Session 13. Biospectroscopy and Oxidative Stress

Lectures

L13.1

Spectroscopic detection of reactive oxygen species and oxidative stress

Izabela Sadowska-Bartosz1,2, Grzegorz Bartosz1,3

University of Rzeszów, 1Department of Biochemistry and Cell Biology, 2Department of Biotechnology and Microbiology, Rzeszów, Poland; 3University of Łódź, Department of Molecular Biophysics, Łódź, Poland

e-mail: Grzegorz Bartosz <gbartosz@biol.uni.lodz.pl>

Reactive oxygen species (ROS) include both free radicals and non-radical compounds. ESR spectroscopy, especially employing spin traps, is the unique technique allowing for detection, identification and quantification of free radicals in vitro and their imaging in vivo. However, the sensitivity of this technique is limited and essays based on the use of more sensitive fluorogenic and luminogenic probes have gained enormous popularity. Classic fluorogenic probes such as 2′,7′-dichlorodihydrofluorescein diacetate or dihydrorhodamine 123 are broadly used for detection of peroxides, but their specificity is low so often they are regarded as general indicators of oxidative stress than detectors of specific ROS. Dihydroethidine has been routinely used for detection of superoxide, but only one of its oxidation products is specific for this radical so precise identification of the product requires HPLC separation. The common problem with fluorogenic and luminogenic probes, especially when used at high concentrations, is their redox cycling; the probes not only detect but also generate ROS. Recently, a variety of new probes, i. a. boronate and BODIPY based, have been introduced, some of them allowing for a more selective detection of ROS. Probes targeted to mitochondria by a positively charged (usually phosphonium) moiety allow for detection of ROS produced by the main endogenous cellular source of ROS. A promising novel approach consists in the use of ROS-reactive fluorescent proteins. HyPer is a unique genetically engineered yellow fluorescent protein (YFP) for ratiometric measurement of hydrogen peroxide; cpYFP protein has been proposed as a superoxide sensor and pn-Green Fluorescent Protein (GFP) as a sensor of peroxynitrite. Other proteins have been constructed for measurement of other indices of oxidative stress like changes in the redox state of main cellular redox systems. RexYFP is sensitive to changes in the NAD+/NADH ratio and rxYFP measures changes in the oxidation state of glutathione. Fluorescent proteins can be targeted to various cell compartments allowing for studies of oxidative stress at the subcellular level. However, they have also drawbacks related, i. a., to the effects of other factors on their fluorescence and possible effects of their presence on cellular redox homeostasis.

L13.2

Advanced optical biosensors for new applications in biology and medicine

Sabato D’Auria, Andrea Scala, Adelia Majoli, Alessandro Capo, Maria Staiano

Institute of Protein Biochemistry, Italian National Research Council, Napoli, Italy

e-mail: Sabato DAuria <s.dauria@ibp.cnr.it>

The know-how for fluorescence protein-sensing is advancing rapidly owing to the continued introduction of new concepts, new fluorophores, and proteins engineered for sensing-specific analytes. Concerns about the reversibility and selectivity of engineered proteins are being addressed by developing biosensors that are based on the utilization of coenzyme-depleted enzymes. Such biomolecules do not consume the substrate and can exhibit conformational changes upon the binding of the analyte, which can be easily detected as fluorescence change. In addition, concerns about the stability of biosensors can be overcome by using thermostable enzymes isolated from thermophilic microorganisms. Finally, the development of new techniques such as polarization-based sensing, anisotropy-based sensing and lifetime-based sensing, all of which can be accomplished with light-emitting diodes as the light source, is prompting the design of a new class of specific and stable biosensors, as has occurred with blood glucose measurement. These biosensors represent a valid alternative to the conventional clinical chemistry diagnostics. Here, we introduce the recent advances in fluorescence biosensors for monitoring celiac disease, a disease present all over the world.
Surface plasmon-coupled emission
Ignacy Gryczynski
Center for Fluorescence Technologies and Nanomedicine, Department of Cell Biology and Immunology, University of North Texas Health Science Center, USA
e-mail: Ignacy Gryczynski <ignacy.gryczynski@unthsc.edu>

Surface Plasmon-Coupled Emission (SPCE) is a plasmonic phenomenon occurring at metallic surfaces attached to a high refraction index glass prism. It converts near-surface fluorescence into a directional radiation. Induced by the excited molecules traveling surface plasmons are out coupled into the glass prism in a form of a hollow cone with very well defined angle. This angle corresponds to a surface plasmon resonance (SPR) angle at the fluorescence emission wavelength. SPCE offers an ultrasensitive detection from a minimal volume of the sample but preserves fluorescence properties of the excited dyes. We will describe advantages of SPCE radiation and its applications in fluorescence spectroscopy/microscopy.

Oral presentations
O13.1
Multiple assay strategy for revealing molecular mechanism of 5’ mRNA cap degradation by Decapping Scavenger enzyme
Zbigniew M. Darżynkiewicz1, Elżbieta Bojarska1, Jan Antosiewicz1, Jacek Jemielity1,2, Edward Darżynkiewicz1,2, Ryszard Stolarski1
1Division of Biophysics at Faculty of Physics, University of Warsaw, Warsaw, Poland; 2Centre of New Technologies, University of Warsaw, Warsaw, Poland
e-mail: Zbigniew Darzynkiewicz <zibi@biogeo.uw.edu.pl>

The 5’ terminal mRNA cap structure is required for premRNA splicing, export, nucleolytic stability and cap-dependent translation. The abundance and lifetime of mRNA is controlled by two major degradation pathways, one of which entails enzymatic hydrolysis of the cap by Decapping Scavenger. The enzyme functions as a homodimer, and is not expected to exist in a stable monomeric form due to large domain-swapping. In addition to the wild-type protein we expressed and purified two heterodimeric mutants with one of the monomers modified at the binding site for loss of its enzymatic activity. The first mutation, H277N, disables cleavage activity, yet the protomer is capable of binding a ligand. The second mutation, N110A / W175A, results in compromised binding capability. Kinetic studies of the cap degradation by the wild-type protein as well as the mutants gave further insight into the enzymatic process, product release and inhibition. Moreover, the cap-binding mechanism was followed using a broad class of non-susceptible, synthetic, cap analogues. Steady-state fluorescence titration provided the protein-ligand equilibrium association constants. Influence of various chemical modifications within the cap structure gave further insight into the details of the enzyme active site that are not visible form the X-ray structure. Pioneer application of dye-labelled cap analogues enabled a ‘reversed’ observation concept, focusing on the ligand emission. Fluorescence time-resolved experiments showed changes in labelled cap analog fluorescence life-times upon binding by protein. Furthermore, novel method of circular dichroism measurements in near UV was applied, in which each aromatic amino-acid type exhibited signal change in a different wavelength. Such effect enabled observations of tertiary structure rearrangements. The overall picture was completed with calorimetric measurements. The latter imply energy effect of the electrostatic contacts, especially H-bonds. Comparison of the results with those previously obtained for the initiation factor eIF4E gave initial data for a broader view of the protein-mRNA interactions.
O13.2

