MARIA M. JEŻEWSKA and ZBIGNIEW W. KAMIŃSKI

UNUSUAL HYPOXANTHINE HYDROXYLATION SYSTEM IN HEPATOPANCREAS OF *HELIX POMATIA* (GASTROPODA)*

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Rakowiecka 36; 02-532 Warszawa, Poland

The enzymatic system in hepatopancreas of *H. pomatia* (terrestrial purinotelic gastropod) hydroxylates hypoxanthine to xanthine and uric acid but fails to hydroxylate adenine, nicotinic acid and 3-methyl-6-hydroxypurine; allopurinol is hydroxylated to oxypurinol 7 times faster than hypoxanthine to xanthine; at concentration of 10^{-6} M it inhibits hydroxylation of hypoxanthine by 55%.

Two protein fractions [precipitated at 0 - 0.30 (I) and 0.30 - 0.45 (II) saturation with (NH₄)₂ SO₄] hydroxylate hypoxanthine with NAD⁺ as a cosubstrate but only fraction I, predominating during the active life, hydroxylates also xanthine and is inhibited by NADH. Protein fraction II, dominant during winter sleep, does not hydroxylate xanthine and its hypoxanthine-hydroxylating activity is not inhibited by NADH. The latter property may enable continuous operation of the protein catabolic pathway under anaerobiosis.

*Helix pomatia*, a purinotelic terrestrial gastropod, excretes xanthine and uric acid as the end-products of protein metabolism (Jeżewska, Gorzkowski & Heller, 1963a). Xanthine oxidase involved in uric acid biosynthesis has been reported in *H. pomatia* hepatopancreas (Balwin & Needham, 1934) and its activity has been found to be most probably NAD⁺-dependent (Barankiewicz & Jeżewska, 1972). The analogous NAD⁺-dependent enzymes from rat and chicken liver are inhibited by NADH (DellaCorte & Stirpe, 1970) and this inhibition, possibly occurring *in vivo* during hypox, attenuates uric acid production from hypoxanthine and results in xanthine accumulation (Kamiński & Jeżewska, 1981). A completely opposite effect of anaerobiosis on purine metabolism has been observed in *H. pomatia*. During winter sleep the snail stores in nephridium mainly uric acid, whereas during the feeding period it excretes more xanthine than uric acid (Jeżewska, Gorzkowski & Heller, 1963b).

The data presented in this paper relate changes in the excretion of purines to the kinetic properties of the hydroxylating enzyme system in *H. pomatia* hepatopancreas.

---

* This work was supported by the Polish Academy of Sciences within the project 09.7.1.

[161]
MATERIALS AND METHODS

*Animals.* *H. pomatia* snails were collected in the vicinity of Warsaw. During the active period (April - September) they were fed burdock leaves. At the end of September the snails withdrew into their shells, the epiphramgm closing the opercula became calcified (October), and the snails were kept buried in soil in a cold room (4°C) till the end of March (hibernation, winter sleep).

*Enzyme preparation.* Hepatopancreas tissue (8 - 10 g) obtained from 8 - 10 snails was washed in cooled 100 mm-Hepps/KOH buffer, pH 8.0, containing 20% of glycerol and 1 mm-phenylmethylsulphonyl fluoride (PMSF), (added before use). The tissue was homogenized in 35 ml of the same buffer in a Potter-Elvehjem glass homogenizer with a teflon pestle, and cooled in an ice-water bath. The homogenate was centrifuged at 20 000 g for 15 min at 4°C, and the resulting supernatant was centrifuged at 160 000 g for 60 min at 4°C. The obtained supernatant was filtered through a GF/C glass filter and was fractionated with 3.8 m-(NH₄)₂SO₄ (Wood, 1976). Protein fractions precipitated at 0.0 - 0.30 (I) and 0.30 - 0.45 (II) saturation with ammonium sulphate were separated, washed with 3.8 m-ammonium sulphate and recentrifuged. The pellets were suspended in 2 and 4 ml, respectively, of 100 mm-Hepps/KOH buffer, pH 8.0, containing 20% of glycerol. Enzymatic activities of fractions I and II were examined.

