to obtained natural pigments are innovative biotechnological techniques for the more environmentally friendly synthesis of different commercially valuable processes. A number of specific or selective reactions have been reported where laccases, the extracellular enzymes produced by many fungal strains, have been used to synthesize products of commercial importance (pharmaceutics, food ingredients, polymers). Laccases (benzenediol:oxygen oxidoreductase, EC 1.10.3.2) are multi-copper-containing enzymes, widely distributed in plants and fungi, that catalyze oxidative conversion of a broad range of substrates such as phenols or lignin-derivatives [7]. Laccase from *Pycnoporus cinnabarinus* catalyzed coupling of 3-(3,4-dihydroxyphenyl) propionic acid with 4-aminobenzoic acid [3]. The synthesis of the actinocin from 4-methyl-3-hydroxyanthranilic acid gave the chromophore of actinomycin antibiotics, conversion of alkaloids or the production of mithramicine there are examples of using laccase to yield biologically active products [4, 5, 6]. Laccases has also ability to induce oxidative coupling reactions of the chemicals, such as phenol derivatives to other phenolic structures, producing intensely coloured products. The uses of laccases in dyes synthesis processes represent a promising alternative to chemical synthesis of existing or new dyes. The aim of this study was to examine the ability of an extracellular laccase produced by a commonly occuring wood-degrading fungus *Cerrena unicolor* to form coloured compounds from simple organic precursors. Screening of 30 different phenolic derivatives such as *o*-, *m*-, and *p*-methoxy, -hydroxy, -sulfonic and aromatic amines were studied in the presence of laccase in liquid media. The findings show that laccase catalyzes the oxidative coupling reaction between selected substrates producing coloured compounds (from yellow by brown to red and blue). Coloured compounds were isolated and analysed firstly by spectrophotometer and secondly by capillary electrophoresis. To check participation of substrates in product formation substrates were added to incubation mixtures in various ratios.

This work was partially supported by EC FP6 Project SOPHIED (NMP2-CT-2004-505899) and the State Committee for Scientific Research (139/E-339/SPB/6. PR UE/DIE 450/2004-2007).

References:

1. Dufosse L, Galaup P, Yaron A, Malis Arad S, Blanc P, Chidambara Murthy KN, Ravishankar GA (2005) *Trends Food Sci Technol* 16: 384-406.
2. Duran N, Teixeira MFS, de Conti R, Esposito E (2002) *Crit Rev Food Sci Nutr* 42: 53-66.
3. Pilz R, Hammer E, Schauer F, Kragl U (2003) *Appl Microbiol Biotechnol* 60: 708-712.
4. Burton SG (2003) *Curr Opin Chem* 7: 1317-1331.
5. Manda K, Hammer E, Mikolasch A, Niedermeyer T, Dec J, Jones A, Benesi AJ, Schauer F, Bollag J-M (2005) *J Mol Cat B: Enzym* 35: 86-92.
6. Osiadacz J, Al-Adhami AJH, Bajraszewska D, Fischer P, Peczynska-Czoch W (1999) *J Biotechnol* 72: 141-149.
7. Thurston CF (1994) *Microbiology* 140: 19-26.

# P13.85

## Influence of vitamin E and extremely low frequency magnetic field on the activity of lactase dehydrogenase of squamous cell carcinoma cells *in vitro*

Renata Polaniak[1], Ewa Birkner[1], Maria Wideł[3], Waldemar Przybyszewski[3], Maria Goliszewska[1], Barbara Filipowska[2], Ewa Grucka-Mamczar[1]

*[1]Department of Biochemistry, Silesian University School of Medicine, Zabrze, Poland; [2]Clinik of Dermatology Silesian University School of Medicine, Zabrze, Poland; [3]Departament of Experimental and Clinical Radiobiology, M.Skłodowska-Curie Memorial Cencer Center and Institute of Oncology, Gliwice Branch, Poland*

Lactase dehydrogenase, LDH, [EC 1.1.1.27] in an catalityc enzyme in the last part of glikolisis. It is cytoplasmic enzyme, marker of many chronic illuess: cardiopathy, hepatopathy and new growth. An influence of vitamin E and ELF magnetic field on activity of lactase dehydrogenase, LDH, of squamous cell carcinoma (SCC) cells of the line AT478 from mous C3H was studies. The squamous cell carcinoma (SCC) is a special line of cell culture that has the ability for megacolonies creation growning in continous contract with ground and other cells. The cells were exposed to ELF magnetic field of complex shape generated by device used in medicine for magnetostimulation. The studies were carried out on six groups:

Group 1 – 30 min exposition per day/24 h
Group 2 – 30 min exposition per day/72 h
Group 3 – only vitamin E /24 h
Group 4 – only vitamin E /72 h
Group 5 – 30 min exposition per day plus vitamin E/24 h
Group 6 – 30 min exposition per day plus vitamin E/72 h.

In two control groups the cells grew without any influence of vitamin E and electromagnetic field. The influence of the vitamin E and the ELF magnetic field on activity of lactase dehydrogenase, LDH, in cells medium

was investigated. Combined incubation with vitamin E and electromagnetic field reveals similar, but slightly less effect. Obtained results suggest that the electromagnetic field can influence on the activity of biochemical nature function of squamous cell carcinoma cells *in vitro*.

## P13.86

### Influence of combined cisplatin and electromagnetic field treatment on antioxidative status in AT478 squamous cell carcinoma line – *in vitro* study

Renata Polaniak[1], Ewa Birkner[1], Monika Adamczyk-Sowa[2], Krystyna Żwirska-Korczala[2], Aleksandra Monsiol[3], Paweł Sowa[2], Katarzyna Birkner[1]

*[1]Department of Biochemistry, Silesian University School of Medicine, Zabrze, Poland; [2]Department of Physiology, Silesian University School of Medicine, Zabrze, Poland; [3]A. Chełkowski Institute of Physics, Department of Medical Physics, University of Silesia, Katowice, Poland*

The presence of electromagnetic field is necessary for the propriate existence of living organisms. In contrast to many other kinds of energy, electromagnetic field penetrates the body, reaching almost all living cells. The electromagnetic field used in magnetotherapy or magnetostymulation reveals analgesic, anti-inflammatory and sedative properties. There is an idea to use those positive properties of electromagnetic field in oncology, therefore the interactions with oncological medications needs further investigations. The aim of the study was to analyze the influence of electromagnetic field and cisplatin (cytostatic drug) used separately or together on oxydoreductive parameters in cancer cells megacolony. The AT478 squamous cell carcinoma line, derived from C3H mice was used in the study. The incubation was performed in standarized pressure and temperature conditions. The following parameters were measured isoenzymes of superoxide dismutase – SOD, [EC 1.15.1.1]: manganese superoxide dismutase – MnSOD, and copper/zinc superoxide dismutase – Cu/ZnSOD activities – estimated according to Oyanagui and expressed in nitrite units/ml of culture medium (NU/ml); glutathion peroxidase – GSH-Px [EC 1.11.1.9] activity – measured after Paglia and Valentine using enzymatic conjuction with glutathione reductase (μmol $NADPH_2$/ml medium). As a marker of lipid peroxidation malondialdehyde – MDA, levels were determined after Ohkawa *et al.* colorimetric methods (μmol MDA/ml medium), using reaction with thiobarbituric acid and calculated by spectrophotometry (515 nm absorbance, 522 nm emission). In our study, electromagnetic field used alone as well as cisplatin alone, caused a significant increase in activity of all measured enzymes and a significant decrease in MDA concentration, compared to control. Combined treatment of electromagnetic field with cisplatin caused a similar effect, but less expressed compared with cisplatin-only treated cells. Our results suggest that cisplatin caused a significant alterations in oxydative enzymes activities and lipid peroxydation. Combined incubation with cisplatin and electromagnetic field reveals similar, but slightly less effect. In conclusion further research is necessary for investigation the influence of electomagnetic field on patients under oncological treatment.

## P13.87

### Resistin, leptin and lipid parameters concentration at different stages of pregnancy and after delivery in rats

Ewa Pruszyńska-Oszmałek, Dawid Szczepankiewicz, Maciej Sassek, Tatiana Wojciechowicz, Przemysław Kaczmarek, Paweł Maćkowiak

*Department of Animal Physiology and Biochemistry, August Cieszkowski University of Agriculture, Poznań, Poland*

Adipocytes produce a great number of peptides including leptin and resistin. Resitin has been suggested to be the link between obesity and type 2 diabetes by producing insulin resistance. Leptin is a hormone that decrease food intake and body weight *via* its receptor in the hypothalamus. In rodents, its also modulates glucose metabolism by increasing insulin sensitivity. Changes in leptin and resistin secretion during pregnancy, especially it regards the level of resistin, are still unclear. Fifty female Wistar rats with initial body weight of 190 ± 10 g were used in the experiment. The pregnant animals were divided into four groups, moreover control group of rats was kept. Rats were sacrificed on 4, 13 and 18 day of gestation and on the first day after delivery. Each group consistent of ten rats. After decapitation blood and liver samples were collected and stored until analysis (–80°C). Leptin and resistin concentrations in serum were assayed using radioimmunological kits, levels of serum lipid parameters (triglycerides, free, estrified, total and HDL cholesterol, free fatty acids) were determined using photocolorimetrical methods. Liver triglycerides content was measured similarly as in serum after extraction of total lipids. The resistin and leptin serum concentrations markedly increased during pregnancy and the highest levels of these hormones were observed in 18th day and after delivery. We also found significant changes in concentration of serum and liver triglycerides, total, HDL and liver cholesterol. However pregnancy did not alter level of free fatty acids, estrified and free cholesterol.

## P13.88

### Alcohol, homocysteine and tripthophan: what are the relationships?

Yu. E. Razvodovsky, Ye. M. Daraschenka,

M. I. Zolotukhin, A. V. Naumov

*Grodno State Medical University, Grodno, Belarus*
*e-mail: razvodovsky@grsmu.by*

Homocysteine (Hcy) is a sulfur-containing amino acid that in recent years has become the subject of great interest. Hcy is formed as an intermediator in methionine metabolism. Alcohol dependence is associated with markedly raised plasma homocysteine levels and homocysteinemia may be risk factor of vascular and intellectual impairment in chronic alcoholics. It is well known that hepatic encephalopathy is a frequent complication of alcoholic cirrhosis. Abnormality of tryptophan metabolism may contribute to its pathogenesis. This study was designed to examine the effect of oral tryptophan load on plasma Hcy level in experimental model of alcoholism. One week alcoholization according to Majchrowizc was associated with marked increase plasma Hcy level. Tryptophan in doses 50 mg/kg intragastrically, when coadministered with alcohol lead to statistically significant increase plasma Hcy level in comparison to control and alcohol treated groups. The results of this study suggest that homocysteinemia coursed by alcohol and tryptophan metabolites may be important underlain factor of organ impairment in alcoholics.

# P13.89

## *In vitro* gastrointestinal digestion study of red cabbage phenolic compounds

Małgorzata Redzynia, Anna Podsedek, Maria Koziołkiewicz

*Institute of Technical Biochemistry, Faculty of Biotechnology and Food Sciences, Technical University of Łódź, Łódź, Poland*
*e-mail: malgosiaredzynia@interia.pl*

The beneficial biological properties of red cabbage (*Brassica oleracea* var. *capitata*) have been partly attributed to their dietary antioxidants, such as vitamins C and E, carotenoids and phenolic compounds. The majority of the antioxidant capacity of red cabbage is from phenolics, especially anthocyanins. Red cabbage contains more than 20 different anthocyanins, which are cyanidin derivatives highly conjugated with sugars (glucose and xylose) and acylated groups (caffeoyl, *p*-coumaroyl, feruloyl, *p*-hydroxybenzoyl, sinapoyl and oxalolyl). Activities of acylated anthocyanins *in vivo* are related to their bioavailability from food matrix and metabolism both in gastrointestinal tract and in tissues. The aim of this study was to determine the stability of red cabbage phenolic compounds during the digestion process. For this purpose, an *in vitro* gastrointestinal digestion method was used in order to simulate physiological conditions of the stomach and small intestine (pH, temperature and enzyme conditions). Subsequently, the antioxidant capacity of the digest was measured *in vitro*. After *in vitro* gastric digestion of raw red cabbage, total phenolics and anthocyanins levels showed increase in comparison to initial sample. The increase of antioxidants concentrations may be consequence of the hydrolysis of phenolic conjugates under gastric pH 2 and/or their releasing from food matrix. On the contrary, anthocyanins level showed decrease about 19%, after gastric digestion of phenolic compounds extract. After the pancreatin-bile salt digestion (simulation of small intestine digestion) decrease in tested compounds were found. The antioxidant capacity of the all digests was negatively affected by both the gastric and intestinal digestion conditions.

