JÓZEF SPYCHAŁA and JAROSŁAW MARSZAŁEK

THE EFFECT OF HIGH PROTEIN DIET ON THE REGULATORY PROPERTIES OF AMP DEAMINASE FROM CHICKEN LIVER

Department of Biochemistry, Academic Medical School
Dębniki 1, 80-211 Gdańsk, Poland

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Feeding high protein diet for 5 days caused a 3.5-fold and 2-fold increase of the activity of xanthine dehydrogenase (EC 1.2.1.37) and 5-nucleotidase (EC 3.1.3.31) respectively, in chicken liver. Six hours after feeding the high protein diet there was no change in either enzyme activity although a 3-fold increase in the level of serum uric acid was observed. High protein diet considerably decreased the activity of AMP deaminase at low, but not at high substrate concentration. The activity ratio, measured at 10.0 and 0.16 mM AMP increased from 14:1 (low protein diet) to 23:1 and 24:1 after 6 h and 5 days of high protein diet, respectively. It has been suggested that feeding birds a high protein diet may cause transformation of liver AMP deaminase (EC 3.5.4.6) from a low $K_m$ form toward a high $K_m$ form.

It is believed that purine ring synthesized de novo in mammalian liver is channeled mainly into adenine nucleotide pool [1]. However, uricotelic animals synthetize purine ring to excrete excess ammonia. Thus, a part of IMP produced in these animals may be converted directly to uric acid without entering the adenylate pool. Several authors presented evidence that the enzyme activities of this pathway in birds are influenced by nitrogen content in the diet [2 - 5] but so far no systematic attempt has been made to correlate the increased de novo purine ring synthesis with the rate of adenine nucleotide turnover. Such an approach could be helpful in evaluating the regulatory mechanism controlling the interconversion and breakdown of purine nucleotides.

The role of AMP deaminase in adenine nucleotide metabolism in chicken liver seems to be of particular importance. This enzyme catalyses the hydrolytic release of ammonia from 5-AMP with a simultaneous generation of IMP.
Multiple metabolism functions have been proposed for AMP deaminase in mammalian liver. Chapman & Atkinson [6] suggested that its main function might be stabilization of adenine nucleotide energy charge; and Van den Berghe et al. [7] showed that this enzyme controls adenine nucleotide breakdown in rat liver. Jackson et al. [8] pointed out that, in the rat hepatomas, AMP deaminase could additionally function as a balancing mechanism for the activities of the adenine and guanine nucleotides in the branches of purine biosynthetic pathways. All these assumptions indicate that, apart from the enzymes of de novo pathway, AMP deaminase seems to control the adenine nucleotide turnover rate.

Since two forms of AMP deaminase exist in adult chicken liver [9, 10] it might be supposed that the regulatory properties of this enzyme are related to uricotelism. If so, the question arises whether and to what extent the AMP deaminase activity in chicken liver is influenced by high protein diet, i.e. how it is related to a high rate of uric acid (IMP) synthesis. This paper is aimed at answering this question.

MATERIAL AND METHODS

Animals and diet. One day old male chicks (White Leghorn, Astra line) were obtained from Gdańskie Zakłady Drobiarskie (Poland). The animals were maintained in wire cages with a 12 h on and 12 h off light cycle and were fed commercial diet (LSM-Bacutil, Poland) containing 17% protein until approximately 10 - 12 weeks of age and 1.5 - 2.0 kg of body weight.

The low protein, high carbohydrate diet (5 g protein and 70 g starch per 100 g of dry weight) consisted of boiled potatoes only and the high protein, low carbohydrate diet (60 g protein and 15 g starch per 100 g of dry weight) was supplemented with casein. Vitamins (Polfa, Poznań, Poland) were provided with tap water during the experiments.

Prior to the experiment chickens were fed low protein, high carbohydrate diet and sample chickens were killed at 0, 6 h, 1 day and 5 days of receiving the high protein, low carbohydrate diet. Food and water were available ad libitum during the whole experiment. Both diets were consumed in similar amounts.

Preparation of crude extract. The chickens were killed by decapitation, samples of 10 ml of blood were collected and freshly excised livers were homogenized in 6 vol. of 2 mM mercaptoethanol in a Teflon-glass, hand-driven homogenizer. The homogenates were centrifuged at 20 000 g for 50 min and the clear supernatant fraction was taken for enzyme assays. The same extract was used for further purification of the enzyme.

The chromatographic procedure. Phosphocellulose column chromatography was preceded by a batch adsorption of the extract on Sigma phosphocellulose. Preliminary experiments had shown that phosphocellulose P-11 (Whatman)
which gives good separation of two molecular forms of chicken liver AMP deaminase [9] exhibited low binding capacity (the yield was about 35%). Therefore, in the subsequent studies phosphocellulose from Sigma was used which allowed to achieve the yield of about 70%. However, as this resin gave much worse separation of the two enzyme forms the step elution procedure permitting to obtain both the optimum reproducibility and optimum yield was applied.