*Chromatography on DEAE-Sephadex.* The suspension (2 ml) of fraction II was passed through a Sephadex G-25 (fine) column (1.5×20 cm) previously equilibrated with 50 mm-Hepps/KOH buffer, pH 8.0. The brown fractions eluted with the same buffer were pooled (4.5 ml) and applied onto a DEAE-Sephadex column (Pharmacia Laboratory Column K9/15, bed height 7.5 cm) previously equilibrated with 250 ml of 50 mm-Hepps/KOH buffer, pH 8.0. The column was washed with 40 ml of the buffer and the yellow-coloured middle fractions were pooled (6 ml). Then the column was washed with 50 mm-Hepps/KOH buffer containing 150 mm-(NH₄)₂SO₄; again the colourless fractions were discarded, and the yellow-coloured fractions were collected (18 ml). Glycerol (20%, v/v) was added and the solution was used in the enzyme assay. Over 50% of the hypoxanthine-hydroxylating activity applied onto the column was recovered and the enzyme was purified about 9-fold as compared with the activity of ammonium sulphate fraction II.

*Protein determination.* This was performed by the method of Bradford (1976).

*Enzyme assays.* The enzymatic activity was examined with hypoxanthine and xanthine as substrates. The standard incubation mixture contained, in a total volume of 3 ml, 50 mm-Tris/HCl buffer, pH 8.0, hypoxanthine or xanthine at the concentration indicated, 175 - 350 μM-NAD⁺, and the enzyme preparation (2 - 20 pkat/ml). Some incubation mixtures contained additionally 40 nkat of lactate dehydrogenase and 0.5 mm-sodium pyruvate. Blanks contained no hydroxypurine. The reaction was monitored at room temperature (20 - 23°C) in quartz cells (light path 1 cm) under aerobic conditions as previously described (Kamiński & Jeżewska, 1979, 1981). During hypoxanthine hydroxylation and the concomitant NADH accumulation, the difference spectra were recorded within the 270 - 350 nm range; when NADH
accumulation was prevented by lactate dehydrogenase, the absorbance was measured at 279 and 302 nm. During xanthine hydroxylation, absorbance was recorded at 340 and 302 nm or only at 302 nm (in the presence of lactate dehydrogenase). A Cary 118C spectrophotometer equipped with a Repetitive Scan was used. The amount of hypoxanthine, xanthine, uric acid and NADH, if present, in the reaction mixture were calculated as previously described (Kamiński & Jęczewska, 1979, 1981) and expressed in nmol/ml of the incubation mixture.

Spectrophotometric determinations of allopurinol hydroxylation to oxipurinol and calculation of the reaction rate were made after Elion et al. (1966) and Johns et al. (1969).

Experiments in vivo on the incorporation of [14C]formate to uric acid were as described by Jęczewska et al. (1964).

Reagents. Hypoxanthine, xanthine, and Tris (2-amino-2-hydroxymethyl-1,3-propanediol), all p.a., were from Serva (Heidelberg, F.R.G.); sucrose, ultrapure, from Schwartz/Mann (Orangeburg, NY, U.S.A.); NAD+, allopurinol, Hepps [4-(2-hydroxyethyl)-1-piperazinopropanesulphonic acid], all A grade, and NADH were products of Calbiochem (San Diego, CA, U.S.A.), and KOH, specially pure, of UCB (Drogenbos, Belgium); lactate dehydrogenase and pyruvate were purchased from Sigma (St. Louis, MI, U.S.A.); Trasylol (inhibitor of proteinases) from Bayer (Leverkusen, F.R.G.); 3-methyl-6-hydroxypurine from Vega-Fox Biochemicals (Tucson, AR, U.S.A.); and nicotinic acid (99% pure) from Carl Roth (Karlsruhe, F.R.G.); (NH₄)₂SO₄, p.a., was a product of Polskie Odczynniki Chemiczne (Gliwice, Poland). The last reagent was purified from traces of heavy metals as previously described (Kamiński & Jęczewska, 1979). Redistilled water was used throughout. Laboratory glassware was soaked in 2% EDTA (w/v) adjusted to pH 10 with K₂CO₃, and then rinsed with redistilled water.