# P13.90

## The influence of testosterone on angiotensin II-dependent changes in tyrosine kinase activity in human prostate cancer cell line DU-145

Elżbieta Rębas[1], Agnieszka Lachowicz[2]

*[1] Department of Molecular Neurochemistry, [2]Department of Comparative Endocrinology, Medical University of Lodz, Lodz, Poland*
*e-mail: elkar@zdn.am.lodz.pl*

Angiotensin II (AngII), the main peptide of the renin-angiotensin system (RAS), is involved in cell proliferation of different types of cells, in normal and pathological as well. The PTKs play important role in growth cell, differentiation and apoptosis. AngII action is dependent on hormonal milieu of the cell, and especially on sex steroid influence. The present study was to examine the non-genomic influence of testosterone on Ang II action on tyrosine kinase (PTKs) activity in androgen-independent humane prostate cancer line DU-145. 7-days old cell cultures of DU-145 human prostate cancer were used as a source of protein tyrosine kinases. These cultures were exposed to different concentrations of Ang II ($10^{-11}$–$10^{-9}$ M). The incubation with testosterone lasted 15 min to estimate the non-genomic effects of steroid. In phosphorylation reaction as a donor of phosphate was used $\gamma^{32}$P-ATP and as substrate served the synthetic peptide – poly Glu, Tyr (4:1). The specific activities of PTKs were defined as pmoles $^{32}$P incorporation into exogenous poly Glu, Tyr/mg of protein/min. Ang II alone had a little effect on PTKs activity in androgen-independent human prostate cancer but after incubation with testosterone this peptide inhibited enzyme activity. The obtained results suggested that testosterone can influence the Ang II action on tyrosine kinase activity in non-genomic way, in spite of fact that the DU-145 cell line is not sensitive to testosterone in classic meaning, and does not posses the androgen receptors.

This work was supported by Medical University Grant Nr 502-16-301 and 503-686-2.

# P13.91

## Effect of medium depth and discrepancy between the observed and expected levels of cytogenetic

**damages caused by ionizing radiation**

Jacek Rogoliński[1], Maria Konopacka[1], Aleksander Sochanik[2], Krzysztof Ślosarek[3]

*[1]Department of Experimental and Clinical Radiobiology, [2]Department of Molecular Biology, [3]Department of Radiotherapy and Brachytherapy Planning, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Poland*
*e-mail: rogolinski@io.gliwice.pl*

Linear electron accelerators which are used in radiotherapy of cancer generate photon and electron radiation characterized by nonmonoenergetic beam. When passing through water (used as equivalent of soft body tissues) such radiation is altered. Interactions with the surrounding milieu increase with greater depth; part of radiation becomes scattered and the proportion of scattered radiation having decreased energy becomes larger with increased depths. Biological efficacy of radiation may be estimated as the number of cells damaged by radiation. The purpose of this study was to assess the numbers of damaged cells with respect to irradiatiation dose at various medium depths. The dose size at selected measurement points was judged from former dosimetric measurements. Human melanoma Sk-mel cells were used in the study. The extent of cytogenetic damages was assessed on the basis of the frequency of cells forming micronuclei or apoptotic cells with characteristic chromatin condensation. As a radiation source, Varian Clinac 2300 CD linear electron accelerator with maximum electron energy of 22 MeV was used. The studied cell cultures were placed in a water phantom at depths varying between 2.5 and 13 cm. Irradiation time was held constant. The number of damaged cells was normalized to the value of damages found at the depth corresponding to the maximum dose. Our measurements show that, at various depths, both the numbers of apoptotic cells and the numbers of cells containing micronuclei were greater than those that should result from the corresponding received dose. This discrepancy between the observed and expected numbers of damaged cells becomes greater with increased medium depth. The effect reported herein is likely to result from the part of scattered radiation since, with increased medium depth, its proportion to the incident beam becomes greater.

## P13.92

**Studies on the interaction of pig heart lactate dehydrogenase (LDH) with anionic phospholipids at low pH conditions; comparison with a muscle form of the enzyme**

Katarzyna Rogozik, Elżbieta Czapińska, Grzegorz Terlecki

*Dapartment of Medical Biochemistry, Wrocław Medical Uniwersity, Wrocław, Poland*
*e-mai:l kasia2@bioch.am.wroc.pl*

Our previous studies showed that muscles form of pig lactate dehydrogenase strongly interacts with cardiolipin-, phosphatidylserine- or other anionic phospholipid-liposomes at pH < 6.5. The binding has electrostatic nature what is in good agreement with the fact that the isoelectric point (pI) value of muscle LDH is 8.6 and at acidic pH molecules of the protein have definitely positive net charge value. LDH from pig heart also interacts with liposomes made of anionic phospholipids and also in acidic pH. However, the interaction is most effective when pH value of environment is about 5.5. Because of pI value of heart form of pig LDH is known to be 5.5 also, it means that the net charge of the protein molecule in such conditions is close to zero. The binding is not observed at high ionic strength and/or neutral pH what indicates its electrostatic nature. For heart LDH-liposomes complexes formation the absence of nicotinamide-nicotine dinucleotides and adenine nucleotides in the interaction environment is necessary. Their presence limits the interaction of heart LDH with liposomes or makes it impossible, in dependence on concentration. This phenomenon is not observed for pig skeletal muscle LDH. The heart LDH-liposomes complexes formed in absence of nicotinamide-nicotine dinucleotides and adenine nucleotides are stable after addition of these substances even to millimolar concentrations. According to effectiveness of the protection of pig heart LDH against interaction with liposomes its substrates and studied nucleotides can be ordered as follows: NADH>NAD>ATP=ADP>AMP>pyruv ate. Phosphorylated form of NAD (NADP), nonadenine nucleotides (GTP, CTP, UTP) and also lactate are uneffective. Obtained results strongly suggest that in lowered pH conditions nucleotide binding domains of pig heart LDH molecule are responsible for the interaction of the enzyme with anionic phospholipids.

## P13.93

**AMP-deaminase from human term placenta – kinetic properties of degradating enzyme**

Anna Roszkowska[1], Magdalena Szydłowka[2], Jerzy Klimek[1], Krystian Kaletha[2]

*[1]Department of Pharmaceutical Biochemistry, [2]Department of Biochemistry, Medical University of Gdańsk, Gdańsk, Poland*
*e-mail: a.koryziak@amg.gda.pl*

AMP-deaminase (AMPD) is a cytosolic enzyme catalyzing deamination of AMP to IMP. The physiological function of AMP-deaminase is connected with the regulation of purine nucleotides pool and stabilization of adenylate energy charge (AEC). The enzyme occurs in the form of three isoenzymes (muscular M, liver L and erythrocytic E) and is coded by the family of three independent genes (*AMPD1*, *AMPD2* and *AMPD3*). In human term placenta, isoenzyme L of AMPD is mainly expressed isoform and its activity depends on pH as well as on the presence of allosteric effectors (ATP, ADP and orthophosphate). AMP-

deaminase from human placenta is an oligomeric enzyme consisting of four identical subunits (homotetramer) of molecular subunit mass about 68 kDa. Experiments with gel filtration on Sepharose CL-6B column revealed that human placenta AMPD can aggregate and different active enzyme aggregates have been detected in the eluate, depending on the presence or absence of potassium ions and some other allosteric effectors in the eluting buffer. During storage of human placenta AMP-deaminase, the activity of the enzyme decreases and changes in its kinetic profile from sigmoidal to hyperbolic one have been observed. This probably reflects the gradual loss of the cooperative interaction between enzyme subunits compared with progressive degradation of AMPD molecules. The decreasing number of active sulphydryl groups of the enzyme observed during enzyme storage seems to support such a conclusion.

## P13.94

### Delta-haemolysins of *Staphylococcus cohnii* – biochemical characterization and cytotoxicity

Małgorzata Różalska[1], Agnieszka Maszewska[2], Paweł Mak[3]

*[1]Department of Pharmaceutical Microbiology, Medical University of Łódź, Poland; [2]Department of Immunobiology of Bacteria, University of Łódź, Poland; [3]Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland*
*e-mail: mrozalska@pharm.am.lodz.pl*

Haemolysins belong to the most important virulence factors of staphylococci. *Staphylococcus aureus*, which is a potentially pathogenic coagulase-positive species, produces four haemolysins – alfa, beta, gamma and delta. On the other hand, only one delta-toxin was found in other several species of coagulase-negative staphylococci (CoNS). Delta-haemolysin of *S. epidermidis* is the most known example and its presence can be found with a simple test of synergistic haemolysis (CAMP). We proved recently that the majority of CoNS strains of both subspecies *S. cohnii* (*S. cohnii* ssp. *cohnii* and *S. cohnii* ssp. *urealyticus*) gave positive CAMP test. The activity of others haemolysins was not found. The same test was positive with electrophoretically pure haemolysins isolated from two selected strains with the highest haemolytic activity. Those samples of haemolysins were obtained by precipitation with ammonium sulphate (0–60% of saturation) of culture supernatants followed by extraction with chlorophorm and methanol mixture. Native PAGE showed high heterogeneity and tendency to aggregation of both toxins, whereas after SDS/PAGE single band of haemolysin with similar molecular weight about 4 kDa was observed. Determination of pI confirmed heterogeneity of both peptides – haemolytic activity demonstrated the acidic as well as the alkaline compound. Moreover, we proved also that *S. cohnii* haemolysins posses cytotoxic activity against human foreskin fibroblasts. Both haemolysins were further subjected to RP-HPLC chromatography and its full amino acid sequence was determined using N-terminal automatic Edman degradation, showing a significant degree of similarity to *S. lugdunensis* hemolysin and *S. saprophyticus* ssp. *saprophyticus* antibacterial peptide.

## P13.95

### Cyclosporine A and platelet procoagulant activity

Tomasz Rusak[1], Marian Tomasiak[1], Halina Stelmach[1], Marek Gacko[2]

*[1]Department of Physical Chemistry, Medical University of Bialystok, Poland; [2]Department of Department of Vascular Surgery and Transplantology, Medical University of Bialystok, Poland*
*e-mail: mtomask@amb.edu.pl*

Cyclosporine A (CsA) is an immunosuppressive drug used to prevent the rejection of transplantated organ and to treat diseases of autoimmune origin. Clinical use of CsA is associated with increased risk of thrombotic episodes. The molecular mechanisms underlying these adverse effects remain unresolved. This study was performed to test the hypothesis that CsA may (nonspecifically) produce platelet procoagulant activity due to its interaction with a plasma membrane. It was found that CsA (1–100 μg/ml) potentiate collagen-evoked platelet procoagulant response (measured as phopholipid-dependend thrombin generation). Platelets treated *in vitro* with CsA (20–200 μg/ml, 20 to 60 min) expressed procoagulant activity. CsA-induced platelet procoagulant response was both dose- and time-related and weaker than that produced by collagen. Flow cytometry studies revealed that CsA treatment results in left shift (decrease) in the forward and side scatter of the entire platelet population. The shift was unimodal, dose dependent and less pronounced than that elicited by collagen. Using flow cytometry and annexin V-FITC as a probe for phosphatidylserine (PS) we demonstrated increased and uniform binding of this marker to small (below 1 μm), normal (1–6 μm) and large (above 6 μm) objects appearing in CsA-treated platelet population. CsA-evoked PS-expression was dose and time dependent and less pronounced than that produced by collagen. CsA at the concentrations similar to that affecting platelet procoagulant response produced LDH release from platelets. These observations indicate that the thrombogenic properties of CsA may result from the alteration of lipid organization in platelet plasma membrane leading to PS externalization and accelerated thrombin generation.