L-Arginine can prevent ethanol-induced oxidative stress in the liver

Alexandr N. Borodinsky, Yury E. Razvodovsky
Grodno State Medical University, Department of Pathological Physiology, Grodno, Belarus
e-mail: Yury E. Razvodovsky <razvodovsky@tut.by>

Background: Alcoholic liver disease is one of the most serious consequences of chronic alcohol abuse [1]. Oxidative stress via generation of reactive oxygen species is suggested to be the major mechanism of alcohol-induced liver injury [2, 3]. Aims: This study tested the hypothesis that ethanol-induced oxidative stress in the liver can be prevented by the administration of L-arginine amino acid in the experimental binge drinking model of alcoholism.

Methods: We investigated the effect of intragastrical administration of L-arginine (500 mg/kg) on oxidative stress and antioxidant status in the liver of male Wistar rats under interrupted alcohol intoxication (8 mg/kg/day (7 days of alcohol intoxication and 7 days of abstinence) during 42 days).

Results: The results revealed that interrupted alcohol treatment caused a significant increase in the level of lipid peroxidation indicated by increase in the levels of superoxiddismutase (SOD)/glutathioneperoxidase (GSH-Px) activities and decrease in the activity of glutathionereductase (GSH-Red). Activation of lipid peroxidation was accompanied by significant increase in serum gamma-glutamyl-transpeptidase (GGTP) activity and decrease in nitric oxid (NO) concentration. Supplementation of L-arginine significantly lowered the activities of SOD and GSH-Px, decreased the level of lipid peroxidation, enhanced the antioxidant status, normalized the activity of biochemical marker of the liver damage (GGDP) and increased the NO concentration.

Conclusions: This study demonstrated that ethanol-induced liver damage is associated with oxidative stress and co-administration of L-arginine may attenuate this damage by decreasing oxidative stress in the experimental binge drinking model of alcoholism.

References:

O13.3

Identification of 2-pyridoxyl-1,3-thiazolidine-4-carboxylic acid, novel metabolite of vitamin B6 and cysteine in human plasma

Rafał Glowacki1, Monika Wyszczelska-Rokiel1, Hieronim Jakubowski2,3
1University of Łódź, Faculty of Chemistry, Department of Environmental Chemistry, Łódź, Poland; 2Department of Microbiology and Molecular Genetics, Rutgers - New Jersey Medical School, Newark, NJ 07103, USA; 3Department of Biochemistry and Biotechnology, University of Life Sciences, Poznań, and Institute of Bioorganic Chemistry, Poznań, Poland
e-mail: Monika Wyszczelska-Rokiel <monikawyszczelska@wp.pl>

Pyridoxal 5’-phosphate (PLP), the active form of vitamin B6 in the human body, is an important co-factor for many enzymatic reactions, including the transsulfuration of homocysteine (Hcy) to cysteine (Cys). The PLP deficiency is the cause of many disorders, such as hypochromic, microcytic or iron-refractory anemia, inflammatory diseases or cancer. PLP is known to react with Cys and Hcy in vitro, forming corresponding 1,3-thiazolidine-4-carboxylic acids and 1,3-tetrahydrothiazine-4-carboxylic acids, respectively. We used HPLC-based methods and chemical tests to identify a novel form of vitamin B6 in human plasma. We provide evidence that 2-pyridoxyl-1,3-thiazolidine-4-carboxylic acid (PTCA), an adduct formed in a reaction of PLP with Cys, is present in human plasma. PTCA was separated from other metabolites present in plasma by an HPLC assay that allows simultaneous quantification of PTCA and low-molecular weight thiols, such as Cys and Hcy. The assay is based on pre-column derivatization with 2-chloro-1-methylquinolinium tetrafluoroborate (CMQT) under acidic conditions, affording a stable UV-absorbing derivative that is separated by HPLC and quantified by monitoring at 355 nm, a characteristic UV maximum of thiol-CMQT derivatives. PTCA was identified by its co-elution with an authentic PTCA standard during HPLC, its sensitivity to base hydrolysis, and the recovery of PLP and Cys after base hydrolysis. PTCA was present in human serum at 44.6±15.8 µM, a concentration similar to plasma reduced Cys, but 1000-fold higher than plasma PLP. In conclusion, our results suggest that PTCA is the major form of vitamin B6 in human plasma and provide methodology for examination of the role of PTCA in human health and disease.

Acknowledgements
**O13.4**

**Isolation and characterization of cancer cell lines resistant to Photodynamic Therapy**

Somayeh Shahmoradi Ghahe¹, Milena Bażlekowa¹, Barbara Tudek¹,²

¹Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Warsaw, Poland; ²Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

e-mail: Somayeh Shahmoradi ghahe <s.shahmoradi@biol.uw.edu.pl>

Photodynamic therapy (PDT) is a medical treatment that utilizes a Photosensitizer (PS) and visible light. PS absorbs light and, in the presence of oxygen, transfers the energy, producing cytotoxic oxygen species that damage cell components and lead to cell death. There are several cellular defense mechanisms against PDT including DNA repair, drug resistance pump, activity of antioxidant detoxifying enzymes and heat shock proteins.

In this study we have grown human cancer cell lines resistant to PDT in order to study their characteristics and resistant markers of PDT. To this aim, eight different cell lines were chosen and exposed for 3 h to 0.6 mM 5-ALA in serum free medium and then irradiated with increasing light dose (635 nm) to achieve survival level in the 4-10% range. From all ALA-PDT treated cell lines, HeLa, human glioblastoma (U-87) and cervical carcinoma (SKG-IIIa) cell lines showed stable resistant features after receiving about 10 cycles of ALA-PDT and two weeks interval between each cycle. Cell doubling time was considerably longer in U87 ALA-PDT resistant cell line compare to parental cell, but no difference in doubling time of other cell lines was observed. SKG-IIIa showed that needed less courses of PDT than other cell lines to become resistant to the treatment. Besides studying some cell characteristics of resistant cells, activity of base excision repair enzymes has been measured and expression of ABCG2, HO-1, OGG1, APE1 and ABH2 genes has been studied by both qRT-PCR and Western blotting that results of which will be shown.