Adsorption of 60 ml of the chicken liver extract on 20 ml of phosphocellulose was carried out under gentle stirring for 30 min at pH 6.8 and 5°C and was terminated by centrifuging the phosphocellulose slurry for 10 s at 2000 g. The slurry was washed three times with 0.25 M KCl containing 2 mM mercaptoethanol and adjusted to pH 7.0, recentrifuged and transferred to Pharmacia K 16/20 column. At this stage additional washing was performed at a 30 ml/h flow rate using 0.65 M KCl containing 2.0 mM mercaptoethanol and adjusted to pH 7.0. AMP deaminase was eluted with 2.0 M KCl at the same flow rate. The enzyme preparations obtained were 400 - 600 fold purified, exhibited the specific activity of 6 - 12 μmol/min per mg protein at 10.0 mM AMP concentration and were essentially free of 5'-nucleotidase and adenosine deaminase activities.

Enzyme assays. AMP deaminase (EC 3.5.4.6) activity was determined calorimetrically from the amount of ammonia liberated at high (10 mM) substrate concentration as described previously [9] and spectrophotometrically from the decrease of absorbance at 265 nm at low (0.16 mM) AMP concentration. The reaction mixture contained 50 mM succinate buffer, pH 6.5, 0.1 M KCl, the appropriate amount of AMP and either 50 μl of crude extract of 25 μl of phosphocellulose eluate in total volume of either 1.0 or 0.5 ml.

5'-Nucleotidase (EC 3.1.3.31) activity was determined by measuring the amount of orthophosphate liberated. The reaction mixture contained 50 mM imidazole/HCl buffer, pH 6.5, 0.1 M KCl, 10 mM MgCl₂ and 5 mM IMP in total volume of 1.0 ml. The reaction was terminated by the addition of 0.5 ml of 15% trichloroacetic acid, and inorganic phosphate was measured by the method of Chen et al. [11].

Xanthine dehydrogenase (EC 1.2.1.37) activity was determined spectrophotometrically at 290 nm as described by Strittmatter [12].

One unit of either enzyme activity was defined as the amount of activity causing the formation of 1 μmol of a product per minute at 25°C. The total activity in 7 ml of the extract is equivalent to 1 g of fresh tissue.

RESULTS

We have used 10 - 12 week old birds to study the effect of high protein diet on the activity of AMP deaminase in chicken liver. Chickens at this
age develop two forms of the enzyme in liver [13]. Other enzymes, such as xanthine dehydrogenase and 5'-nucleotidase, very sensitive to protein content in the diet [2], were applied as markers.

The data given in Table 1 show that short-term feeding of high protein, low carbohydrate diet did not influence the activity of any marker enzyme but on feeding prolonged to 5 days the activities of both xanthine dehydrogenase and 5'-nucleotidase increased 3-4 and 2-fold, respectively. The level of uric acid in blood serum increased almost 3-fold as early as after 6 h, and on the fifth day it amounted to 12.5 mg per 100 ml of chicken blood serum (190% of control). Also the results presented show that the activity of liver AMP deaminase decreased after 6 h of the high protein, low carbohydrate diet and remained low on the fifth day. The decrease is more pronounced when the activity is measured at 0.16 mM AMP concentration.

**Table 1**

*The effect of low and high protein diets on the plasma uric acid and on the activity of xanthine dehydrogenase, 5'-nucleotidase and AMP deaminase in chicken liver extracts*

Low protein diet had been withdrawn 12 h before the high protein diet was given. The values represent mean, ±SD, from a number of experiments indicated in the brackets. The statistical significance of differences from the low protein diet group was tested by Student t test. The results are expressed as \(\mu\)mol × min\(^{-1}\) × g\(^{-1}\) of fresh tissue

<table>
<thead>
<tr>
<th>Diet</th>
<th>Uric acid</th>
<th>Xanthine dehydrogenase</th>
<th>5'-Nucleotidase</th>
<th>AMP deaminase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.16 mM AMP</td>
</tr>
<tr>
<td>Low protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>diet</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>5 days</td>
<td>6.6 ± 3.0</td>
<td>1.40 ± 0.23</td>
<td>0.53 ± 0.14</td>
<td>0.37 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(7)</td>
<td>(7)</td>
<td>(5)</td>
</tr>
<tr>
<td>High protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 hours</td>
<td>17.5 ± 7.2</td>
<td>1.32 ± 0.14</td>
<td>0.44 ± 0.11</td>
<td>0.07 ± 0.03*</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>High protein</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>diet</td>
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<tr>
<td>5 days</td>
<td>12.5 ± 5.3</td>
<td>4.53 ± 0.85</td>
<td>0.94 ± 0.19</td>
<td>0.14 ± 0.08*</td>
</tr>
<tr>
<td></td>
<td>(3)</td>
<td>(4)</td>
<td>(4)</td>
<td>(5)</td>
</tr>
</tbody>
</table>

* \(p < 0.001\)

The ratio of the reaction velocity at 10 mM AMP to that at 0.16 mM increased considerably in the livers of the animals fed high protein diet.