RESULTS AND DISCUSSION

It is known that many factors: oxidizing agents, proteolytic enzymes and freeze-thawing treatment damage structure of xanthine oxidoreductase and change their activity towards hydroxypurines and electron acceptors. In preparation of the H. pomatia enzyme, special attention must be paid to proteinases of the intestinal juice which could leak during separation of the intestine from hepatopancreatic lobes, and also to intracellular proteinases of hepatopancreas itself (otherwise called "digestive gland"). To stabilize the enzyme and prevent its destruction, a proteinase inhibitor, PMSF, and glycerol were added to the buffer used for homogenization of hepatopancreas (Trasylol was found to be less efficient). On the other hand, dithiothreitol used for prevention of a loss of activity with NAD⁺ as electron acceptor, had no effect on stoichiometry between the purine substrate utilization and NADH production.

Differences in protein fractionation related to the two distinct periods of snail life. Depending on the snail life periods (activity during the warm seasons and hibernation in winter), the protein fractionation gave different results. During the feeding period
two fractions I and II precipitated at 0.0 - 0.30 and 0.30 - 0.45 ammonium sulphate saturation, respectively. At the early stage of hibernation (October - December) fraction I gradually disappeared and at the later stages of hibernation (January - March) there was virtually no protein fraction I, and only fraction II was obtained. After awakening (April), protein fraction I reappeared.

**Annual cycle of changes in oxypurine-hydroxylating activity.** The hypoxanthine-hydroxylating activity during the feeding period was mostly present in protein fraction I, although fraction II was also active (only traces of activity were found in July). At early stages of hibernation when fraction I gradually diminished, the bulk of hypoxanthine-hydroxylating activity was recovered in fraction II. At the later stages of hibernation, total activity was present in fraction II (protein fraction I was practically absent). During this displacement of enzymatic protein from fraction I (feeding period) to fraction II (winter sleep) the total hypoxanthine-hydroxylating activity was diminished by half.

Annual changes in the xanthine-hydroxylating activity were much more pronounced. This activity was found only in protein fraction I, thus it diminished and increased with the disappearance and reappearance of this fraction. Therefore, in comparison with the hypoxanthine-hydroxylating activity, the activity towards xanthine diminished faster during winter sleep, fell nearly to zero in February and increased faster after awakening (Fig. 1).

The above differences suggested that there might be two hydroxylating enzymes: one of the xanthine dehydrogenase type, acting with two oxypurines, and another of the aldehyde oxidase or purine hydroxylase II type, both hydroxylating specifically.

---

**Fig. 1.** Annual cycle of hydroxylating activity in *H. pomatia* with xanthine as substrate, expressed as percentage of the activity towards hypoxanthine. The enzymatic protein precipitated at 0.0 - 0.45 \((\text{NH}_4)_2\text{SO}_4\) sat. was used. The values are mean from 3 - 7 experiments (10 snails for each experiment). October: entering into winter sleep (calcification of epihragm closing the operculum); November - March: winter sleep (hypoxy); April: awakening; April - September: feeding period.
hypoxanthine (Rajagopalan & Handler, 1964; Scanzochio & Sealy-Lewis, 1978, respectively). However, it is of considerable interest that protein fraction II, practically devoid of the activity towards xanthine when freshly obtained, exhibited this activity after prolonged storage (1 month) at —20°C (Table 1). Moreover, this activity appeared in the fraction II purified 9-fold on DEAE-Sephael column. In this preparation, the xanthine-hydroxylating activity accounted for 28% of the hypoxanthine-hydroxylating activity (Table 1). The recovery of the hypoxanthine-hydroxylating activity after DEAE-Sephael chromatography was about 50%. Since no other fraction collected exhibited this activity, this increase in the affinity towards xanthine could not result from separation of another hypoxanthine-hydroxylating enzyme. It seems more likely that some changes in the enzyme protein during purification and storage are responsible for the increase in the activity towards xanthine, and that the same enzyme protein, but with modified catalytic specificity, occurs in protein fractions I and II.