---

**O13.5**

**Enzymatic generation of highly fluorescent nucleoside analogues using various forms of purine-nucleoside phosphorylase (reverse reaction)**

Alicja Stachelska-Wierzchowska¹, Jacek Wierzchowski¹, Beata Wielgus-Kutrowska², Goran Mikleuševic³

¹Department of Physics and Biophysics, University of Varmia & Mazur in Olsztyn, Poland; ²Division of Biophysics, Institute of Experimental Physics, University of Warsaw, Poland; ³Division of Physical Chemistry, Ruder Boskovic Institute, Zagreb, Croatia

e-mail: Alicja Stachelska-Wierzchowska <alicja.stachelska@uwm.edu.pl>

Mammalian and bacterial forms of purine-nucleoside phosphorylase (PNP) as well as their variants effectively catalyze syntheses of a variety of highly fluorescent nucleoside analogs, potentially useful as fluorescent probes of the enzyme active site. In particular, ribosides of 2,6-diamino-8-azapurine can be synthesized using a wild-type and the Asn204Asp mutant of the calf PNP, while the *E. coli* PNP can be used to effectively synthesize highly fluorescent adenosine and inosine analogs such as 1,N6-etheno-adenosine and lin-benzo-inosine. In some instances, the non-typical N8- and/or N7-ribosides can be obtained as well. Kinetic parameters of the synthetic and/or phosphorolytic reactions suggest strong interaction between enzymes and substrates. Potential applications for analytical and/or mechanistic studies will be discussed.
Posters

P13.1

Structural and spectroscopic studies of nanosystems based on gemini surfactants as innovative delivery systems in gene therapy

Weronika Andrzejewska, Maciej Kozak

Department of Molecular Physics, Faculty of Physics, Adam Mickiewicz University, Poznań, Poland
e-mail: Weronika.Andrzejewska <andrzejewskaw@gmail.com>

The process of complex formation between cationic gemini surfactants and nucleic acids is very interesting and important from the point of view of gene therapy. This new method of treatment of genetic diseases based on the application of a transgene introduced into a pathogenic cell and this is the hope for successful treating neurodegenerative disorders or cancer. The most important thing is to find the best vector for gene delivery into infected cell. Perfectly suitable for this purpose are non-viral vectors - delivery system based on surfactants. These compounds have specific properties such as good solubility, low toxicity, biocompatibility and ability to form ordered structures (e.g. hexagonal and regular shape). With the help of spectroscopic and structural studies we try to determine the optimal parameters for such systems [1-3].

In particular, the aim of this study was to determine the possibility to use gemini surfactants as complexing agents for nucleic acids [4, 5].

References:

Acknowledgments

The research was supported by research grant (UMO-2011/01/B/STS/00846) from National Science Centre (Poland).

P13.2

Lipoic acid, a well-known antioxidant, is able to restore aldehyde dehydrogenase activity blocked by nitroglycerin. Implications for nitrate tolerance

Anna Bilska-Wilkosz, Magdalena Górný, Danuta Kowalczyk-Pachel, Małgorzata Iciek, Maria Sokolowska-Jeżewicz, Lidia Włodek

Jagiellonian University, Medical College, Chair of Medical Biochemistry, Kraków, Poland
e-mail: Anna.Bilska-Wilkosz <anna.bilska@uj.edu.pl>

A link between lipoic acid (LA; 1,2-dithiolane 3-pentanoic acid), its reduced form dihydrolipoic acid (DHLA; 6,8-dimercapto-octanoic acid) and aldehyde dehydrogenase (ALDH) activity is a new hypothesis postulated in the literature of recent years in the context of studies on the enzymatic mechanisms underlying bioactivation of organic nitrates and tolerance to these compounds [1,2].

In the present study we investigated the ability of LA, DHLA and dithiothreitol (DTT): (1) to prevent ALDH inactivation by nitroglycerin (glyceryl trinitrate, GTN) and (2) to restore GTN-inhibited ALDH activity.

The studies were conducted with the use of yeast ALDH. The activity of ALDH was determined in the presence of 1 mM NAD and 8 μM propionaldehyde as a substrate. The enzyme was preincubated in three ways (1) with 0.1 mM GTN in 50 mM sodium phosphate buffer (pH 8) for 20 min; (2) with 0.1 mM GTN for 5 min, then with addition of DHLA/LA (1mM) for further 15 min; (3) with DHLA/LA (1mM) for 5 min, then with addition of 0.1 mM GTN for further 15 min. Catalytic activity of ALDH was determined by the spectrophotometric method based on the test utilizing optical properties of NAD(P)/NAD(P)H/H+. Data are presented as a percentage relative to the control sample containing only ALDH without GTN, LA and DHLA (100%).

The obtained results indicated that ALDH activity in the presence of GTN decreases to 12.1%. When the enzyme was earlier preincubated with GTN and then with LA or DHLA, ALDH activity was 31.6% and 71.9%, respectively. On the other hand, when ALDH was earlier preincubated with LA/DHLA and then GTN was added the activity was 68.6% and 106.9%, respectively. This means that LA and DHLA were able to restore ALDH activity blocked by GTN. It was also shown that GTN did not inhibit ALDH activity when the enzyme was earlier preincubated with DHLA.

References:

Acknowledgments

This work was supported by the National Science Centre, Kraków, Poland, grant no. 2011/01/B/NZ4/01675.

Warsaw, September 9th–12th 2014
Amphotericin B (AmB) due to its chemotherapeutic properties occupies a special place among the antifungal agents. It has been considered the gold standard in the treatment of systemic fungal infections. The physicochemical mechanism of AmB action is related to the formation of transmembrane channel structures by this antibiotic. The chemical mechanism is associated with destructive effects of the antibiotic on the cell membrane through the oxidative effect. As a result of free radical reactions, different severe peroxidation of phospholipids occurs in the fungal cell membrane. Since the mechanism of increased permeability of the membrane due to the formation of channels by AmB not only explains its fungicidal activity (only fungistatic), some authors have shown that the oxidative mechanism is responsible for the killing effect of the antibiotic. Also AmB toxicity against eukaryotic cells may be associated with oxidative processes mentioned above. Due to possessing in the molecule the system of seven conjugated double bonds (chromophore), AmB may be subject to oxidation processes. In the experimental conditions the oxidation of AmB molecule was induced by free iron (iron (III) chloride, pH 2.5) and by heme iron (methemoglobin, pH 7.0). The effects of interaction of iron (III) with AmB was analyzed by electron spectroscopy (absorption and fluorescence) and oscillation (FTIR and Raman). The use of the chelator 1,10 phenanthroline clearly defined oxidative effect of iron (III) on AmB molecule. The observed spectral changes may indicate the selective oxidation of conjugated double bonds in the chromophore of AmB molecule by ferrous ions (III). Presented spectral changes in the spectra of AmB were first observed in our previous studies where the typical oxidant - KMnO₄ was used. In the present work, we obtained the same spectroscopic effects, but induced by a significant from a biological point of view oxidant - iron (III) ions. In this case, the observed spectral changes suggest further selective oxidation of the conjugated double bonds in the chromophore of AmB molecule by iron (III) ions.