To study the effect of high protein diet on the kinetic properties of the purified AMP deaminase, the enzyme was purified using phosphocellulose column chromatography. Since the two forms of chicken liver AMP deaminase exhibit significant differences in their kinetic properties [9, 13], i.e. form II
Table 2

*The effect of subsequent feeding of low and high protein diet on the velocity ratio of the purified chicken liver AMP deaminase*

Low protein diet had been withdrawn 12 h before high protein diet was given. The significance of differences from the low protein diet group was tested by Student *t* test. The values represent mean, ±SD from n experiments.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Ratio (\frac{v \text{ at } 10.0 \text{ mM AMP}}{v \text{ at } 0.16 \text{ mM AMP}})</th>
<th>n</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low protein diet</td>
<td>14.4 ± 1.4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>5 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High protein diet</td>
<td>22.8 ± 5.0</td>
<td>5</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>6 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High protein diet</td>
<td>25.9 ± 3.9</td>
<td>4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>1 day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High protein diet</td>
<td>25.2 ± 2.2</td>
<td>4</td>
<td>&lt; 0.001</td>
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<tr>
<td>5 days</td>
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</table>

is entirely inactive at low (0.16 mM) substrate concentration, the ratio of the reaction velocity at 10.0 mM AMP and at 0.16 mM may be assumed to measure the proportion of the two enzyme forms. Data presented in Table 2 show that this ratio, similarly as that presented for the liver extracts, significantly increased after 6 h of high protein feeding and remained fairly unchanged up to the fifth day under these conditions. The lower values of the ratio found in the crude liver extracts may result from a slight overestimation (15%) of the AMP deaminase activity due to the combined activity of 5'-nucleotidase and adenosine deaminase, especially at low substrate concentration.

**DISCUSSION**

It is believed that in rat liver the bulk of IMP synthetized by the *de novo* pathway enters either the adenine or the guanine nucleotide pool [1, 14] and most of the uric acid produced by man, or the sum of uric acid and allantoin in the rat, derives from the biodegradation of adenine nucleotides.

However, a different situation exists in chicken liver. In this uricotelic species the majority of IMP synthetized *de novo* is probably channeled directly into the catabolic pathway without entering the adenylate pool. It is reasonable to assume that under conditions of high rate of IMP synthesis, a protecting mechanism would emerge which might slow down
both the entry of purine ring into the adenine nucleotide pool and the rate of AMP catabolism. This view is supported by the observation presented in this paper that the increased production of uric acid is accompanied by the decrease of AMP deaminase activity measured at physiological AMP concentration (Table 1).

The change of the activity ratio after feeding the high protein diet indicates that under these conditions a transformation of AMP deaminase from a low $K_m$ form toward a high $K_m$ form might have occurred; the following evidence favours this assumption: (i) Two molecular forms of AMP deaminase were found in chicken liver [9, 10, 13]. (ii) The difference in the ratio of the reaction velocity at saturating to that at low substrate concentration has been shown to represent the kinetic differences between the two enzyme forms. As the total enzyme activity measured at 0.16 mM AMP decreased by a factor of 5 after 6 h of high protein diet feeding, the reasonable explanation could be that a part of the low $K_m$ form underwent conversion into the high $K_m$ form. (iii) The change of the activity ratio was observed both with crude extract and the purified enzyme; therefore, it is unlikely to be due to the presence of effector(s).

The possible molecular nature of the interconversion phenomenon is unknown, however. This is the first report in which the metabolic significance of this phenomenon is presented. Welch & Rudolph [15] presented evidence that in chicken liver after acute estradiol treatment there was no change of AMP deaminase activity either in the presence and or the absence of ATP, a known enzyme activator. However, if their results are presented in a manner similar to ours, i.e. if the activity in the presence of ATP would be considered as $V_{\text{max}}$ and that in the absence of ATP as measured at low substrate concentration, 24 h after estradiol injection their calculated ratio would diminish from of 3.1 to 2.0, i.e. by about 35%. Concomitantly, there was a significant increase of incorporation of formate into cellular purines [15]. Thus, an increase of the adenine nucleotide turnover rate seems to be accompanied probably by the AMP deaminase interconversion from a more ATP dependent (high $K_m$) form toward a less ATP dependent (low $K_m$) form.

We propose that AMP deaminase interconversion phenomenon may be a part of a more general regulatory mechanism, which slows down the turnover of adenylates in the liver of uricotelic species in order to uncouple the conversion of IMP into adenine nucleotides from its de novo synthesis. A separation of the adenine nucleotide pool from the pathway of uric acid synthesis would require, moreover, the regulation of the IMP conversion to AMP, and not only the suggested control of degradation of AMP, constantly kept in equilibrium with ADP and ATP by adenylate kinase. Whether a similar or some other control mechanism operates also at the level of purine ring entry into the adenine nucleotide pool, remains to be
established. Operation of such a mechanism would be advantageous for maintaining the liver adenine nucleotides at a constant level, independently of the environmental conditions, also including composition of the diet.

REFERENCES