**Table 1**

*Substrate specificity of protein fractions I and II towards hypoxanthine and xanthine*

The incubation mixture was as described in Methods. Initial concentration of the substrates was 20 - 25 μM. The protein fractions were added in the amounts giving approximately the same initial rate of hypoxanthine hydroxylation.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Initial hydroxylation rate (nmol • min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hypoxanthine</td>
</tr>
<tr>
<td>Protein fraction I</td>
<td>0.99</td>
</tr>
<tr>
<td>Protein fraction II</td>
<td></td>
</tr>
<tr>
<td>— freshly obtained</td>
<td>0.98</td>
</tr>
<tr>
<td>— after storage for 1 month at —20°C</td>
<td>0.99</td>
</tr>
<tr>
<td>— after DEAE-Sephael chromatography</td>
<td>0.88</td>
</tr>
</tbody>
</table>

*Hypoxanthine hydroxylation by fractions I and II.* Both protein fractions: I, isolated from actively feeding snails, and II, obtained from the snails during winter sleep, hydroxylated hypoxanthine to the intermediate, xanthine, and further to uric acid (Figs. 2A and 3), although fraction II exhibited practically no activity towards xanthine used as substrate. The ability of the hibernating snail to produce uric acid in February (when the enzymatic activity towards xanthine falls to zero, Fig. 1) was confirmed *in vivo*: one week after an injection of [¹⁴C]formate into hepatopancreas of hibernating snails, radioactive uric acid was isolated from nephridium; its radioactivity accounted for 4% of the radioactivity of formate injected.
Fig. 2. Time-course of hypoxanthine→xanthine→uric acid (A) and of xanthine→uric acid (B) hydroxylation by the protein fraction I (0.30 ammonium sulphate sat.) from hepatopancreas of actively feeding snail *H. pomatia*. The hydroxylation process was followed spectrophotometrically under conditions of NADH accumulation (closed symbols) and reoxidation by the lactate dehydrogenase- pyruvate system (open symbols). The reaction mixture contained 350 μM-NADH, 5 pkat of the enzymatic activity/ml of incubation mixture, and 21.6 μM-hypoxanthine or 24.8 μM-xanthine in 50 mm-Tris/HCl buffer, pH 8.0. Utilization of hypoxanthine (□, ■); formation of: xanthine (△, ▲), uric acid (○, ●) and NADH (▼).

The time-course of hypoxanthine hydroxylation could be more exactly followed spectrophotometrically when fraction II was used, because this fraction contained less of the substances interfering in the spectrophotometric measurements. When the activity of this fraction was examined (Fig. 3) it became clearly evident that hypoxanthine utilization and accumulation of the intermediate, xanthine, were approximately linear till hypoxanthine concentration decreased by half; also uric acid was formed at a constant rate, even till the total exhaustion of hypoxanthine. This linearity of the curves suggests that hypoxanthine and xanthine do not compete for the active site of the hydroxylating enzyme system in the snail hepatopancreas. If such competition between those two substrates existed, the respective progress-curves would be parabolic and hyperbolic, as it has been found for xanthine oxidoreductases from milk (Jeżewska, 1973), turkey liver (Cleere et al., 1975) and rat liver (Kamiński & Jeżewska, 1981).