Reference:

Acknowledgements
This research was financed by the National Science Centre of Poland on the basis of decision no. DEC-2012/05/B/NZ1/00037.
### P13.6

**Oxidative modification of protein in pediatric cystic fibrosis patients with bacterial infections**

Sabina Galiníak1, Marta Rachel1, Grzegorz Bartosz2, Iżabela Sadowska-Bartosz2

1Department of Biochemistry and Cell Biology, University of Rzeszów, Rzeszów, Poland; 2Department of Molecular Biophysics, University of Łódź, Łódź, Poland; 3Institute of Physiotherapy, Faculty of Medicine, University of Rzeszów, Rzeszów, Poland; 4Department of Biotechnology and Microbiology, University of Rzeszów, Rzeszów, Poland

e-mail: sabina.galinia@wp.pl

**Introduction:** Cystic fibrosis (CF) is a systemic and genetic disease, involving the disorder of secretion of exocrine glands, causing changes in the respiratory and digestive tract. The patient’s body produces too sticky mucus that causes abnormalities in the internal organs. Well-characterized genetic cause of this recessive disease is one of 1500 mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) including the most common known as ΔF508. Lungs of patients with CF, due to the presence of viscous mucus already in infancy and early childhood, are colonized by *Staphylococcus aureus* and *Haemophilus influenzae*. These bacteria contribute to the damage of epithelium lining the airways and subsequent colonization with *Pseudomonas aeruginosa* or *Burkholderia cepacia* complex.

**Aim of study:** This study was aimed at comparison of the usefulness of plasma protein oxidation parameters: carbonyl and sulphhydril group content, AOPP and AGEs; dityrosine, N*-formylkynureine, kynurenine, tryptophan fluorescence and Amadori products, as well as erythrocyte carbonyl and sulfhydryl group content; AOPP and AGEs.

**Results and conclusions:** Elevated glycophore (4.22±0.91 and 4.19±1.04 vs control 3.18±0.53 fluorescence units (FU)/mg protein; p<0.05) and carbonyl group levels (1.9±0.64, 1.87±0.45 vs control 0.94±0.19 nmol/mg protein; p<0.05) as well as increased glutathione S-transferase activity (2.5±0.88 and 2.57±0.79 U/g Hb vs 0.77±0.16 U/g Hb; p<0.05) were noted in *Pseudomonas aerugi- nosa* and *Staphylococcus aureus* infected CF. Kynurenine level (4.91±1.22 vs 3.89±0.54 FU/mg protein; p<0.05) was elevated only in *Staphylococcus aureus* infected CF. These results confirm oxidative stress in CF and demonstrate the usefulness of the glycophore level and protein carbonyl groups as markers of oxidative modifications of plasma proteins in this disease.

**Acknowledgments**

This work was financially supported by the NCN, Poland under Decision No DEC-2012/05/ZZ2/01618 (M.S.); DEC-2012/05/B/ST6/05472 (S.S.) and by grant BMW / 514 /RAU-1/2013 x 26 (K.G.), and also ERA SyLabBio project CSys, ANR (Grant No. ANR-2009-SYTB-002-02) and through the “Investments for the Future” LABEX SIGNALIFE program ANR-11-LABX-0028-01 (ED.).

---

### P13.5

**Intercellular differences in cell cycle progression observed with FUCCI reporter system**

Karolina Gajda1, Magdalena Skonieczna1, Patryk Bil1, Sebastian Student1, Ryszard Smolarczyk2, Céline Feillet2, Franck Deaunay2, Patrick Martin2, Joanna Rzeszowska-Wolny1

1Institute of Automatic Control, BioSystems Group, Silesian University of Technology, Gliwice, Poland; 2Institut de Biologie Valrose, Université Nice-Sophia Antipolis, CNRS UMR7277/INSERM U1091 Parc Valrose, 06108 Nice cedex 2, France; 3Institute of Oncology, Maria Skłodowska-Curie Memorial Cancer Center and Gliwice Branch, Center for Translational Research and Molecular Biology of Cancer, Gliwice, Poland

e-mail: Karolina Gajda <k.gajda86@wp.pl>

Genotoxic stress causes changes in cell cycle progression and cancer cells exposed to ionizing radiation usually arrest cell cycle in G2 phase. However observation of single living cells using fluorescent microscopy shows that the individual cells sometimes differ in the length of cell cycle phases. The aim of the present study was to compare the cell cycle progression by the individual control and exposed to ionizing radiation HCT116 and HEPA1-6 cancer cells. FUCCI (fluorescent ubiquitination-based cell-cycle indicator) reporter system was used to visualize cell cycle. Both cell lines were transfected with FUCCI reporter genes coding for red (mKO2) and a green (GFP) fluorescent protein fused with cell cycle specific proteins Cdt1 and geminin under the control of their promoters. In transfected cells Cdt1 protein tagged with mKO2 is expressed identifying G1 cells with red fluorescent nuclei. In the S, G2, and M phases geminin tagged with GFP is expressed and cells in these phases are marked with green fluorescent nuclei. HCT 116 cells were observed in vivo and images were captured every 1 hour after IR, by time-lapse fluorescence microscope at 37°C and 5% CO2. To examine irradiation effects cells were exposed to 4 Gy dose of X-rays immediately before transfer to microscopy.

Observation performed on un-irradiated HEPA1-6 cells showed the presence of cells whose daughter cells were characterized by significantly different times of cell cycle progression. Irradiation caused strong cell cycle inhibition in G2 in HCT116 cells 12 hours after irradiation and showed time-lapsed dynamics in comparison to control cells. The FUCCI system allowed for the detection of asymmetry in behavior of the cells descending from the same mother cell and characterization of cell cycle dynamics in different cell types.

**Acknowledgments**

This work was financially supported by the NCN, Poland under Decision No DEC-2012/05/NN2/01618 (M.S.); DEC-2012/05/B/ST6/05472 (S.S.) and by grant BMW / 514 /RAU-1/2013 x 26 (K.G.), and also ERA SyLabBio project CSys, ANR (Grant No. ANR-2009-SYTB-002-02) and through the "Investments for the Future" LABEX SIGNALIFE program ANR-11-LABX-0028-01 (ED.).