## P13.96

### Measurement of activity, cell-type specificity and changes of expression of P-glycoprotein in

**response to perfluorinated organic acids (PFOA)**

Izabela Rusiecka, Andrzej C. Składanowski

*Department of Molecular Enzymology, Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Gdansk, Poland*
*e-mail: izabelarusiecka@amg.gda.pl*

The Multixenobiotic Resistance (MXR) system plays an important role in eukaryotic cells. It consists of efflux pump, P-glycoprotein (P-gp), and multiple resistance-associated protein (MRP). Measuring range of MXR induction may be used as biomarker of a cell/organism exposition for foreign substances. The plasma membrane P-gp is able to block influx or increase efflux of many low-molecular hydrophobic agents. We tested various cell lines for the presence and induction of this system. Activity was indirectly estimated with Rhodamine B, a fluorescent P-gp substrate, and measuring intensity of individual cells in the presence of PFOA. HeLa have been found as expressing a high level of P-gp. Changes of MXR components expression were measured by Western blotting using monoclonal antibodies C219. In the control experiment, a 5-fold increase of P-gp (130 kDa subunit) was detected after 8 days incubation of cell culture with micromolar concentrations of $Cd^{2+}$. The exposure on perfluorodecanoic acid resulted in 2-fold increase of the P-gp level. The results were confirmed by more specific flow cytometry method. Preliminary assays show the structure-quantity-of-P-gp dependence for a set of perfluorinated organic acids. Cells or whole organisms, after exposure on pollutants, can increase both activity and expression of MXR. We observed a concentration or structure dependent changes in activity of the transporter. Testing a potential toxicity by measuring MXR changes *in vitro*, can be done with relatively low error at a high diversity of compounds. We suggest that this method may be a good and economically viable for screening toxicity of newly introduced chemical compounds.

# P13.97

**Distribution of resistin protein in different rat tissues**

Maciej Sassek, Ewa Pruszyńska-Oszmałek, Piotr Wilczek, Paweł Maćkowiak

*Department of Animal Physiology and Biochemistry, August Cieszkowski University of Agriculture, Poznań, Poland*

Adipocytes are well known as cells demonstrating intensive physiological activity and production a great number of factors such as leptin, adiponectin, many interleukins, and resistin. Resistin has been discovered in 2001 and belongs to a family of resistin-like molecules. This is unknown family of protein before and the remaining members are called RELM alpha, beta and gamma. In rodents resistin is the 114 amino acids protein which is rich in cysteine. In the physiological conditions resistin forms a homodimer. This compound seems to be a hormone which exerts wide action on metabolism. However, structure of resistin receptors is until unknown. It is highly probable that this protein, secreted from adipose tissue, is involved in the induction of resistance of tissues for insulin. Physiological investigations indicate that serum concentration of this hormone increases in obese mice and decreases after treatment with anti-diabetic drugs. mRNA for resistin is decreasing at restricted diet and enhances in the old ages. Injection of resistin to healthy mice impairs insulin action while administration of anti-resistin antibody enhances sensitivity to insulin and improves its action. These facts suggest that resistin is a hormone that can be links obesity with insulin resistance and finally diabetes mellitus type II. We analyzed the distribution of resistin protein in different rat tissues by the RIA kit obtained from Linco. Tissues was homogenized then centrifuged 30 min by 30 000 × *g* 50 μl of clear natant was used in RIA measurements.

# P13.98

**Monitoring of chimerism in patients after allogeneic bone marrow transplantation employing AmpF*l*STR SGM Plus kit**

J. Sawecka[1], J. Skulimowska[1], K. Hałaburda[2], B. Mariańska[2], J. Kościelak[1]

[1]*Department of Biochemistry, Institute of Hematology and Blood Transfusion, Warszawa, Poland;* [2]*Department of Bone Marrow Transplantation, Institute of Hematology and Blood Transfusion, Warszawa, Poland*
*e-mail: jsawecka@ihit.waw.pl*

Examination of chimerism after allogeneic bone marrow transplantation (allo BMT) allows for early detection of a successful engraftment or graft rejection or disease relapse. Chimerism in 20 patients who received allo BMT was monitored at different time intervals, usually every 30 days during the first year, and thereafter every 90 days. To determine chimerism we analysed short tandem repeats (STR) at 10 different *loci* (D3S1358, vWA, D16S539, D2S1228, D8S117, D21S11, D18S51, D19S433, THO1, FGA) and the gene for amelogenin. Appropriate fragments of DNA were amplified using one fluorescent primer for each locus (5-FAM, JOE or NED). The amplified material was electrophoretically separated under denaturing conditions in an ABI PRISM 377 sequenator and thereafter analysed employing Gene Scan and Genotyper software. In all we perfomed 97 analyses and genetically differentiated 20 donor/recipient pairs. We were unable to differentiate two pairs of twin sisters who showed identity at each locus analysed. In 12 patients subjected to myeloablative BMT, DNA of donor BM replaced in 100% that of recipient BM on day 30 i.e total chimerism was achieved. In two patients total chimerism occurred after two weeks and in three patients after three weeks

since allo BMT. All these patients are alive and well for 1–3 years. A mixed chimerism was observed in three patients subjected to non-myeloablative BMT. In one patient the mixed chimerism persisted for 6 weeks at 70% level and subsequently rose to 100% on the 9th week. In the second patient the mixed chimerism was still observed in the fourth week but reached the 100% level in the 5th week. The third patient, in whom the mixed chimerism amounted to 75% in the 7th week, subsequently died in the 8th week.

## P13.99

### Significance and role of heat shock proteins in ischemic brain stroke

Wojciech Sawuła[1], Leszek Kadziński[1], Zyta Banecka-Majkutewicz[2], Walenty Nyka[2], Bogdan Banecki[1]

*[1]Department of Molecular and Cellular Biology, Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk, Gdańsk, Poland; [2]Department of Neurology, Medical University of Gdańsk, Gdańsk, Poland*

Brain stroke is one of the diseases connected mainly with the elderly part of the population. It has been postulated that heat shock proteins may play a significant role in ischemic brain stroke. Most of the theories explaining their mode of action in this disease are based on the abnormally high heat shock protein levels in atherosclerotic plaque. The explanations are twofold, as heat shock proteins can either be present in order to oppose the effects of cellular shock, or their presence may contribute to the development of atherosclerotic processes. In our research we have decided to investigate both theories. In order to study the influence on the development of ischemic stroke we measured the level of antibodies against several bacterial and human heat shock proteins in the plasma of stroke patients and members of the control group. Interestingly, analysis of the level of antibodies points at the essential role of a heat shock protein from *E. coli* in the development of ischemic stroke. In order to study the protective role, we have studied the interactions between the Hsp70 system and an enzyme involved in the metabolism of homocysteine, one of the risk factors for ischemic stroke. Proteins of the Hsp70 system protect the studied enzyme from the effects of heat shock and are able to partially restore its activity after thermal denaturation.

## P13.100

### Direct detection of N-homocysteinylated albumin in human serum

Marta Sikora[1], Łukasz Marczak[2], Tomasz Twardowski[1], Hieronim Jakubowski[1]

*[1]Protein Biosynthesis Group, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland; [2]Plant Secondary Metabolites Biochemistry Group , Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland*
*e-mail: martas@ibch.poznan.pl*

Homocysteine-thiolactone (HcyTL) is a naturally occurring metabolite. Its level positively correlates with the level of homocysteine, a known independent risk factor for cardiovascular and neurodegenerative diseases in humans. HcyTL freely diffuses through cellular membranes and accumulates in extracellular fluids. HcyTL is chemically reactive and forms adducts with protein, N-Hcy- protein, in which the carboxyl group of Hcy is linked by an amide bond with epsilon-amino group of a protein lysine residue. This reaction impairs protein structure and function. The bulk of Hcy circulating in human blood is N-linked to hemoglobin and albumin. It has been recently found that Lys 525 is a predominant site of N-homocysteinylation in human serum albumin *in vitro* and *in vivo*. Here, we describe a method that allows direct monitoring of N-Hcy-albumin in human serum. Native and HTL-modified human serum were analyzed by proteomic approaches. Samples of human serum were reduced with DTT, modified with IAA and digested with trypsin. Peptides were purified by HPLC and subjected to mass spectrometric analysis (MALDI-TOF). A peptide with a mass of 1301.5, corresponding to the mass of N-Hcy-Lys 525-containing peptide, was present in a MALDI-TOF spectrum. In conclusion, N-homocysteinylated albumin (Lys 525) is easy to detect directly from human serum, which is promising for the future as a new diagnostic tool for diseases associated with elevated levels of plasma Hcy.

## P13.101

### Isolation and functional characterization of soluble NTPases involved in metabolism of NTPs other than ATP in bovine tissues

Viktor Sivuk[1], Iryna Rusina[2], Tatyana Luchko[1], Irina Gulyai[1], Alexander Makarchikov[1]

*[1]Institute of Biochemistry, NAS of Belarus, Grodno, Belarus; [2]Grodno State Agricultural University, Grodno, Belarus*
*e-mail: a_makarchikov@yahoo.com*

ATP hydrolysis is eventually a driving force for most energy dependent processes of cellular activity. Yet, in some biosyntheses UTP, GTP and CTP are also used. The present communication deals with soluble NTPases involved in metabolism of NTPs in bovine tissues. Two soluble $Me^{2+}$ dependent NTPases with *m* 146 and 60 kDa have been revealed in bovine kidney and partially purified to give specific activities of 80.2 and 30.3 μmol/min/mg respectively. The 146 kDa NTPase was found to have rather strict substrate specificity catalyzing the cleavage of ITP, UTP, GTP and XTP, while neither other NTPs nor NDPs

could serve as substrates. The enzyme shows a maximal activity at pH 6.5–7.5 and obeys hyperbolic kinetics giving the apparent $K_m$ of 0.584 ± 0.02 mM for ITP. The NTPase of 60 kDa was capable of hydrolyzing all NTPs and NDPs examined, with relative rates ranging from 12.4% for ATP to 100% for UTP. The enzyme exhibited a pH-optimum of 7.5, the apparent $K_m$ for ITP was evaluated to be 24.0 ± 3.4 μM. When cytosolic fraction from the kidney was subjected to analytical gel-filtration, two NTPase activity peaks emerged, with the first one corresponding to *m* of 146 kDa and the second peak of 60 kDa. Thus, the enzymes seem to be localized in the cytosol. The 60 kDa NTPase occurs in various tissues, while liver, kidney and small intestine were found to be the richest sources of the high *m* enzyme.

# P13.102

## Physical and biochemical research of oligonucleotides influence on HPV16 DNA transcription and replication

Lidia Skibinska[1], Anna Goździcka-Józefiak[2], Łukasz Durzyński[2]

*[1]Faculty of Physics, Institute of Physics, Molecular Biophysics Department, Adam Mickiewicz University, Poznań, Poland; [2]Faculty of Biology, Institute of Experimental Biology, Department of Molecular Virology Adam Mickiewicz University, Poznań, Poland*
*e-mail: lidkaski@amu.edu.pl*

Human papillomaviruses (HPV) cause warts and have been well established as sexually transmitted agent that lead to invasive cervical cancer and associated precancerous lesions. The HPV virion has a double-stranded, circular DNA genom of approximately 8 kbp. The regulation of HPV genes expression is complex and is controlled by cellular and viral transcription factors. The E2HPV protein is the viral factor critical for viral replication and transcription. Short (20bp) oligonucleotides complementary to two different fragments of virus E2DNA were used in FCS experiment. Observations of fluorescence fluctuation of labeled oligonucleotides – virus E2DNA (non-labeled) solution can indicate interactions between those molecules. In the experiment some changes in correlation function and diffusion coefficient of labeled ssDNA in virus E2DNA solution were observed. Simultaneous changes in diffusion time and two components autocorrelation function reveal two kinds of fluorescence molecules: either free ones or E2DNA associated oligonucleotides. Those results imply that the 20-base DNA and E2DNA interact. Such interactions could be responsible for blocking further expression of virus genom. The process was also investigated by analysis of E2DNA replication (PCR technique) in the presence of those specific oligonucleotides. The purpose of the study is to obtain such specific oligonucleotides which would block virus transcription and replication of HPV.

# P13.103

## Genetic background of primary ciliary dyskinesia and Kartagener syndrome (PCD/KS) in Polish patients – analysis of mutations in the *DNAH5* gene and search for possible association with the major mutation in the *CFTR* gene

U. Skrzypczak, K. Voelkel, K. Modrzynska, E. Rutkiewicz, M. Witt[1], E. Zietkiewicz

*Institute of Human Genetics, Polish Acad Sci, Poznań; [1]also: Intl Inst Molec and Cell Biol, Warsaw*

Genetic background of primary ciliary dyskinesia (PCD), a rare genetic disorder caused by defects in the ultrastructure and motility of cilia, is not clear. Clinical symptoms affect airways and male reproductive system and in 50% cases are associated with situs inversus (Kartagener syndrome, KS). PCD, mainly inherited as an autosomal recessive trait, is genetically highly heterogeneous. Mutations in *DNAI1* and *DNAH5*, the only two genes proven to be involved in PCD pathogenesis, are responsible for less than ¼ of the cases. *DNAH5* (5p15.2) is characterized by high allelic heterogeneity, with over 40 mutations discovered so far in patients with immotile cilia and lack of ODA. Here, mutation screening of *DNAH5* was performed in ~100 Polish PCD/KS. Twneny-nine of 79 exons were examined so far. Thirteen mutations were found in 18 families, both PCD and KS; in all but 4 families, only one mutation was identified so far. Mutations (STOP and missense absent from ~160 control chromosomes), included 4 already known and 9 not previously described; 5 were found more than once, others were "private" for one family, confirming high allelic heterogeneity in *DNAH5.* Distribution of the identified mutations corroborates mutation clustering in exons 34, 34, 49. Participation of modifier genes, among them *CFTR,* has been postulated in PCD. PCD patients were therefore screened for the presence of the most frequent *CFTR* gene mutation, F508del. The mutation was found in 3 out of 124 PCD/KS patients, i.e. at the frequency not significantly different than in the general Caucasian population (~7/600 chromosomes), indicating no involvement of F508del in PCD/KS phenotype. Occurrence of two other *CFTR* mutations (2,3del21 kb, 3849+10 kbC > T) among PCD/KS patients is currently under investigation.