Slow accumulation of uric acid seemed to be another characteristic feature of the hypoxanthine-hydroxylating system of hepatopancreas. The level of uric acid in the reaction mixture was 3 - 5 times lower (Figs. 2A and 3) than that of xanthine till the total exhaustion of hypoxanthine, and irrespective of the protein fraction examined. In contrast to the snail enzyme, more uric acid and less xanthine accumulate during hypoxanthine hydroxylation by hydroxylating enzymes from other sources (Jeżewska, 1973; Cleere et al., 1975; Kamiński & Jeżewska, 1981).
Fig. 3. Time-course of hypoxanthine→xanthine→uric acid hydroxylation by the protein fraction II (0.30 - 0.45 ammonium sulphate sat.) from hepatopancreas of snail *H. pomatia* during winter sleep. The hydroxylation process was followed spectrophotometrically under conditions of NADH accumulation (closed symbols) and reoxidation by the lactate dehydrogenase - pyruvate system (open symbols). The reaction mixture contained 350 μM-NAD⁺, 15 pkat of the enzymatic activity/ml of incubation mixture, and 32.5 μM-hypoxanthine in 50 mM-Tris/HCl buffer, pH 8.0. Utilization of hypoxanthine (□, ■); formation of: xanthine (△, ○), uric acid (○, ●) and NADH (▼).

*Hypoxanthine hydroxylation and purine excretion*. It is known that at the end of hibernation the nephridium of *H. pomatia* contains about 5 times more uric acid than xanthine (Jeżewska et al., 1963b). This xanthine uric acid ratio is opposite to the ratio of these products found during hypoxanthine hydroxylation (Fig. 3). Therefore, accumulation of xanthine and uric acid was studied in relation to hypoxanthine concentration using protein fraction II (Fig. 4). The initial rate of xanthine accumulation increased with the increase of hypoxanthine concentration up to 75 μM and at higher concentration remained practically constant. In contrast, the initial rate of uric acid accumulation was constant within the hypoxanthine concentration range of 20 - 75 μM, and at lower concentrations this rate even exceeded the rate of xanthine accumulation. This shift towards uric acid in the pool of the hypoxanthine hydroxylation products is in accordance with the accumulation of uric acid during winter sleep, when the metabolic flux of hypoxanthine is reduced in consequence of a 5-fold attenuation of protein metabolism (Jeżewska, 1969). In turn, the increased accumulation of xanthine with the rise of hypoxanthine concentration
Fig. 4. Effect of hypoxanthine concentration on the hypoxanthine→xanthine→uric acid hydroxylation by the protein fraction II (0.30 - 0.40 ammonium sulphate sat.) from hepatopancreas of snail *H. pomatia* during winter sleep. The hydroxylation was followed spectrophotometrically. The incubation mixture contained 350 μM-NAD⁺, 20 pkat of enzymatic activity/ml of the incubation mixture, 8 - 120 μM-hypoxanthine, and the lactate dehydrogenase - pyruvate system, in 50 mM-Tris/HCl buffer, pH 8.0. Initial rate (nmol·min⁻¹/ml of incubation mixture) of xanthine formation (△) and of uric acid formation (○).

is consistent with predominance of xanthine over uric acid in the nephridial excreta during the feeding period (Jeżewska et al., 1963b), when the metabolic flux of hypoxanthine increases with the enhancement of protein metabolism (Jeżewska, 1969). The effect of the modified affinity towards xanthine on changes of the xanthine/uric acid ratio during the annual cycle of the snail remains to be elucidated.