---

**Warsaw, September 9th–12th 2014**
P13.7

The effect of lipoic acid on the activity of glyceraldehyde-3-phosphate dehydrogenase in vitro

Magdalena Górny, Małgorzata Iciek, Anna Bilska-Wilkosz, Danuta Kowalczyk-Pachel, Lidia Włodek
Jagiellonian University, Medical College, Chair of Medical Biochemistry, Kraków, Poland

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), an enzyme which plays a crucial role in glycolysis, is a protein whose activity is affected largely by oxidative modification. The activity of GAPDH is mainly dependent on the presence of Cys-152 in the active center of the enzyme. Reactive -SH group of cysteine can undergo the processes of S-nitrosylation, S-glutathionylation, S-sulfhydration and oxidation to sulfenic, sulfinic and sulfonic acid. Most of these reactions leads to a loss of the enzymatic activity of GAPDH [1-3].

In this study, we investigated the effect of lipoic acid (LA) on the skeletal muscle–derived glyceraldehyde-3-phosphate dehydrogenase activity in vitro. Lipoic acid, its oxidized form (disulfide) is used as adjunctive therapy in the treatment of diabetes and diabetic complications.

The enzymatic activity of GAPDH was monitored by the increase in NADH concentration ($\varepsilon_{340} (NADH) = 6.220 \text{ M}^{-1}\text{cm}^{-1}$). The control mixture contained 50 mM Tris-HCl buffer (pH 7.5) with 5 mM EDTA, 20 mM potassium phosphate, 1mM glyceraldehyde-3-phosphate (GAP), 1 mM NAD+ and 25 ug/ml GAPDH. The assay mixture contained additionally 1 mM LA.

Preliminary studies showed that lipoic acid inhibited GAPDH activity by more than 50% as compared to controls, suggesting that the LA modifies thiol groups of GAPDH by S-lipoylation. It seems that these results are essential to supplement our knowledge of the regulation of this enzyme's activity in the glycolytic pathway. On the other hand, they show additional biological aspects of lipoic acid used in therapy of many diseases (diabetes, neurodegenerative diseases, diseases of joint) [4].

Reference:

P13.8

Evaluation of the impact of coenzyme Q and vitamin A and E on the activity of protein metabolism enzymes of liver of rats intoxicated with sodium fluoride

Natalia Grzegorzak1, Barbara Stawiarska-Pięta1, Monika Sojka1, Agata Kabała-Dzik1, Jolanta Zalejska-Fiolka2, Magdalena Wyszyńska1, Ewa Birkner2
1Department of Pathology, Faculty of Pharmacy, Silesian Medical University in Katowice, Sosnowiec, Poland; 2Department of General Biochemistry, Faculty of Medicine in Zabrze, Silesian Medical University in Katowice, Zabrze, Poland

e-mail: Natalia Grzegorzak <ngrzegorzak.natalia@gmail.com>

The studies have shown that exposure to fluoride causes oxidative stress, which is one of the causes of metabolic disorders in various organs. Thus, recent researches have confirmed that various antioxidant can stop oxidative changes after exposure to fluoride. The aim of this study was to assess the impact of co-administration of CoQ with vitamins A and E on the activity of protein metabolism enzymes and TAS in liver of rats that were exposed to NaF. The study was conducted on 18 female Wistar rats. The animals were divided into 3 groups with 6 animals in each. Rats in the control group received distilled water. In the I and II study groups animals received 2.5 mg NaF/rat/24 h. Additionally, animals in the group II were given 200 μg CoQ/rat/24 h, 3 mg vitamin E/rat/24 h and 250 IU vitamin A/rat/24 h.

The experiment lasted 5 weeks. Then the animals were anesthetized by intraperitoneal injection of 0.5 ml of 1% heksobarbital/100g of body mass. At necropsy the liver was taken. The activities of aspartate aminotransferase (AST) (EC 2.6.1.1), alanine aminotransferase (ALT) (EC 2.6.1.2) and γ-glutamyltranspeptidase (GGTP) (EC 2.3.2.2) were determined in liver homogenates. Total antioxidative status (TAS) and protein concentration was also determined.

The studies showed a statistically significant decrease in AST activity in the liver of experimental animals. In contrast, ALT and GGTP activity was slightly reduced, indicating a fluoride ions interference in the metabolism of proteins. Co-administration of fluoride and antioxidants showed beneficial effects on strengthening of the total antioxidant capacity of the liver, and the activity of the assayed protein metabolism enzymes. The values of these parameters were similar to those in the control group.

In the summary, the results indicate the possibility of preventing changes related to exposure to fluoride by strengthening the antioxidant defense system.
P13.9

**Amifostine — investigation of the prodrug transformation process into the biologically active thiol form**

Małgorzata Iciek, Magdalena Górný, Anna Bilska-Wilkosz, Danuta Kowalczyk-Pachel, Lidia Włodek

Chair of Medical Biochemistry, Medical College of Jagiellonian University, Kraków, Poland

e-mail: Małgorzata Iciek <malgorzata.iciek@uj.edu.pl>

Amifostine (S-2-(3-aminopropylamino)-ethylphosphoro-thioate; WR2721; Ethyol) is one of a few drugs able to protect healthy tissues during radio- and chemotherapy. The drug is administered intravenously or subcutaneously in the inactive form in which the thiol group is phosphorylated. In endothelial cells, amifostine is dephosphorylated in the reaction catalyzed by alkaline phosphatase (ALP) to the active metabolite with a free sulfhydryl group (-SH) ([2-(3-aminopropylamino)-ethanethiol; WR1065]). This compound acts mostly as a reactive oxygen species (ROS) scavenger. Amifostine selectively protects normal tissues without reducing the toxic action of radio- and chemotherapy due to low ALP activity in cancer cells.

The aim of the present study was to develop a new in vitro method for examination of kinetics of ALP-catalyzed transformation of the inactive drug form into the active form. The method of determination of -SH groups with the Ellman’s reagent (5,5′-dithiobis-2-nitrobenzoic acid (DTNB)) is not reliable under these conditions because DTNB is unstable and decomposes non-enzymatically in alkaline environment. Moreover, the Ellman’s method is not useful for determination the active form of WR1065 in biological material due to the presence of other thiols (e.g., glutathione).

The studies were conducted with the use of endothelial ALP from bovine intestine and amifostine manufactured by LKT laboratories. The studies led to development of the method for monitoring of ALP-catalyzed reaction of WR2721 phosphorylation to WR1065. It consists in a direct measurement of absorbance at a wavelength of 233 nm selectively absorbed by WR1065. Thus, the methods allows for a direct continuous monitoring of an increase in product concentration. This method was used for determination of kinetic parameters (K_m and V_max), however, despite that a wide range of amifostine concentrations was used, saturation of the enzyme with the substrate was not achieved. Similar situation was described for WR2721 and ALP from the canine liver.