Supported by KBN 3PO5E 038-24.

# P13.104

## Purification of adenosine 5′-phosphoramidate hydrolase from yellow lupin (*Lupinus luteus*) seeds to homogeneity

Małgorzata Słocińska, Elżbieta Starzyńska, Andrzej Guranowski

*Department of Biochemistry and Biotechnology, University of Agriculture, Poznań, Poland*
*e-mail: mslocinska@yahoo.com*

Adenosine 5′-phosphoramidate is distributed in a wide variety of organisms including higher plants. It can play role as a regulatory molecule of some enzymes. Moreover it was identified as a substrate of rabbit Hint- and yeast Hnt1-proteins that act as hydrolases and are related to histidine triad proteins superfamily. Adenosine 5′-phosphoramidate can be synthesized from adenosine 5′-phosphosulfate (APS) and ammonia by adenylylsulfate:ammonia adenylyl transferase (EC 2.7.7.51) (Fankhauser *et al.* (1981) *Biochem J* 195: 545-560) and hydrolyzed to AMP and ammonia by adenosine 5′-phosphoramidate hydrolase (EC 3.9.1.). It is little known about the latter enzyme and its physiological role in living systems. So far adenosine 5′-phosphoramidate hydrolases have been purified from *Dictyostelium discoideum* and rat liver, and our study demonstrates for the first time the occurrence of its activity in plants. We have found adenosine 5′-phosphoramidate hydrolase activity in extracts from yellow lupin seeds and purified the protein to electrophoretic homogeneity by ammonium sulfate fractionation (0–40%), ion-exchange (DEAE-Sephacel), exclusion (Sephadex G-100), adsorption (hydroxylapatite) chromatographies followed by preparative electrophoresis. On the SDS/PAGE the purified protein showed one band of molecular mass around 32 kDa. Basic characterization of that protein will be presented.

# P13.105

### Phosducin family proteins in ciliates

K. Sobierajska, H. Fabczak, B. Groszyńska, A. Czechmański, S. Fabczak

*Department of Cell Biology, Nencki Institute of Experimental Biology, Warsaw, Poland*
*e-mail: k.sobierajska@nencki.gov.pl*

Phosducin (Phd) and related phosducin-like protein (PhLPs) were originally thought to down-regulate G-protein pathways. Indeed there is growing evidence that PhLPs may have other function in the cell. PhLPs were shown to modulate function of chaperonin CCT and mediate actin and tubulin folding as well is essential for G$\beta\gamma$ dimer assembly. Recently we have demonstrated that proteins analogous to both Phd and PhLPs also exist in ciliate *Blepharisma*. While the ciliate Phd is dephosphorylated in light-dependent manner, protein displaying immunoanalogy to PhLP exhibits no dependence of phosphorylation upon light. The ciliate Phd in its dephosphorylated state forms a complex with G$\beta\gamma$. In addition, using primers recognizing conserved domain of Phd we found fragment of *Blepharisma* Phd homologue sharing a significant degree of identity with mammalian Phd. To test whether these proteins are present in other ciliates we have analysed immunochemically *Paramecium* and *Tetrahymena*. The examination showed that 40 kDa protein was recognized by antibody against PhLP in both ciliates. Moreover, we have screened both *Tetrahymena* and *Paramecium* genomic data base with the collection of *PhLP* sequences from different organisms. It reveals presence of one gene in ciliates encoding the protein sharing high degree of identity with *PhLP*s. By using PCR with the primers of the putative *PhLP* gene (GWSUNIT00176487001) in *Paramecium*, this strategy resulted in the cloning and sequencing gene transcript of *Blepharisma*. The deduced amino acid sequence of the transcript displayed a significant degree of homology to *Paramecium* PhLP.

Supported by the grants 2P04C 01427 and 2P04C 01330 of the Ministry of Education and Science.

# P13.106

### Evaluation of the lipophilicity of purine derivatives by experimental and computational method

Jolanta Sochacka[1], Alicja Kowalska[2]

[1]*Department of General and Analytical Chemistry,*
[2]*Department of Organic Chemistry, Faculty of Pharmacy, Medical University of Silesia, Sosnowiec, Poland*
*e-mail: jsochacka@slam.katowice.pl*

Lipophilicity as one of the fundamental physico-chemical properties of the compounds decided about its biological activity and plays a key role in rationally projection of drugs process. Experimental method, the standard shake flask used to determination of lipophilicity expressed as log *P* have some limitations. The alternative techniques are the chromatographic methods. Lipophilicity parameters can be also estimated by a computer method. The aim of this study was to examine the lipophilicity of the series of purine derivatives: *I* – 2,6-dialkoxy (or 2,6-dialkylthio)-7-methylpurines, *II* – 2,6-dialkoxy (or 2,6-dialkylthio)-7,9-dimehyliodides, and *III* – bis [2-alkoxy (or 2-alkylthio)-7-methyl-6-purynyl]disulfides with the potential cytotoxic and cytostatic activity. The lipophilicity parameter log $P_{TLC}$ was determined by use of the RP TLC (RP-18$F_{254}$ plates, mobile phase: acetone, buffer pH 7.0). The clog *P* values were calculated using five computer programs. The experimental log $P_{TLC}$ values were compared with theoretical clog *P*. The effects of substituents on lipophilicity in compounds *I* – *III* were also be evaluated.

# P13.107

### Antioxidant activity of proline-rich peptides from colostrum of different mammalian species

Agata Sokołowska[1], Renata Bednarz[2], Antoni Polanowski[1]

[1]*Institute of Biochemistry and Molecular Biology, University of Wroclaw, Poland;* [2]*Iraferon, Poland*
*e-mail: Agata.Sokolowska@mars.bf.uni.wroc.pl*

Proline-rich polypeptide complex (PRP-complex) iso-

lated from ovine colostrum, known as Colostrinin®, has shown efficacy in treatment of Alzheimer's Disease after oral administration. Despite of therapeutic importance the mechanism of action of Colostrinin® is still unknown. The recent results pointed out the antioxidant activity of Colostrinin®. It significantly reduces 4HNE-mediated cellular damage and inhibits superoxide anion and nitric oxide production. It was found that the maximal amount of ABTS• free radical scavenged by 1.25 μg of PRP-complex ranged from 8–16 μM concentration under the test conditions, using modified TEAC assay, depending on colostrum's source and method of purification. Moreover the comparison of scavenging properties among several dairy baby-food available on Polish market was made. PRP-complex derived from human colostrum scavenged ABTS• at maximal average concentration (*C*) 15.89 μM whereas that of formula designated for newborns at the age of 0–5 months (made by Nutricia, Humana, Nestle) reached 3.39, 4.13, 3.95 μM, respectively. This fact underlined importance of newborns' feeding with first mother's milk. In the case of milk for children at the age from 1 to 3 years old ("Junior" made by Nestle) averaged ability to scavenge free radicals was almost twice higher (5.71 μM) and was closer to 8 μM reached for PRP-complex from bovine milk ("Łowickie"). The TEAC assay revealed that of over 30 component peptides of Colostrinin® synthetic analogues of B1, A2, B3, A4 peptides are the best scavengers of free radicals.

# P13.108

## Global changes in nuclear gene expression in response to elevated irradiance in *Arabidopsis thaliana*

[1]Małgorzata Solińska, [3]Maria Drath, [4]Małgorzata Oczko, [2]Paulina Piontek, [2]Artur Jarmołowski, [3]Włodzimierz Krzyżosiak, [1]Grzegorz Jackowski

*[1]Department of Plant Physiology, [2]Department of Expression of Genes, Adam Mickiewicz University, Poznań, Poland; [3]Laboratory of Cancer Genetics, Institute of Biiorganic Chemistry, Polish Academy of Sciences, Poznań; [4]Department of Nuclear Medicine and Clinical Endocrinology, Maria Curie-Skłodowska Oncological Center, Gliwice, Poland*
*e-mail: msolin@poczta.onet.pl*

Plants can perform profound changes in their structures and functions in response to fluctuating environmental conditions. Variations in irradiance have a potential to elicit responses at the whole plant, leaf and chloroplast level which may involve changes in gene expression patterns. To investigate global changes in expression patterns of nuclear genes of *Arabidopsis thaliana* exposed to elevated irradiance we took the advantage of DNA microarray technology. Expression profiles of 24000 nuclear genes represented by Affymetrix ATH1 genome array platform were monitored in plants grown in low light conditions and acclimated for 5 h to elevated irradiations generating various photosystem II (PS II) functional states (determined by PAM fluorimetry) reflecting various levels of risk of PS II photodamage (medium light – ML, high light – HL and excessive light – EL). Among the genes analysed 268 were revealed to be significantly down- or up-regulated (defined as ≥ 2-fold change) in plants exposed to all elevated irradiances. Totally 524, 690 and 1069 genes were differentially expressed in response to ML, HL and EL conditions, respectively. The most affected groups of genes have been functionally classified as metabolism and protein synthesis ones according to MIPS classification scheme.

# P13.109

## The Sgt1 protein – studies of its co-chaperone properties

Magdalena Spiechowicz[1], Jacek Kuznicki [1,2], Anna Filipek [1]

*[1]Nencki Institute of Experimental Biology, Warsaw, Poland; [2]International Institute of Molecular and Cell Biology, Warszawa, Poland*
*e-mail: a.filipek@nencki.gov.pl*

Sgt1 is a protein required for the activity of kinetochore and of ubiquitin ligase complexes in yeast cells (Kitagawa *et al.*, 1999). Sgt1 was also identified in other organisms including mammals where it was found at high level in the brain (Niikura and Kitagawa, 2003; Spiechowicz and Filipek, 2005). Immunohistochemical staining of human brain sections showed that Sgt1 is present in multiple neurons of various brain areas. Morphometric analyses revealed that the density of Sgt1-immunopositive neurons in the cortex of healthy aged and Alzheimer's disease (AD) brain sections is different. In AD a significant decrease in Sgt1-immunopositive neurons was found in some cortical areas such as temporal, angular and posterior cingulate cortex (Spiechowicz *et al.*, 2006). The presence of Sgt1 in postmitotic neurons and its decreased immunostaining in AD might suggest the involvement of Sgt1 in SCF-mediated ubiquitination or in regulation of some other multiprotein complexes. It has been already shown that mammalian Sgt1 binds to Hsp90 (Lee *et al.*, 2004). To check whether Sgt1 might be a component of a multi-chaperone complex, in this work, we studied the interaction of Sgt1 with several heat shock proteins. Our data showed that Sgt1 shares some properties of co-chaperone proteins and that it binds not only to Hsp90 but also to some other Hsp90–related proteins. Futher studies concerning the role of Sgt1 in a multi-chaperone complex are currently underway in our laboratory.

This work was supported by grants: MNiSW to A.F. and FIRCA/NIH to J.K.