*NAD⁺*-dependence and *NADH* inhibition of fractions I and II. Under aerobic conditions in the presence of *NAD⁺*, fraction I (freshly obtained) hydroxylated hypoxanthine and xanthine with simultaneous production of NADH (Figs. 2A and B) in the amount approximately equivalent to that of the purine substrate utilized; similar stoichiometry was observed during hypoxanthine hydroxylation by protein fraction II (Fig. 3). This suggests that the native oxypurine-hydroxylating activity in *H. pomatia* hepatopancreas is entirely *NAD⁺*-dependent. After storage at –20°C, stoichiometry of the reactions catalysed was disturbed, the number of NADH molecules formed was lower than that of oxypurine molecules utilized. However, since the enzyme did not acquire the ability to react with oxygen as electron acceptor, and the enzyme fraction did not exhibit the NADH oxidase activity, the cause of this discrepancy remains obscure.

*NADH* is a known inhibitor of xanthine dehydrogenases from rat and chick liver (Della Corte & Stirpe, 1970). Therefore, the hydroxylation rate under conditions of NADH accumulation and NADH reoxidation (lactate dehydrogenase and pyru-
vate added to the reaction mixture) were compared using the hydroxylating enzyme system from *H. pomatia* hepatopancreas. It was found that fractions I and II differed in sensitivity to NADH inhibition. The hydroxylating activity of fraction I was inhibited by NADH even upon its accumulation at micromolar concentration (Fig. 2A). The initially high inhibition of hypoxanthine utilization in the presence of NADH diminished with the progress of the reaction (55 and 34% of inhibition at 30 and 72 min, respectively, of the catalytic process), whereas uric acid formation from the intermediate, xanthine, was lower by a half throughout the reaction time (Fig. 2A). Under the same conditions the utilization of xanthine used as substrate was diminished by about 40% during 72 min of the reaction (Fig. 2B). In contrast, the hypoxanthine-hydroxylating activity of fraction II (inactive towards xanthine) was insensitive to NADH inhibition (Fig. 3). The cause of this difference in sensitivity to NADH between the two enzyme fractions remains unknown.

It has been reported that *H. pomatia* snails differ in the lactate metabolism (Wieser, 1978) during the aerobic and hypoxic periods of the annual life cycle. This difference could be connected with changes in the NAD\(^+\)/NADH ratio in snail tissues. If so, it could be that under conditions of anaerobiosis during winter sleep the insensitivity of the hypoxanthine-hydroxylating activity to NADH inhibition may enable continuous operation of the protein catabolic pathway in this purinotelic gastropod.

**Comparison of hypoxanthine-hydroxylating enzyme from *H. pomatia* with xanthine oxidoreductases and aldehyde oxidase.** Xanthine oxidoreductases have been reported to occur in several species of snails and bivalves (cf. Campbell & Bishop, 1970). On the other hand, a lack of xanthine oxidoreductases and the presence of aldehyde oxidase (an enzyme hydroxylating hypoxanthine but not xanthine; Rajagopalan & Handler, 1964) has been reported for several molluscs including uricotelic species (Wurzinger & Hartenstein, 1974). The enzyme from *H. pomatia* hepatopancreas seems to differ from xanthine oxidoreductases known so far, as well as from aldehyde oxidase. Protein fractions I and II did not use oxygen as electron acceptor and did not hydroxylate 3-methyl-6-hydroxypurine, a good substrate for aldehyde oxidase (Krenitsky et al., 1972). They were also inactive towards nicotinate, a substrate for purine hydroxylase II, another enzyme hydroxylating hypoxanthine but not xanthine (Scacciochio & Sealy-Lewis, 1978).

Allopurinol, a potent inhibitor of mammalian and avian xanthine oxidoreductases (Elion, 1966; Ní Fhaoláin & Coughlan, 1978) but not of aldehyde oxidase (Johns et al., 1969), inhibited also the *H. pomatia* enzyme. In the presence of 3 \(\mu\)M-allopurinol, hydroxylation of hypoxanthine and xanthine by protein fraction I decreased by 30-40% (reaction time 15 min). When protein fraction II purified on DEAE-Sephadex column was preincubated with allopurinol at concentration of 2 \(\mu\)M for 10-30 min, hypoxanthine hydroxylation was inhibited by about 55%. These results suggest that allopurinol is not a "tight-bound" inhibitor for the snail enzyme. Moreover, it turned out that allopurinol itself was actively hydroxylated by the purified protein fraction II (Fig. 5). The rate of oxipurinol formation decreased with time (0.355, 0.099 and 0.057 nmol·min\(^{-1}\) on the first, fifth and sixteenth minute
of the reaction). This decrease in the rate of allopurinol hydroxylation suggests that the enzyme was gradually inhibited by the oxipurinol formed, whereas the hypoxanthine hydroxylation rate was linear for 20 min and kept linear till substrate concentration decreased by half (Fig. 3).