Monitoring of the reaction of amifostine transformation into its active thiol form is important for further studies aimed to widen its use. WR1065 can be oxidized to a disulfide form or can form mixed disulfides in this way influencing cellular thiol status.

P13.10

**Expression of antioxidative enzymes and lipids peroxidation in kidneys of frog exposed to heavy metal ions**

Marta Kaczor-Kamińska1, Piotr Sura2, Maria Wróbel1

1Jagiellonian University, Collegium Medicum, Chair of Medical Biochemistry, Kraków, Poland
2Jagiellonian University, Collegium Medicum, Chair of Medical Biology, Kraków, Poland

e-mail: Marta.Kaczor-Kaminska <martak.kaczor@uj.edu.pl>

e-mail: Maria.Wrobel <maria.wrobel@uj.edu.pl>

Heavy metal ions (Ca^{2+}, Hg^{2+}, Pb^{2+}) are environmentally persistent toxins. Toxic effect exerted by these metal ions is multidirectional. There are three main reasons for their high toxicity in living organisms: 1) high affinity for thiols, amino- and carboxyl groups of amino acid residues in proteins (change of the structural and catalytic properties of proteins), 2) the effect on the antioxidant potential of cells — a reduced level of glutathione can ultimately leads to the generation of oxidative stress, 3) they compete with divalent cations of metals such as copper or zinc in binding for active sites of proteins what leads to the inhibition or modification of their activity.

Main goal of the experiment was the determination of malone dialdehyde concentration (one of lipid peroxidation products) and a change of gene expression (glutathione peroxidase, catalase and superoxide dismutase — cytoplasmatic and mitochondrial form) in the kidney of *Xenopus tropicalis*. Sulfurtransferases genes expression were also assayed. The animals had been exposed to lead ions (28 mg/l), cadmium (40 mg/l), and mercury (1.353 mg/l) for 10 days.

The exposition to heavy metal ions caused an increase in amount of mRNA for all sulfurtransferases (γ-cystathionase, 3-mercaptoppyruvate sulfurtransferase and rhodanese) with one exception for mercucic ions which caused a decreased expression of 3-mercaptoppyruvate sulfurtransferase. Expression of glutathione peroxidase, catalase and cytoplasmic form of superoxide dismutase was also increased. The amount of mRNA for γ-cystathionase and mitochondrial superoxide dismutase remained unchanged. The exposition to heavy metal ions was without any effect on lipid peroxidation under the experimental conditions.

Obtained results suggested that the exposure to heavy metal ions generates oxidative stress in the frog's kidney. That effect was associated with the increased expression of antioxidative enzymes in the kidney.
Polyphenolic compounds exhibit a series of beneficial effects on human health. For this reason, new substances of high biological activity, and without side effects, are sought for. Leaves of mini-kiwi are a rich source of polyphenols, but are not used in health protection mostly due to scanty reports on their biological activity. The results of our earlier studies showed that substances contained in kiwi leaves do not exert any destructive effect on the erythrocyte membrane but, on the contrary, make it stronger and more resistant to changes in the medium toxicity. When in contact with membranes, the substances become imbedded in the hydrophilic region of the membrane, which makes them potential, effective scavengers of free radicals that arise in membrane vicinity. In addition, the changes in physical properties of the erythrocyte membrane inducted by the extract may suggest that the compounds interact strongly not only with membrane lipids but can also modify the membrane proteins.

The aim of the study was to determine the biological activity of a mini-kiwi (Actinidia arguta) leaf extract. In particular, the ability of the extract compounds to protect erythrocytes and their membranes against hemolysis and peroxidation induced by various physicochemical agents. In addition, the investigation was to examine the effect of the extract on activity of the protein responsible for multidrug resistance of L5178 mouse lymphoma T cells transfected by MDR gene.

The spectrophotometry and cytometry methods were used in the investigation. The antioxidant activity of the extract was determined based on the concentration of malondialdehyde formed during lipid peroxidation. The extract’s anthermolytic activity was found based on the concentration of hemoglobin released as a result of the oxidative damage of erythrocytes. The extract’s effect on activity of the MDA protein (glycoprotein P1) was determined on the basis of the accumulation of 123 rhodamine in MDR treated and parental cell lines.

The present investigation showed that the mini-kiwi extract is effective in protecting the erythrocyte membrane against oxidation induced by UVC and UVB radiation. Moreover, the compounds contained in extract protect erythrocytes against hemolysis induced by free radicals. A study on the effect of extract on MDR protein activity in cells of mouse lymphadenoma showed that the extract modifies the protein’s activity only slightly.

Substances contained in kiwi leaf extract are effective in protecting erythrocytes and their membranes against the harmful action of free radicals and practically do not influence multidrug resistance of tumor cells.
Factors influencing compact-extended structure equilibrium in oligomers of Aβ1–40 peptide — an ion mobility mass spectrometry study

Marcin Kłoniecki1, Ewa Sitkiewicz1, Jarosław Poznański1, Wojciech Bal1, Michał Dadlez1,2
1Institute of Biochemistry and Biophysics PAS, Biophysics, Warsaw, Poland; 2Institute of Genetics and Biotechnology, Biology, Warsaw, Poland
e-mail: Marcin Kłoniecki <marcinkloniecki@wp.pl>

Oligomers formed by amyloid beta peptide (Aβ) are widely believed to be the main neurotoxic agent in Alzheimer’s disease. Studies discovered a broad variety of oligomeric forms, which display different levels of toxicity. Some of these forms may further assemble into mature fibrils, while others might be off-pathway from conversion to fibrils and assemble into alternative forms. To better understand a relationship between the structure and toxicity of Aβ oligomers, systematic characterization and classification of all possible forms is required, facilitating rational design of the beneficial modifiers of their activity. In previous ion-mobility analysis of Aβ1–40 oligomers, we have detected the coexistence of two alternative structural forms (compact and extended) in a pool of low-order Aβ1–40 oligomers. These forms may represent two pathways of the oligomer evolution, either leading to fibrils or to off-pathway oligomers, potential candidates for the neurotoxic species. Here, we have analyzed the impact of incubation time, the presence of selected metal ions and the effect of a series of point mutations on mutual population of alternative forms. We have shown that a salt bridge D23K28 provides stabilization of the compact form whereas G25 is required for the existence of the extended form. We have found that binding of metal ions also stabilizes the compact form. These results improve our understanding of the possible molecular mechanism of the bifurcation of structural evolution of non-monomeric Aβ species into an off-fibril pathway, ultimately leading to the formation of potentially neurotoxic species.