# P13.110

## Effects of ochratoxin A on DNA repair

### in cultures of pig lymphocytes and porcine foetal kidney cells

Jan Stec

*Department of Biochemistry, National Veterinary Research Institute, Pulawy, Poland*
*e-mail: stec@piwet.pulawy.pl*

In cultured peripheral blood lymphocytes the mycotoxin ochratoxin A (OTA) induced unscheduled DNA synthesis (UDS) only in a narrow concentration range. Using a culture medium supplemented with 10% fetal calf serum, at 2 μM OTA a week induction and 25 μM OTA a marked induction of DNA repair was observed (15 ± 11% and 45 ± 21% respectively). Concentration > 25 μM OTA were cytotoxic, and < 2 μM no induction occurred. In cultures of porcine foetal kidney cells, a target organ of the mycotoxin OTA induced UDS in a concentration dependent manner. At concentration between 1 μM and 5 μM OTA a concentration dependent increase of repair was observed. Above 5 μM OTA, the cytotoxic concentration, a maximum of DNA repair intensity 60 ± 12% was found. This amount is comparable to control cultures incubated with 0.1 or 0.2 μM aflatoxin $B_1$ ($AFB_1$) (40 ± 15 and 60 ± 10% of repair intensity, respectively. These results shown that in cultured pig lymphocytes induction of UDS is relatively week whereas in foetal cells this effect was significant. In generally the foetal cells express very high activity of enzyme systems responsible for metabolism of xenobiotics as compared to cell from grown up animals. Whether this effect is also due to OTA metabolites formed locally in the kidney cells can not be excluded since the blood lymphocytes have been shown to be able to metabolize xenobiotics independently from the kidney or the liver. The results presented here strongly support the view that OTA is actually a genotoxic substance a fact which should be regarded in risk assessment for decision concerning this substance.

## P13.111

### The role of the $Na^+/K^+$-ATPase in the generation of procoagulant activity in blood platelets

Halina Stelmach, Marian Tomasiak, Tomasz Rusak, Michał Ciborowski

*Department of Physical Chemistry, Medical University of Bialystok, Poland*
*e-mail: mtomask@amb.edu.pl*

In circulation platelets may remain in contact both with exogenous and endogenously produced inhibitors of $Na^+/K^+$-ATPase. This study was performed to test how the inhibition of $Na^+/K^+$-ATPase by ouabain affects platelet procoagulant response. It was shown that platelets treated *in vitro* by ouabain (20–200 μM for 20 to 60 min) express procoagulant activity. Ouabain-induced procoagulant response was dose- and time-dependent, less pronounced than that evoked by collagen, similar to that evoked by gramicidin, not affected by EDTA or aspirin and strongly reduced in the absence of extracellular $Na^+$ ions or by the hyperosmolarity. Platelets treated by ouabain accumulated $^{22}Na^+$ in a dose dependent manner. Flow cytometry studies revealed that ouabain treatment results in the appearance of both degranulated and swollen cells. The ouabain-evoked rise in size and granularity heterogeneity was dose- and time dependent, similar to that produced by gramicidin and slightly affected by aspirin. Ouabain- and gramicidin- treated platelets express phosphatidylserine (PS). The ouabain-evoked PS expression was dose- and time dependent, weaker than that produced by collagen, similar to that produced by gramicidin and slightly affected by ASA. Electronic cell sizing measurements showed a time dependent increase in mean platelet volume upon treatment with ouabain and gramicidin. Hyposmotically evoked platelet swelling resulted in the development of procoagulant response. The obtained results indicate, that the rise in intracellular $Na^+$ concentration evoked by inefficiently operated $Na^+/K^+$-ATPase may produce platelet swelling, loss of plasma membrane asymmetry, and as a consequence- generation of procoagulant activity.

## P13.112

### Mutagenization as a method for increase the hydrolytic enzymes biosynthesis by *Geotrichum candidum* PH1 yeasts strain

Regina Stempniewicz, Michał Piegza, Danuta Witkowska, Anna Banasiak

*Department of Biotechnology and Food Microbiology, Agricultural University of Wroclaw, Wroclaw, Poland*
*e-mail: wit@ozi.ar.wroc.pl*

*Geotrichum candidum* yeasts synthesize many hydrolytic enzymes, which can be useful in food industry. The purpose of presented research was to improve the *G. candidum* PH1 strain by UV mutagenization in order to increase biosynthesis hydrolytic enzymes, which can degrade the non-starch polysaccharides. The strain was previously tested as a starter culture in barley malting process. The arthrospore suspension of *G. candidum* PH1 was exposed to UV light for 10 s, and after this the agar plate with Saunders medium with cellulose, xylan and pectin, as a enzymes inductor was inoculated. The same medium with glucose was used as a control. 5 days incubation in 30°C allowed to select 25 strains characterized by the good growth and differentiation of colony size between the plates with different substrates. UV-mutants and the parent strain were cultivated in MGP medium with sugar beet pulp at 28°C for 7 days, at 168 rpm (Gyratory Shaker). After culture the activity of cellulase, xylanase and polygalacturonase was determined in supernatants. Mutants of the parent strain expressed the 2–6 times high extracellular hy-

drolases activity than *G. candidum* PH1 strain. Among tested yeasts 10 strains synthesized effectively all of hydrolases, 9 strains – two of examineted enzymes and 2 strains only one. The most effective mutants were: *G. candidum* Pc3/d, which synthesized cellulase at 4.62 nKat/mg, *G. candidum* C2/d, which synthesized xylanase at 7.10 nKat/mg and *G. candidum* K3/d, which synthesized polygalacturonase at 16.74 nKat/mg level. The parent strain *G. candidum* PH1 had corresponding activity of 1.24 nKat/mg, 1.51 nKat/mg, and 3.07 nKat/mg level. It was shown, that mutagenization with UV light could be a good method for improvement the *G. candidum* strain in increasing the biosynthesis the important enzymes in malting, and the new strains could be much more effective in degradation of non-starch polysaccharides of barley.

# P13.113

## Cellobiose dehydrogenase in white wood rot fungi

Justyna Sulej, Tomasz Deptuła, Jerzy Rogalski

*Department of Biochemistry, Maria Curie-Skłodowska University (UMCS), Lublin, Poland*
*e-mail: justynasulej@o2.pl*

Cellobiose dehydrogenase (CDH) is an extracellular oxyreductase produced by organisms participating in the biodegradation of lignin cellulose. The enzyme possesses two prosthetic groups: heme and flavine. It is produced by many white and brown wood rot fungi as well as by certain bacteria. Cellobiose dehydrogenase is an enzyme possessive of potentially interesting qualities with some possible practical applications. The enzyme can be used in amperometric biosensors, which are utilized in biotechnology, food technology, agriculture, health service, medicine and sewage monitoring [1]. Currently, biosensors with CDH are used in detecting lactose in food. On the basis of the available CDH gene sequences, 24-nucleotyde starter sequences were designed, specific for cellobiose dehydrogenase. From the white root fungi collection, belonging to the Department of Biochemistry, UMCS, deoxyribonucleic acid was isolated, which was used in the PCR method identification of the CDH gene. The strains with the gene present were then grown on the liquid substrate with crystalline cellulose as the only carbon source and investigated for the dynamics of cellobiose dehydrogenase production. The strains *Phanerochaete chrysosporium, Schizophylum commune* and *Phlebia radiata* proved to be the most efficient organisms.

References:

1. Timur S, Pazarlioglu N, Pilloton R, Telefoncu A (2004) Thick film sensors based on laccases from different sources immobilized in polianiline matrix. *Sensors and Actuators B* 97: 132-136.

# P13.114

## Proline iminopeptidase of triticale seedlings

Urszula Szawłowska, Wiesław Bielawski

*Department of Biochemistry, Warsaw Agricultural University (SGGW), Warsaw, Poland*
*e-mail: ulaszawlowska@wp.pl*

Aminnopeptidases are important enzymes in plant growth and development, leaf senescence and proteolytic processes. Proline iminopeptidase (EC 3.4.13.8-9) is an aminopeptidase which can release specifically an amino-terminal proline residue from short peptides. Proline iminopeptidase, one of the four major aminopeptidases in the *xTriticosecale* Wittm. was partially purified. Its molecular weight was estimated to be about 398000 by gel filtration chromatography. Iminopeptidase hydrolyzed proline-β-naphthylamide more efficiently than the naphthylamides of phenylanine and alanine, and only weakly hydrolyzed the naphthylamides of arginine, leucine, tyrosine, and glycine. $K_m$ value for activity against proline-β-naphthylamide was $2.5 \times 10^{-7}$ M. The optimal pH for the hydrolysis of proline-2-naphthylamide was 7.8. The optimal temperature for iminopeptidase was 37°C, and enzyme was stable up to 37°C. The activity of proline iminopeptidase was inhibited by the *N*-(trans-Epoxysuccinyl)-L-leucine [E-64], but was also inhibited by phenylmethanesulphonyl fluoride and diisopropyl fluorphosphate. These results imply that the enzyme cysteine and serine residues play a possible part in the enzyme activity.

# P13.115

## Changes of ghrelin and growth hormone expression in hypothalamus – pituitary axis during pregnancy in the rat

D. Szczepankiewicz, M. Skrzypski, E. Pruszyńska-Oszmałek, P. Kaczmarek, T. Wojciechowicz, K. W. Nowak

*Department of Animal Physiology and Biochemistry, August Cieszkowski University of Agriculture, Poznań, Poland*

Ghrelin is a hormone regulating, above all, growth hormone secretion. However, ghrelin also regulates food intake and energy metabolism. Ghrelin acts *via* its receptors GHSR (Growth Hormone Secretagogue Receptor), mainly by active isoform GHSR-1a. It was found that ghrelin from hypothalamus and pituitary and hypothalamic GHRH (Growth Hormone Releasing Hormone) are connected and they both regulate growth hormone release. It was also suggested that ghrelin may indirectly influence energy balance by growth hormone release. In this experiment, growth hormone and active ghrelin level in serum were measured. We studied expression of ghrelin and GHSR-1a in hypothalamus and pituitary as well as growth hormone releasing hormone and growth hor-

mone receptor (GHR) in hypothalamus, growth hormone releasing hormone receptor (GHRH-R) and growth hormone in pituitary. Pregnant female Wistar rats were used according to the methodology described previously. Leptin and growth hormone in serum were measured by radioimmunoassay (RIA). Total RNA was used to perform real – time analysis of gene expression. In our study we found that active grelin and growth hormone increased during pregnancy and decreased after delivery. Moreover, growth hormone gene expression increased during pregnancy in pituitary and these changes were connected with higher expression of ghrelin in hypothalamus as well as its receptor GHSR-1a in pituitary. GHRH and its receptor expression decreased during pregnancy, so this mechanism is not likely to be resposible for GH release in this physiological state.

## P13.116

### Vasopressin-induced platelet procoagulant response

Marian Tomasiak, Halina Stelmach, Tomasz Rusak, Michał Ciborowski

*Department of Physical Chemistry, Medical University of Bialystok, Poland*
*e-mail: mtomask@amb.edu.pl*

Arginine vasopressin (AVP) is known to activate $Na^+/H^+$ exchanger (NHE) in human platelets. Activated NHE has been reported to mediate platelet procoagulant response. We therefore investigated whether *in vitro* treatment of platelets with AVP may results in procoagulant response. It was found that AVP (50–200 nM)-treated platelets express procoagulant activity (measured as phospholipid-dependent thrombin generation). AVP-evoked procoagulant response was dose and time dependent and weaker then that produced by collagen or monensin (mimics $Na^+/H^+$ antiport). Both the AVP- and collagen-produced procoagulant responses where less pronounced in the presence of EIPA (a NHE inhibitor), and genistein (tyrosine kinase inhibitor). Flow cytometry studies revealed that *in vitro* platelet treatment with AVP was associated with the appearance of both degranulated and swollen cells. AVP-evoked rise in size and granularity heterogeneity was similar to that produced by monensin and was reduced in the presence of EIPA or genistein. Using flow cytometry and annexin V-FITC as a probe for phosphatidylserine (PS) we demonstrated increased and uniform binding of this marker to all subsets of AVP-treated platelet population. AVP-evoked PS-expression was dose dependent, weaker than that exerted by collagen and inhibited by EIPA or genistein. As judged by optical swelling assay, AVP in a dose dependent manner produced a rise in platelet volume. The swelling was inhibited by EIPA and its kinetics was similar to that observed in the presence of monensin. Electronic cell sizing measurements showed an increase in mean platelet volume (MPV) following AVP-treatment. Rise in MPV was reduced by EIPA or genistein. It is concluded that in human platelets AVP is able to elicit procoagulant response, which is mediated by NHE exchanger.