![Graph showing allopurinol hydroxylation](image)

**Fig. 5.** Allopurinol hydroxylation by the purified protein fraction II from *H. pomatia*. Hydroxylation was followed spectrophotometrically at 285 nm, under conditions of NADH reoxidation. The reaction mixture contained 350 μM-NAD⁺, 60 μM-allopurinol, enzyme solution (1 pkat/ml), and the lactate dehydrogenase-pyruvate system in 50 mM-Tris/HCl buffer, pH 8.0. The blank sample contained no allopurinol.

The rates of oxipurinol formation accounted for 725, 202 and 116% of the initial rate of hydroxylation of 40 μM-hypoxanthine (0.049 nmol·min⁻¹) measured under the same experimental conditions. The higher hydroxylation rate with allopurinol than with hypoxanthine is characteristic for aldehyde oxidase, whereas milk xanthine oxidase hydroxylates hypoxanthine faster than allopurinol (Krenitsky *et al.*, 1972). The conversion of allopurinol to oxipurinol, occurring in liver of various mammalian species has been attributed rather to the activity of aldehyde oxidase than of xanthine oxidoreductase (Huh *et al.*, 1976).

Adenine, which is a poor substrate for xanthine oxidase and aldehyde oxidase (Krenitsky *et al.*, 1972) as well as for turkey liver xanthine dehydrogenase (Cleere *et al.*, 1975) was not hydroxylated by the purified protein fraction II.

The data presented provide evidence that the enzyme from *H. pomatia* hepatopancreas, though showing some properties of xanthine oxidoreductase as well as of aldehyde oxidase, is different in many respects from the hypoxanthine-hydroxylation enzymes known to date.

The drawing of Figures by Mr Daniel Mumot is gratefully acknowledged.
REFERENCES


NIEZWYKŁY UKŁAD ENZYMATYCZNY HYDROKSYLUJĄCY HIPOKSANTYNĘ
DO KSANTYNY I Kwasu Moczowego W WĄTROBOTRZUSTCE
HELIX POMATIA (GASTROPODA)

Streszczenie

Preparat enzymatyczny z wątrobotrzustki purynotelicznego ślimaka Helix pomatia, hydroksylujący hipoksantynę do ksantyny i kwasu mocowowego, nie hydroksylował adeniny, kwasu nikotynowego i 3-metylo-6-hydroksypuryny, natomiast hydroksylował allopurynol do oksypurynolu siedem razy szybciej niż hipoksantynę do ksantyny. Allopurynol w stężeniu $10^{-6}$ m hamował hydroksylację hipoksantyny w 55%.

Dwie frakcje białkowe (wytracające się w zakresach 0 - 0.30 (I) i 0.30 - 0.45 (II) nasycenia siarczanem amonu) hydroksylowały hipoksantynę przy współudziale NAD+, lecz tylko frakcja I, przeważająca podczas aktywnego życia ślimaka, hydroksylowała także ksantynę i była hamowana przez nagromadzający się NADH. Frakcja II, przeważająca podczas snu zimowego, nie hydroksylowała ksantyny, a jej aktywność względem hipoksantyny nie była hamowana przez NADH. Ta ostatnia właściwość umożliwia, być może, nieprzerwany katabolizm białek w warunkach anaero-biozy podczas snu zimowego ślimaka.

Received 15 November, 1983