Degradation of vitamin E derivatives modulated by UVB light

Grażyna Neunert1, Przemysław Siejak1, Piotr Wałejko2, Stanisław Witkowski2, Krzysztof Polewski1
1Poznan University of Life Sciences, Department of Physics, Poznań, Poland; 2University of Białystok, Institute of Chemistry, Białystok, Poland
e-mail: Grażyna Neunert <neunert@up.poznan.pl>

In the past few years, most research on tocopherols has focused on structural variations of vitamin E (α-Toc) with the aim to improve the proapoptotic potency of these agents and simultaneously less susceptible to oxidation than α-Toc. The most studied member of these developed compounds is α-tocopheryl succinate (TS).

In search of synthetic derivatives of vitamin E with strong antitumorigenic potency and more resistant to influence of light, we examined the irradiation decomposition process of two novel esters: di-α-Tocopheryl maleate (TMA) and α-Tocopheryl malonate (TMO), and commercially available α-Tocopheryl succinate (TS). To our knowledge, this study presents the first time the efficacy and mechanism of photodegradation of vitamin E ester derivatives induced by UVB light in a single study.

The photodecomposition of α-Toc and its esters in some organic solvents was monitored by absorption spectrosopy. Upon UV illumination of α-Toc the absorption bands at 223 and 295 nm become less pronounced indicating the consumption of vitamin E. Simultaneously, a new absorption band appears around 250 nm attributed to α-tocopherol quinone. The absorbance bands of studied esters peaking at 279 nm and 285 nm for similar irradiation conditions as for α-Toc, decreased with irradiation time, while in the ranges 240-270 nm and 290-310 nm the absorbance increased. During irradiation the isosbestic points in the absorption spectra at 230 nm, 274 and 290 nm for investigated esters and α-Toc were observed. The spectral and kinetic changes in the other investigated solvents are similar to those obtained in methanol.

The α-Toc absorbance at 295 nm plotted as a function of irradiation time can be well fitted to a single exponential decay with a rate constant of $k = 0.723 \text{ s}^{-1}$. For all tested esters the absorption changes at 285 nm can be fitted to a linear function. Our results shown that α-Toc esters degraded through direct absorbance of UVB radiation. The different kinetics of degradation of α-Toc esters indicate that mechanism of oxidation of vitamin E induced by UVB light have been changed as a result of ester-linked moiety attached to the position-6 oxygen atom of the phenolic ring of the chroman head.
**P13.15**

**The impact of propofol on oxidative-antioxidant balance in the experimental model of Parkinson’s disease in Wistar rats**

Wioletta Szczurek, Ewa Romuk, Artur Biernacki, Krystyna Młynarek, Ewa Birkner

Medical University of Silesia, School of Medicine with the Division of Dentistry, Department of Biochemistry, Zabrze, Poland

e-mail: Ewa Romuk <eromuk@gmail.com>

**Introduction:** Propofol is a medication used in general anesthesia, which has antioxidant properties that’s why it appears that propofol may have protective effects in neurodegenerative diseases by influence the oxidation-antioxidant balance.

**Aim:** Evaluation of selected parameters of the oxidation-antioxidant system in the liver, kidney and blood serum in an experimental model of Parkinson’s disease in Wistar rats treated with propofol.

**Materials and Methods:** 6-hydroxydopamine (6-OHDA) was given to neonatal rats to destroy the dopaminergic central nervous system. The tests were performed on 32 rats divided into 4 groups: 1: control, 2: 6-OHDA, 3: control of propofol, 4: 6-OHDA propofol. The rats were decapitated at 45 days of age, organs and blood serum were collected. In blood serum concentrations of malondialdehyde (MDA), total antioxidant capacity (TAC) and total oxidative status (TOS) were measured. Homogenates of the liver and kidneys were performed, the enzyme activity of catalase, glutathione peroxidase (GPX), glutathione transferase, glutathione reductase and the concentration of TAC, TOS, MDA were assessed.

**Results:** The results of liver: in group 4 a statistically significant increase in the activity of glutathione reductase, catalase, and TAC and statistically significant reduction in the concentration of TOS and MDA comparing to group 2 was found. Statistically significant increase in the activity of catalase, the TAC and a significant decrease in the concentration of MDA was observed in group 3 compared to 1. The special correlation between increase in catalase activity and administered propofol was found.

In the kidney group 4 was characterized by the statistically significance increased GPX activity and reduced MDA concentration compared with group 2.

In the blood serum a statistically significant reduction of MDA concentration, LPS and TOS in group 4 comparing with group 3 was observed. A similar trend is also demonstrated in comparing group 2 and 4.

**Conclusions:** Propofol stimulates the activity of antioxidant enzymes and reduces the level of oxidative stress in individuals with Parkinson’s disease.

In the liver catalase plays a main role in oxidative stress reduction, whereas in the kidney glutathione peroxidase.

The administration of propofol individuals with lesions of the central dopaminergic system restores proper balance of oxidative-antioxidative system to a level comparable with healthy individuals.

**References:**

---

**P13.16**

**The impact of estrus induction on bactericidal activity of peripheral blood cells in dairy cows**

Radosław Skórzewski1, Wiesław Krumrych2, Marek Gehrke3, Janusz Zbylut4, Jan Grajewski1, Magdalena Twaruzek1

1Kazimierz Wielki University Bydgoszcz, Institute of Experimental Biology, Division of Physiology and Toxicology, Poland; 2Kazimierz Wielki University Bydgoszcz, Institute of Experimental Biology, Department of Biochemistry and Cell Biology, Poland; 3Poznan University of Life Sciences, Faculty of Animal Breeding and Biology, Institute of Veterinary, Poland; 4Private Veterinary Practice, Osielsko, Poland

e-mail: Radoslaw Skorzewski <pracowniabiochemii@tlen.pl>

**Research shows that in case of inflammations and tumors** [1-3] **as well as after physical exercises** [4] **the increase of blood chemiluminescence (CL) can be observed both in humans and animals. It is worth pointing out that very few studies have been carried out in cow and if so, they mainly concerned the animals with mastitis** [5].

The aim of the study was the evaluation of the impact of hormonal induction of estrus on activity of blood neutrophils in dairy cows.

The study was carried out on 36 clinically healthy animals of Holstein-Friesian breed. Only the animals over 50 days after the parturition, with no traces of uterus asymmetry in the per rectum examination or pathological vaginal discharge in the per vaginam examination and corpus luteum in the ovary were qualified for the induction program. The health condition of the animals was determined on the basis of palpation and routine haematological examinations.

The estrus was induced with a single intramuscular cloprostenol injection (a synthetic analogue PGF2α) at a dosage of 500 μg/animal. Blood from the external jugular vein was taken from each cow twice, prior to the injection and on the 3rd/4th day after the injection. The animals were observed for 7 days after the injection and the day of estrus occurrence was marked.