## P13.117

### Genotyping intragenic neutral markers allows genetic homogenization of primary ciliary dyskinesia (PCD) families before screening for causative mutations in the *DNAH5* gene

A. Tomaszewska, K. Voelkel, E. Rutkiewicz, M. Witt[1], E. Zietkiewicz

*Institute of Human Genetics, Polish Academy of Sciences, Poznań; [1]also: International Institute of Molecular and Cell Biology, Warsaw, Poland*

Primary ciliary dyskinesia (PCD) is a rare genetic disorder caused by defects in the ultrastructure and motility of cilia. Clinical symptoms include chronic sinusitis, bronchiectasis and male infertility, in 50% cases associated with situs inversus (Kartagener syndrome, KS). PCD, mainly inherited as an autosomal recessive disease. So far, mutations in only two genes (*DNAI1* and *DNAH5)* have been identified to underlie ultrastructural cilia defects in PCD patients; they account for less than ¼ of the analyzed cases and are characterized by high allelic heterogeneity. Because of clinical and genetic heterogeneity of PCD, screening for causative mutations in the large *DNAH5* gene (79 exons) is not cost-effective. The aim of this research was to identify families, in which mutations in *DNAH5* are not involved in the pathogenesis of PCD/KS. Sixty-eight Polish PCD families (probands, their parents and siblings) were analyzed: 31 CDO (ciliary dysfunction only) and 37 KS families. Their DNA was analyzed for the consistency in the inheritance of neutral intragenic SNPs (in exons: 28, 44 and one in intron 74) and the disease phenotype. In addition, the diversity of centromeric microsatellite D5S1991 marker was analyzed. Some statistically significant differences between PCD/KS patients and healthy controls were noted in the frequency of 3-position SNP haplotype variants, but not of the allele frequency of particular SNPs. In some of the families, different genotypes were seen in affected siblings, in others both affected and non-affected siblings of the heterozygous parents had the same genotypes. *DNAH5* was hence classified as not involved in pathogenesis of the disease in 13 families with KS and 8 with CDO. This accounts for 1/3 of the informative (with more than one child) PCD families, which can be excluded from further screening for mutations in *DNAH5.*

Supported by KBN 3PO5E 038-24.

## P13.118

### The activity of the antioxidative enzymes in *Nicotiana tabaccum*

### Samsun nn infected with TMV

Monika Tomaszewska[3], Agnieszka Bajda[1], Dorota Konopka[2], Tadeusz Chojnacki[1], Ewa Swiezewska[1], Maria Drążkiewicz[3], Ewa Skórzyńska-Polit[3]

*[1]Department of Lipid Biochemistry; [2]Plant Pathogenesis Group, Institute of Biochemistry and Biophysics, PAS, Warszawa, Poland; [3]Department of Plant Physiology, Maria Curie-Skłodowska University, Lublin, Poland*
*e-mail: Ewa.Skorzynska@umcs.lublin.pl*

In our experiments *Nicotiana tabaccum* Samsun nn, infected with Tobacco Mosaic Virus (TMV) was used. Samsun nn plants are not resistant to TMV infection. Three neighboring fully expanded leaves of one-month-old plants were infected. Samples were collected at the zero time point, three and seven days after infection from infected plastron and mock plants (treated with water). Determination of the activity of antioxidative enzymes such as: ascorbate peroxidase (APX), guaiacol peroxidase (POX), superoxide dismutase (SOD), glutathione reductase and catalase (CAT) were carried out spectrophotometrically. The same method was also used to find pH optima of the activity of lipoxygenase (LOX). Among antioxidative enzymes POX activity was found to be significantly increased in leaves of *N. tobaccum* on the third and seventh day after TMV infection in comparison to mock plants. SOD and RG activities were strongly decreased in plants infected with TMV as compared to mock plants. Two optima at pH 7.5 and 8.5 for LOX activity were found in mock plants while in leaves of infected plants LOX activity at pH 8.5 was mainly increased. Changes in the activity of studied enzymes showed quick response to TMV infection.

This study was supported by the Committee for Scientific Research (KBN) grant No. PBZ-KBN-110/P04/2004.

## P13.119

### In search of short RNAs which influence the enzymatic activities of two ribonucleases: HIV-1 RT and human Dicer

Agata Tyczewska, Zofia Pietrusiewicz, Tomasz Twardowski, Marek Figlarowicz

*Institute of Bioorganic Chemistry Polish Academy of Sciences, Poznań, Poland*
*e-mail: agatat@ibch.poznan.pl*

Alongside proteins, small RNAs are main factors regulating gene expression. Through specific RNA-RNA or RNA-DNA interactions they control transcription, RNA stability, translation, structure of genes and host-virus interactions. The questions arise whether small RNAs could also regulate the functions of proteins (through specific RNA-protein interactions) and how such molecules could be encoded in the genome? We put forward the hypothesis that in organisms there might exist short RNA molecules that specifically bind proteins and influence their biological activities. The main goal of the undertaken project is to identify short RNA molecules that influence the enzymatic activities of two proteins involved in RNA degradation; namely: HIV-1 reverse transcriptase (it has three activities – polymerase, recombination and RNase H) and human ribonuclease Dicer. We have employed SELEX method to identify RNAs that bind target proteins with high affinity and specificity. After the selection procedure we used bioinformatic methods to check whether obtained RNAs are encoded in the transcribed parts of the human genome. So far we have finished the selection of 32 nt single-stranded RNAs specifically binding HIV-1 RT. We are now testing how the selected aptamers affect reverse transcriptase enzymatic activities. We are also ending the selection of 20 nt RNAs binding the human ribonuclease Dicer. After the 5th cycle of SELEX we performed test sequencing. The bioinformatic analysis showed that RNAs form imperfect hairpins with apical loops. So far we have not found any RNA which perfectly matches a transcribable fragment of the human genome.

## P13.120

### Changes in activity and kinetic properties of some key enzymes in rat tissues after the anabolic steroid treatment

Adam Tylicki, Agata Kawałko, Justyna Sokólska, Sławomir Strumiło

*Department of Animal Biochemistry, Institute of Biology, University of Białystok, Białystok, Poland*
*e-mail: atyl@uwb.edu.pl*

Effects of anabolic steroids are mainly based on their ability to induce protein synthesis in muscle tissue. However a possible influence of anabolic steroids on the activity and kinetic properties of key enzymes of metabolism has not been really studied. Therefore, we examined the effect of the known anabolic steroid Deca-Durabolin (DD) on enzymes which supply anabolic processes with NADPH and ribose phosphate and participate in bioenergetics. It was established by us that DD treatment (20 mg/kg) causes an activation of pyruvate dehydrogenase complex, glucose-6-phosphate and 6-phosphogluconate dehydrogenases, malic enzyme, and NADP-linked isocitrate dehydrogenase in the rat heart. About 7-fold increase of activity of pyruvate dehydrogenase complex may be based on its dephosphorylation by specific phosphatase. Moreover, 6-phosphogluconate dehydrogenase from the hearts of DD-treated rats showed higher affinity (lower Michaelis constant value) for its substrate, in comparison with control. In the liver and skeletal muscle only a few enzymes were sensitive to DD treatment. Activation of transketolase by DD in the liver was accompanied by inhibition of glucose-6-phosphate and 6-phosphogluconate dehydrogenases in this tissue. Body and liver weights of the rats were unchanged, but heart weight was increased 10 days after DD injection. Electrocardiographic data

showed additionally that a small prolongation of the QRS complex took place after DD treatment. It may be concluded that DD exhibits tropic and general stimulatory action *via* key enzymes in the heart muscle more distinct than in other tissues.

## P13.121

### Real-time quantitative PCR in monitoring minimal residual disease in follicular lymphoma patients

Andrzej Tysarowski[1], Jadwiga Kulik[1], Ewa Paszkiewicz-Kozik[2], Anna Fabisiewicz[1], Jan Walewski[2], Janusz A. Siedlecki[1]

*[1]Department of Molecular Biology, [2]Hemato-Oncology Intensive Care Unit, Lymphoma Department, Cancer Center-Institute, Warsaw, Poland*
*e-mail: a.tysarowski@coi.waw.pl*

Follicular lymphoma (FL) is a neoplasm of follicle center B cells. The t(14;18)(q32;q21) translocation is a characteristic aberration in follicular lymphoma and is detectable in 85% of cases. As a consequence, the anti-apoptotic *BCL-2* gene comes under the control of the *IgH* chain enhancer what leads to the overexpression of functional BCL-2 protein. Minimal residual disease – MRD is caused by a very low number of malignant cells which survived in different body compartment, despite the therapy, and is responsible for the relapse of the disease. Detection of t(14;18) by nested PCR, due to its high degree of accuracy, is used as a diagnostic tool in FL. In comparison real-time quantitative PCR (qPCR) enables the measurement of the number of circulating cells bearing t(14;18). It has been shown previously that detection of the t(14,18) in post-autologous bone marrow transplantation and chemotherapy treated patients correlated with relapse but t(14;18) positive cells were also detectable in patients during long-term clinical remissions. Presented work contains exemplary data collected from 23 individuals, who were investigated from 24 to 60 months of clinical treatment. The results demonstrate that nested PCR is enough sensitive to detect MRD but only qPCR permit to follow the decreasing number of FL-cells during the treatment. Therefore both PCR may be used to detect MDR but only qPCR may be helpful for clinicists to evaluate whether the patient is at high risk of relapse or in complete molecular remission.

## P13.122

### Noninvasive method of estimation of amino acid state of nursing mothers by the content of amino acids in breast milk

Magdalena Velichko[1], Ludmila Gutikova[2]

*[1]Chair of Physiology and Biochemistry, Grodno State Agrarian University, Grodno, Belarus; [2]Chair of Obstetrics and Gynaecology, Grodno State Medical University, Belarus*
*e-mail: klam@tut.by*

There was made a comparative quantitative and qualitative identification of free amino acids of breast milk and blood serum of puerperas (96 healthy and 249 suffered from preeclampsia during pregnancy: 1 group – light degree, 2 – medium, 3 – severe) on the 6 day of postnatal period applying amino acid analyzer KNAUER-Aminosis A 200 (USA) using postcolumnar derivatization of amino acids with ninhydrin. Sequentially eluated and determined: taurine, aspartate, threonine, serine, proline, glycine, alanine, valine, methionine, isoleucine, leucine, lysine, histidine, arginine, threonine, glutamate, phenylalanine, tyrosine. Results of the research work were processed statistically using the programme "ANOVA". There was revealed sharp direct correlation between the content of essential amino acids in breast milk and blood serum ($r = 0.57$; 0.59; 0.63; 0.61; $P = 0.02$); total quantity of all amino acids ($r = 0.75$; 0.89; 0.67 and 0.69; $P \leq 0.01$) in the screening group and main groups correspondingly. Provided adequate taking (in the morning, on an empty stomach) and processing of the sample of breast milk (centrifugation and deproteinization) this biological liquid can be highly competitive in diagnostic self-descriptiveness and value of samples of blood serum and can be used as noninvasive method of estimation of amino acid state of nursing mothers. The use of breast milk in clinic laboratory diagnostics allows to estimate indexes of amino acid metabolism of organism of puerperas quickly, repeat analyses many times, avoiding the probability of infection of examined women.

## P13.123

### Neutral haplotype diversity in the *CFTR* gene in healthy Polish population and in cystic fibrosis patients carrying four most frequent CF mutations

K. Voelkel, A. Jóskowiak, M. Nawracala, E. Rutkiewicz, U. Skrzypczak, M. Witt[1], E. Zietkiewicz

*Institute of Human Genetics, Polish Academy of Science, Poznań; [1]also: International Institute of Molecular and Cell Biology, Warsaw, Poland*

The aim of the study was to characterize neutral haplotype diversity in the *CFTR* gene in general Polish population and in Polish cystic fibrosis patients carrying the most frequent *CFTR* mutations: F508del, 2,3del21kb, 3849+10kbC>T and G542X. Four marker sequences within the *CFTR* gene were analyzed: two SNPs (T854 and TUB20), one two-allelic STRP (IVS6aGATT) and one multiallelic STRP (IVS17bTA). The analysis (PCR-RFLP, ASO hybridization and denaturing PAGE) was carried out on DNA samples from 125 CF patients and a reference group of 85 individuals. The most common IVS6a-

GATT-T854-TUB20 haplotype in healthy Polish population is 7-1-2 (54%; 59% in Europeans); it is associated with many IVS17bTA alleles (mainly 30, 31, 32). The second common haplotype is 7-2-1 (21%), only with allele 7 of IVS17bTA (in Europeans – 17%). Next frequent are: 6-2-2 (10%), 6-1-2 (7%), 7-2-2 (6%) – each associated with several IVS17bTA alleles; 7-1-1, 6-1-1 and 6-2-1 are very rare (< 1%); their distribution closely resembles that in the European population. The F508del mutation occurred on the background of 6-1-2 haplotype, most commonly with IVS17bTA alleles 31 and 32. Haplotype 6-1-2 with allele 33 was the most common background of the G542X mutation; in single chromosomes, G542X was found also on 7-1-2 background (with alleles 31 and 33) and on 6/7-2-1/2 background. Over 90% of the chromosomes with the 3849+10kbC>T mutation had 7-2-1 haplotype, associated exclusively with the allele 7. In several patients, the 3849+10kbC>T mutation occurred on 6-1-2 (with alleles 31 and 7) and 7-1-2 (with 46). The majority (over 90%) of the chromosomes with 2,3del21kb mutation occurred mainly on 7-1-2 (80% with allele 33 and 10% with 32); 10% mutations were found on the less frequent haplotypes (7-2-2 and 6-1-2, with 33). Possible explanations of the observed pattern of disease chromosomes distribution in populations are discussed.