Spontaneous (WS) and stimulated by opsonized zymosan (OZ) luminal dependent CL was used to measure neutrophil oxygen metabolism in whole blood. The assessment was performed using a microplate luminometer (Thermolabsystems, Finland). The kinetic method was used in the assay measuring CL for 60 min at 38°C. The results were presented as a value of CL integration in relative light units. The animals were divided into 3 groups: the 1st being the cows in which clinical estrus occurred after the hormone injection and pregnancy was confirmed on the 42nd/43rd day following the insemination; the 2nd being the cows in which clinical estrus occurred after the hormone injection and pregnancy was not confirmed and the 3rd the cows in which clinical estrus symptoms did not occur.

On the 3rd/4th day after the hormone injection, WS and OZ CL increase was observed in relation to the CL levels prior the injection. The increase of CL was statistically significant in all groups, while the increase of CL OZ was significant only in the 1st and 2nd group.

Studies showed the increase of oxygen-dependent bactericidal activity of blood granulocytes in dairy cows after the induction of the estrus. Therefore, this reaction should be taken into consideration when CL measurements are used for diagnostic purposes.

**References:**
Fluorescent dyes in studies on the mechanism of antifungal action of Galleria mellonella lysozyme against Candida albicans

Aneta Sowa-Jasień, Sylwia Stączek, Agnieszka Zdybicka-Barabas, Jerzy Wydrych, Paweł Mak, Małgorzata Cytryńska

1Department of Immunobiology, 2Department of Comparative Anatomy and Anthropology, Faculty of Biology and Biochemistry, Maria Curie-Skłodowska University, Lublin, Poland; 3Department of Analytical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland

e-mail: Aneta Sowa-Jasień <ca.sowajasien@gmail.com>

Fluorescent dyes are particularly advantageous in biological research because they combine very high sensitivity and selectivity in target detection with low toxicity. As such, they have become a method of choice for tracing and localization of proteins, detection of specific molecules in cells and tissues, and staining of cellular organelles. Changes in the potential of cell membranes, the presence of reactive oxygen species in cells, cell death by apoptosis can be also visualized using a fluorescent dye.

Our research focuses on explaining the mechanism of antifungal action of Galleria mellonella lysozyme using Candida albicans. Lysozyme plays an important role in innate immunity against invading pathogens in animals. This protein is well known antimicrobial polypeptide exhibiting antibacterial and antifungal activities. Antibacterial action of lysozyme is related to enzymatic muramidase activity as well as to non-enzymatic activity resembling a mode of action of cationic defense peptides. However, the mechanism of lysozyme fungistatic and/or fungicidal activity is not clear. Our previous study revealed that purified G. mellonella lysozyme, which like the human lysozyme belongs to c-type family of lysozymes, bound to the cell surface of different yeasts and filamentous fungi. Moreover, G. mellonella lysozyme inhibited C. albicans growth at a relatively low concentration (0.5 µM).

To characterize the effect of lysozyme action on the fungal cells we used a number of methods based on fluorescent dyes and imaging of cells in a laser scanning confocal microscope. Using FITC-labeled G. mellonella lysozyme we showed directly that lysozyme binds to the surface of C. albicans cells. The metabolic activity of C. albicans cells after incubation with 0.5 µM lysozyme was tested by LIVE/DEAD staining using FUN-1 dye. These experiments revealed reduction of metabolic activity of cells treated with G. mellonella lysozyme. Furthermore, Calceofluor White staining of the lysozyme-exposed C. albicans cells showed no changes in the cell wall in comparison with the control cells. These observations, together with the inability of the lysozyme to degrade standard chitinase substrates, suggested that G. mellonella lysozyme could reduce the fungal growth through a non-enzymatic mode of action. Staining of C. albicans protoplasts with FITC-conjugated Annexin V and Propidium Iodide after incubation with 0.5 µM lysozyme showed that G. mellonella lysozyme can induce apoptosis in C. albicans cells. The use of fluorescent dyes in our study allowed to obtain information relevant to explain the mechanism of antifungal action of G. mellonella lysozyme.

Acknowledgements

The work was supported by the Grant No 2012/05/B/NZ1/00033 (Decision No: UMO-2012/05/B/NZ1/00033) from NCN, Poland.
The influence of anthocyanidin and UVB radiation on keratinocytes elasticity and viability

A. Sobiepanek, M. Milner-Krawczyk, T. Kobiela

Institute of Biotechnology, Faculty of Chemistry, Warsaw University of Technology, Poland

e-mail: Anna Sobiepanek <annaw.sobiepanek@gmail.com>

The ultraviolet B radiation is a common treatment for numerous skin diseases. On the other hand, a chronic exposure to the solar UVB radiation advances the ageing process of the skin, causes oxidative stress and the formation of reactive oxygen species (ROS). It also may lead to the development of melanoma and non-melanoma skin cancer [1]. As a result, a lot of research was made about the influence of photochemopreventive agents on the reduction of the occurrence of skin cancer, especially the botanical antioxidants like delphinidin [2]. However there are no investigations presented regarding the surface morphology and elasticity of irradiated living keratinocytes. There is an increasing evidence that the changes in the mechanical properties of living cells may regulate different processes and the survival of the cells, but also it can be used as an early diagnosis of several diseases [3]. Moreover, the decrease of the cell stiffness for living epidermal keratinocytes treated with sodium lauryl sulphate was presented [4]. Therefore, we examined the effect of UVB radiation and the addition of delphinidin on the mechanical properties of keratinocytes using the atomic force microscopy (AFM) correlated with the fluorescent microscopy and the viability test.

For the presented studies, we used the immortalized human keratinocyte (HaCaT) cell line. We examined the influence of delphinidin on HaCaT cells applying different concentration (5-10 μM; both pretreatment and post-treatment) After 24 h of the incubation, the cells were radiated with a dose of 100 mJ/cm². MTT assay was applied to define the number of living cells after the UVB radiation. The AFM measurements of the keratinocytes’ stiffness and the visualization of the actin filaments were carried out after 24 h from the UVB radiation. All determined Young’s modulus values were normalized to the value obtained for the control measurements.

The ultraviolet B radiation decreased the viability of HaCaT cells and the stiffness of cells measured by the AFM. Likewise, the fluorescence microscopy revealed changes in the organization of actin filaments in the radiated cells. However, the stiffness of irradiated cells were at level of control for pre-treatment with 10 μM delphinidin and post-treatment with 5 μM delphinidin.

These results show that UVB radiation has a significant influence on the viability and mechanical properties of HaCaT cells. The combined AFM/fluorescence microscopy study presents the photochemopreventive effect of delphinidin. This approach can be used to track chemically-induced changes in cell elastic properties under physiological conditions.