Supported by KBN 3PO5E 038-24.

# P13.124

## Apoptotic effect of phytic acid in intestinal epithelial cells Caco-2

Ludmiła Węglarz[1], Joanna Wawszczyk[1], Arkadiusz Orchel[2], Zofia Dzierżewicz[2]

*[1]Department of Biochemistry, [2]Department of Biopharmacy, Medical University of Silesia, Sosnowiec, Poland*
*e-mail: lweglarz@slam.katowice.pl*

Phytic acid (PA), an essential component of high fiber diet, is physiologically present in human large gut at concentrations reaching 4 mM. It has revealed promising efficacy against various cancers including colon cancer but molecular mechanisms underlying its anti-malignant action are not fully understood. It has been suggested that PA could induce apoptosis of cancer cells, however, molecular mechanisms by which it activates apoptotic machinery in tumor cells remain unclear. The aim of the study was to determine the influence of phytic acid (1, 5 and 10 mM) on apoptosis of intestinal epithelial cells Caco-2. Induction of apoptosis by PA was examined in two ways: by analyzing expression of pro- and anti-apoptotic genes *bax* and *bcl-2* at the transcription level (after 12 h and 72 h PA treatment) and by measurement of caspase-3 activity in cells incubated with PA for 24 h and 48 h. A number of studies have revealed that apoptosis induced by many agents is mediated through an increase of the Bax/Bcl-2 ratio. Incubation of Caco-2 cells with 1 mM, 5 mM and 10 mM PA for 12 h caused 1.1-, 1.4- and 1.7-fold increased in Bax/Bcl-2 ratio, respectively. Treatment with 1 mM PA for 72h resulted in 1.9-fold increase in the Bax/Bcl-2 ratio, which did not change following incubation with higher PA doses. Phytic acid, both 5 and 10 mM induced caspase-3 activity in Caco-2 cells which suggests involvement of caspase-3 in PA-induced apoptotic events. Our results suggest that PA could induce apoptosis of Caco-2 cells by up-regulating the ratio of Bax/Bcl-2 and by activating apoptotic machinery through caspase signaling cascade.

# P13.125

## Enzymatic activities analysis of *Conidiobolus coronatus* colonies with high and low pathogenicity to *Galleria mellonella* larvae

Wioletta Wieloch, Mieczysława I. Boguś

*The W. Stefański Institute of Parasitology, Polish Academy of Sciences, Warsaw, Poland*
*e-mail: wiolaw@twarda.pan.pl*

*Conidiobolus coronatus* is a pathogenic fungus to different insect species. The fungal invasion occurs through penetration of the host integument by physical pressure and excretion of enzymes digesting insect cuticle like proteases, chitinases and lipases. Death of the host occurs as a result of the production of toxins by this fungus. Colonies of *Conidiobolus coronatus* derived from single spores of isolate 04:427 differed in their pathogenicity towards *Galleria mellonella* last instar larvae. It was examined that those colonies differ in their genetic profile by AFLP analysis. The aim of the study was to examine enzymatic activities of colonies in post incubation medium. Colonies MC9 (70% pathogenicity), MC39 (0% pathogenicity) and 04:427 (parent strain) were grown in LB medium for seven days. Medium was then filtered through paper filter and stored in –20°C until use. The enzymatic activities of post incubation medium were measured spectrophotometrically or fluorimetrically with the use of synthetic substrates suitable for elastase, chymotrypsin, chitinase, *N*-acetyl glucosaminidase and lipase assessments. Comparison of results revealed that colony MC9 shows the highest chymotrypsin, chitinase and *N*-acetyl glucosaminidase activities. Elastase and lipase activities were higher in colony MC39. Parent colony exhibited medial values of enzymatic activities. It seems that chitinolytic enzymes (chitinase and *N*-acetyl glucosaminidase) play crucial role in efficient fungal invasion into insect body. Protease activity is also required, but without synergistic activities of chitinolytic enzymes is not sufficient.

This work was supported by the Ministry of Education and Science, grant No. 2P04C 002 28.

# P13.126

## EPR studies of paramagnetic centers

### in streptomycin and erythromycin irradiated by protons

Sławomir Wilczyński[1], Janusz Lekki[2], Wojciech Kwiatek[2], Ewa Chodurek[3], Barbara Pilawa[1]

*[1]Department of Biophysics, School of Pharmacy, Medical University of Silesia, Poland; [2]Institute of Nuclear Physics, Polish Academy of Science, Cracow, Poland; [3]Department of Biopharmacy, School of Pharmacy, Medical University of Silesia, Sosnowiec, Poland*
*e-mail: swilczynski@slam.katowice.pl*

Type, properties and concentration of paramagnetic centers in streptomycin and erythromycin irradiated by protons were examined. Knowledge about changes of molecular structures in irradiated drugs are important for optimal sterilization processes. In our studies the induction of free radicals was carried out by an irradiation with a 2 MeV proton beam from the IFJ PAN Van de Graaff accelerator. The area of the irradiating beam spot on a tablet surface was about 0.12–0.14 $mm^2$ and every pill was irradiated in several (4–8) spots. The total proton beam charge per single pill varied from about 100 nC to 300 nC. The total dose deposited in the whole irradiated volume in a single pill was in the range of at least $4 \times 10^6$ Gy. A proton-induced characteristic X-ray yield was registered (PIXE method) by a Si(Li) detector (Princeton Gamma Tech). Detection and estimation of concentration of free radicals were carried out using electron paramagnetic resonance spectroscopy in powdered samples. Electron paramagnetic resonance measurements at room temperature were performed using an X-band (9.3 GHz) EPR spectrometer with modulation of magnetic field of 100 kHz. The microwave frequency was recorded. EPR spectra were measured with low microwave power ~0.7 mW to avoid microwave saturation of resonance absorption curves. Free radical concentration in the samples (N), g-factor and linewidths ($\Delta B_{pp}$) of EPR spectra, were measured. The influence of microwave power on amplitude and linewidth of the resonance absorption curves of the irradiated samples were determined.

## P13.127

### DNA microarray application for gene expression profiling in plants

Wiesława Włoszczak[1], Luiza Handschuh[2], Marek Figlerowicz[1], Michał M. Sikorski[1]

*[1]Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland; [2]University of Medical Sciences, Poznań, Poland*
*e-mail: wieslawa@ibch.poznan.pl*

DNA microarrays are one of the most powerful tools for gene expression studies enabling thousands of genes to be simultaneously analyzed in a single experiment. Herein, we have used an oligonucleotide microarray to monitor the expression of selected genes, encoding *pr-10* genes, ferritin genes, leghemoglobin genes and tRNA glutamine synthetase from yellow lupine. We designed forty two 24–28-nt-long oligonucleotide probes; two specific for each gene and two universal for each gene family. The probes were chemically synthesized and spotted in 8 replicates on the Corning Epoxide Slides with the SpotArray 24 (PE Life Sciences). Total RNA was extracted from the following yellow lupine organs: roots, leaves, seeds and pods, according to the Chomczyński and Sacchi protocol. DNA contaminants were removed by selective RNA precipitation with 2 M LiCl. After optimization of the procedure of target preparation we applied a method where total double-stranded cDNA was amplified by using oligo(dT) and 5′universal primers. Biotinylated cRNA probes were subsequently prepared from cDNA, fragmentized and used for microarray hybridization in a HybArray12 automatic hybridization station (PerkinElmer). Following hybridization, the microarrays were washed and incubated with streptavidin-Cy3. The microarrays were scanned with a ScanArray® Express (PE Life Sciences). The data were quantified and analyzed with the ScanArray Express software, using Adaptive Circle quantification method. Obtained results showed that the expression level of individual analyzed genes differs in studied samples. In addition, the hybridization signal generated by probes designed for the same gene may indicate the presence of other unknown homologues in examined organs.

## P13.128

### Optimization of reaction conditions to determine mycelial elastolytic activity of the entomopathogenic fungus *Conidiobolus coronatus*

Emilia Włóka, Mieczysława I. Boguś

*Institute of Parasitology, Polish Academy of Science, Warszawa, Poland*
*e-mail: milka@twarda.pan.pl*

Objectives: The relatively little-investigated parasitic fungus *Conidiobolus coronatus* (Entomophthorales) kills host insects rapidly and efficiently. Fungal entomopathogens produce an array of extracellular digestive enzymes: proteases, chitinases and lipases which are involved in degrading insect cuticle components. Among the enzymes taking part in breaching the cuticle, the proteinases are believed to play a major role and have been consider as a significant factor of fungal virulence. The major goal of this study, optimization of reaction conditions allowing to measure elastase activity in *C. coronatus* mycelia homogenates, is prerequisite for further studies concerning role played by elastase in *C. coronatus* virulence. Methods: Elastolytic activity in mycelial homogenate of *C. coronatus* propagated in LB medium was measured spectrophotometrically at 410 nm. *N*-succinyl-Ala-Ala-Pro-Leu *p*-nitroanilide was used as a chromogenic substrate. In exper-

iments the 100 mM Tris/HCl buffer pH range 4–11 with addition of 20 mM $CaCl_2$ was used. Results: The optimal parameters of enzymatic reaction are as follows: reaction time (5 min), total reaction volume (0.5 ml), amount of tested homogenat (0.350 mg of fungal proteins/ml), substrate concentration (10 mM). Elastase present in *C. coronatus* mycelium showed higher activity in 30°C representing the optimal temperature to cultivate the insect host *Galleria mellonella* (elastase activity 0.094 ± 0.001 dA/min) than in 20°C representing optimal temperature to propagate *C. coronatus* (elastase activity 0.079 ± 0.007 dA/min). Conclusion: Elastase activity is present in mycelial homogenates of *C. coronatus*. Further investigations are necessary to establish the role of *C. coronatus* elastase in insect cuticle degradation upon mycosis.

# P13.129

### Comparison of total antioxidant capacity of cell culture media

Maciej Wnuk[1], Anna Lewińska[2], Ewa Słota[3], Grzegorz Bartosz[2,4]

*[1]Department of Genetics, University of Rzeszów, Rzeszów, Poland; [2]Department of Biochemistry and Cell Biology, University of Rzeszów, Rzeszów, Poland; [3]Department of Immuno- and Cytogenetics, National Research Institute of Animal Production, Balice, Poland; [4]Department of Molecular Biophysics, University of Łódź, Łódź, Poland*
*e-mail: mawnuk@o2.pl*

Synthetic cell culture media contain such components as amino acids, carbohydrates, microelements, necessary growth factors and many more which may have influence results of *in vitro* studies. Their ingredients may have its pro- or antioxidant properties thus their could have great impact on cell redox homeostasis. Total antioxidant capacity (TAC) can thus be an important parameter of redox equilibrium of cell culture media. We evaluated TAC of common used mammalian, yeast and bacterial culture media like RPMI 1640, Iscove's Modified Dulbecco's Medium (IMDM), Dulbecco's Modified Eagle Medium (D-MEM) contains GlutaMAX I, MEM EAGLE'S 1959 with Earle's salts, DMEM, Parker medium 199 with Hanks salts; bacterial medium Luria Bertani (LB); yeast extract peptone-glucose (YPG) and yeast nitrogen base (YNB) adopting $ABTS^{\bullet+}$ decolorization assay and ferric ion reducing antioxidant power assay (FRAP). The obtained results suggest that tyrosine can be an important contributor to the antioxidant properties of mammalian cell culture media. We also found that other amino acids e.g. tryptophan and arginine may exert antioxidant properties. We observed that arginine may act directly as antioxidant and, moreover, it may enhance antioxidant effect of other compounds present in medium. Additionally, glucose and Phenol Red may be contribute to TAC of yeast and mammalian cell culture media, respectively.

# P13.130

### Leptin is involved in the porcine ovary endocrine function – Ob-Rb expression in the ovary during estrous cycle and granulosa cells steroidogenesis *in vitro* studies

T. Wojciechowicz, D. Szczepankiewicz, P. Kaczmarek, K.W. Nowak

*Department of Animal Physiology and Biochemistry, Agricultural University, Poznan, Poland*

Leptin is a protein hormone produced by the adipose tissue and, as a "satiety hormone", decreases appetite and increases energy expenditure in the organism. The main role for leptin is to control food intake and energy metabolism, however, leptin plays an important role in regulation of the reproductive system. Several lines of evidence indicate that leptin acts directly on the ovary: granulosa and thecal cells have high affinity receptors for leptin, moreover, leptin's receptor mRNA identified in porcine ovary has one of the highest level when compared to other organs. Ob-R mRNA has been identified in thecal and interstitial cells, whole ovary, corpora lutea and in luteinized *in vitro* granulosa cells of the human and porcine ovary. The aim of our study was to investigate the possibility that leptin may have direct effect on the porcine ovary. To probe this hypothesis we analyzed long form of the leptin receptor (Ob-Rb) expression in granulosa and thecal cells during the estorus cycle of the porcine ovary using the semiquantitative RT-PCR assay. Second, we analyzed the leptin action on progesterone secretion in cultured granulosa cells isolated from the porcine preovulatory follicles. The results obtained in our experiments indicate that leptin has direct action in porcine follicles: (i) leptin can exert biological effects on porcine granulosa and thecal cells *via* Ob-Rb receptor, (ii) Ob-Rb expression vary during follicle growth in granulosa cells – is expressed in a relatively high level in granulosa cells obtained from small follicles (iii) leptin decrease progesterone secretion from cultured granulosa cells and, therefore, can be the ovarian steroidogenesis regulator.

# P13.131

### Substrate specificity phenol monooxygenase isolated from *Stenotrophomonas maltophilia* KB2 strain

Danuta Wojcieszyńska, Izabela Greń, Sylwia Łabużek, Monika Respondek

*Department of Biochemistry, University of Silesia, Katowice, Poland*
*e-mail: dmanka@us.edu.pl*

Several bacterial strains use phenols substrates as their primary sources of carbon and energy. The wide range of substrates that can be transformed by these microorgan-

isms make them a powerful tool for the bioremediation of environmentally harmful substances. Flavin-dependent monooxygenases play an important role in the mineralization of phenolic compounds. In the present study we investigated substrate specificity phenol monooxygenase isolated from *Stenotrophomonas maltophilia* KB2 strain after induction different phenols. The bacterium was cultured in mineral medium. The carbon source (one of phenolic compounds) was added to a final concentration of 5 mM. The crude extract was assayed for phenol monooxygenase activity. We tested transformation of 13 phenols. The phenol monooxygenase activity was monitored spectrophotometrically following the oxygenation of NADH at 340 nm. Protein concentration in crude extract was determined by the Bradford's method. The catalytic parameters of phenol monooxygenase: $K_m$, $V_{max}$ were calculated by iteration using SigmaPlot 9.0 software. The catalytic parameters of phenol monooxygenase were estimated. Based on the obtained results $K_m$, $V_{max}$ were estimated to be 1.62 μM, 6.39 μM/min, respectively. The phenol monooxygenase shows maximal activity at pH 7.0 and 30°C. The phenol-induced enzyme from *Stenotrophomonas maltophilia* KB2 manifested a wide substrate range in transforming aromatic compounds. All of 13 phenols were transformed. When the cells were induced by methylphenols the monooxygenase activity was the highest. 4-nitrophenol- or 3-nitrophenol-induced enzyme transformed only 7 of investigated phenols. When the cells were induced by 4-aminophenol than the activity of monooxygenase was the lowest.

## P13.132

### Stabilizing effect of molecular oxygen complexed to myoglobin on the protein structure

Edyta Woźniak-Celmer[1], Artur Giełdoń[2], Michał Woźniak[1]

*[1]Department of Medical Chemistry, Medical University of Gdańsk, Gdańsk, Poland; [2]CERM, University of Florence, Via Sacconi, 6, 50019 Sesto Fiorentino (FI), Italy*
*e-mail: edyta@amg.gda.pl*

Myoglobin (Mb) is a small monomeric enzyme with molecular mass 17 kD. The primary Mb function is to bind and release molecular oxygen ($O_2$). Myoglobin works also as a facilitator of the diffusion of $O_2$ from capillaries to the mitochondria [1], in a working muscle Mb is a storage of dioxygen. Insight the Mb active site heme group containing protoporphiryn ring with centrally located iron ion on +2 oxidation state. Fe(II) ion has tetragonal bipyramid geometry and is coordinated by 4 N-atoms from protoporphiryn ring and two histidine residues – proximal (His93) and distal (His64). In the present work molecular modeling (MM) and molecular dynamics (MD) techniques were used to study theoretical model of horse heart myoglobin. The protein model was build using Mb crystal structure of Mb (Brookhaven Protein Data Bank file code (1azi)) [2] as a template. Following the nonprotein fragments present in the original pdb file were eliminated. Subsequently heme group parameterized on the template of protoporphiryn IX [3,4] using GAMESS program [5] was immersed in the active site of Mb. All simulations were done using Amber 7.0 force fileld [6], with following conditions: 1 fs time step, anisotropic scaling pressure bath of $p_0$ = 1 at, T = 300 K and 1 ns total simulation time. The results shows high termodynamical stability of horse heart Mb with non-occupied active site/active center occupied by heme group (HEM)/ active center occupied by heme group with inorganic ligand $O_2$ (HEM+$O_2$). In each computed model after the simulation the topology of all 8 (A-H) $\alpha$ helices and overall protein structure was maintained well. In the Mb model with flat heme group docked in the active site, the non-occupied space between iron ion Fe(II) from protoporphiryne ring and distal histidine (His63) was visible. It is probably the first state of "preparation" for binding of small ligand (e.q. $O_2$). The Mb active center non-occupied/occupied by heme group HEM is wider and shallower than in model complexed with heme group and dioxygen. Those changes show that presence of small inorganic ligand in the active site of Mb stabilizes structure of protein. Two histidine residues – distal and proximal from E7 and F8 helices, respectively play an additional stabilization role of the Mb structure, especially in model with $O_2$. The observed vertical and horizontal conformation of distal and proximal histidine residue (in respect order) are confirmed by the literature data. We found also, that presence of dioxygen in active center of Mb indicate its deeper penetration by the heme ring.

References:

1. Blomgerg LM, Blomberg MRA, Siegbahn PEM (2005) *J In Biochem* 99: 949-958.
2. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissing H, Shindyalov IN, Bourne PE (2000) The Protein Data Bank. *Nucleic Acids Res* 28: 235-242.
3. Little RG, Dymock KR, Ibers JA (1975) *J Am Chem Soc* 97: 4532-4539.
4. Koening D (1965) *Acta Cryst* 18: 663-673.
5. Schmidt MW, Baldridge KK, Boatz JA, Elbert ST, Gordon MS, Jensen JH, Koseki S, Matsunaga N, Nguyen KA, Su S, Windus TL, Dupuis M, Montgomery JA (1993) *J Comput Chem* 14: 1347-1363.
6. Case DA *et al.* (2002) Amber v7.0. University of California.

## P13.133

### Phosphate deficiency affects acid phosphatases activity and growth of two wheat varieties

Ewa Wyszołmirska[1], Agnieszka Szczygła, Iwona Ciereszko[1]

*[1]Department of Plant Physiology, Institute of Biology, University of Białystok, Białystok, Poland*
*e-mail: ewaw@uwb.edu.pl*

Phosphate deficiency is considered to be one of the major crop limitation factor. Therefore plants have evolved various strategies, including root architecture modifications, organic acid exudation or enzymes secretion that enhance Pi uptake in limiting conditions. The influence of phosphate deficiency on plant growth and activity of acid phosphatases (extracellular and intracellular) in roots and shoots were investigated. Two wheat varieties (*Triticum aestivum* L. *vs* Bryza and Opatka) were grown for 1–3 weeks on complete nutrient medium (+P) and without phosphate (–P). Pi deficiency changed growth of all studied plants: significantly decreased shoot growth, however not affected root elongation. The root/shoot ratio in Pi deficient plants was higher as compared to the control. Pi content decreased in the leaves and roots of all the studied plants grown in the –P media. Phosphate starvation significantly increased the activity of extracellular and intracellular acid phosphatases. The increase of phosphatase activity was generally more significant in Bryza cultivar as compared to Opatka. The highest acid phosphatase activity was observed in shoots of Pi deprived plants. Our results indicate an important role of acid phosphatases in the acclimation of both wheat cultivars to early Pi deficiency.

# P13.134

## RPGR mutation in the patient with overlapping X-linked retinitis pigmentosa and primary ciliary dyskinesia symptoms apparently disrupts transport of the inner dynein arm component into the cilium

E. Zietkiewicz[1], M. Geremek[1,6], H. Omran[2], S. Amselem[3], A. Pogorzelski[4], W. Biczysko[5], M. Witt[1,7]

*[1]Institute Human Genetics, Polish Academy of Sciences, Poznan; [2]University Hospital, Freiburg; [3]INSERM, Creteil; [4]Institute Tuberculosis and Lung Diseases, Rabka; [5]University Medical Sciences, Department of Pathomorphology, Poznan; [6]Polish Academy of Sciences, Medical Research Center, Warsaw; [7]International Institute Molecular and Cell Biology, Warsaw, Poland*

RPGR (retinitis pigmentosa GTPase regulator) protein has been localized to connecting cilia of photoreceptor rods and cones and in the transitional zone of motile cilia. Mutations in the *RPGR* gene on Xp21.1, responsible for 70% of the X-linked retinitis pigmentosa (RP), have been reported to be associated with pigmentary retinopathy, hearing loss, and bronchosinusitis. Recently, mutation in *RPGR* has been reported in a family with overlapping phenotypes of the X-linked RP and primary ciliary dyskinesia (PCD). Here, we analyzed the *RPGR* in a multi-generation Polish family with previously reported overlapping XLRP and PCD. All cilia in the proband were immotile and electron microscopy analysis revealed numerous abnormal cilia, with inner dynein arms (IDA), but not ODA, missing. A missense G52R mutation in the last nucleotide of *RPGR* exon 2 was found upon direct PCR sequencing of 19 exons, ORF15, and their surrounding introns in the proband. G52R is an example of exonic mutation disrupting splicing process and resulting in the frameshift and premature termination. X-linked inheritance of this mutation in the family was confirmed by ASO hybridization. Immunostaining with fluorescent detection was performed on epithelial cells from nasal brushing. Predictably, mutated *RPGR* was not present in the transitional zone of cilia in the patient. Another antibody, targeting a component of inner dynein arms, revealed defect of those structures in patients cilia. Results indicate that RPGR plays an important role in the transport of inner dynein arms components into the cilium. Defect of this process may cause overlapping symptoms of X-linked RP and PCD.

Supported by by KBN PBZ 122/P05.

# P13.135

## Melatonin and relative compounds in the brain after tryptophan administration to rats with shifted light-dark cycle

M. Zolotukhin, Ya. Darashenka

*Grodno State Medical University, Grodno, Belarus*

Aim of study: We investigated the possibility to reverse diurnal rhythms of central synthesis of biogenic amines and relative compounds in rats by rapid reversing of light-dark cycle and the effects of tryptophan (Trp). Methods: Male Wistar rats were synchronized by artificial light-dark cycle for 14 days followed by rapid reversing the phases of the cycle. After next 14 days tryptophan was administered intragastrically, 100 mg/kg, 3 h after the beginning of the dark phase (100 mg/kg). Rats were sacrificed 1.5 h after the Trp administration. The concentrations of biogenic amines and their metabolites were determined by HPLC. Results: We found significant increase of the levels of Trp, 5-HTP, 5-HT, 5-HIAA, Nacetyltryptophan (NAT), tryptamine (Trn), melatonin in the striatum, Trp, 5-HTP, 5-HIAA, and NAT in the hypothalamus and brain hemispheres, 5-HIAA and NAT, but not Trp, in the midbrain, and Trp and 5-HT (11-fold) in the pineal gland. The last changes were accompanied by remarkable elevation of neither melatonin nor NAS. The results obtained suggest that the forced exchanging the light and dark phases by 14 days is not appropriate to induce corresponding changes in the diurnal rhythms of melatonin synthesis. Despite the strong increase in the synthesis and turnover of serotonin, the administration of Trp did not lead to the increase in the melatonin content in the pineal gland and all brain regions except the striatum. The pineal melatonin level was found to be 4.59 ± 1.37 pmol/g after Trp administration and 6.86 ± 0.94 pmol/g in control rats. Both values are close to normal light-phase levels. Thus, the excessive intake of tryptophan in high doses, which can induce remarkable acceleration of serotonin synthesis, is still unable to increase of melatonin synthesis.