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TWO FORMS OF AMP DEAMINASE FROM THE LIZARD (*LACERTA AGILIS*) LIVER

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Abstract—1. Two molecular forms of AMP deaminase have been revealed by phosphocellulose column chromatography in the liver of uricotelic lizard.

2. The calculated $S_{0.5}$ value of the purified lizard liver AMP deaminase was 2.5 ± 0.1 for the form I and 3.6 ± 0.4 for the form II.

3. Both forms of the enzyme were activated by ATP and ADP but the form II to a much higher extent. GTP activated only the form II and inorganic phosphate inhibited both forms.

4. The occurrence of multiple forms of liver AMP deaminase in uricotelic species, as well as its difference from the mammalian enzyme regulation by GTP is suggested to be connected with the uricotelism in these animals.

INTRODUCTION

Two forms of AMP deaminase have been recently found in the liver of uricotelic chicken (Spychała and Makarewicz, 1983). On the other hand only single form of this enzyme has been reported to be present in the liver of ureotelic rat, turtle and frog (Ogasawara *et al.*, 1975; Smith *et al.*, 1977; Spychała *et al.*, 1983; Spychała, 1984). In view of these findings the assumption has been put forward that the interspecies differences of liver AMP deamination may be connected with the intensity of the overall purine nucleotides metabolism which in turn could be related to either urico- or ureotelism (Spychała, 1984).

However only a limited number of species have been investigated so far and therefore this study was undertaken in order to disclose whether in the liver of lizard two forms of AMP deaminase are present and whether their regulatory properties differ from those reported for chicken liver enzyme. *Lacerta agilis* belongs to Squamata reptiles which are entirely uricotelic as they have been reported to lack a functional urea cycle (Brown and Cohen, 1960).

MATERIALS AND METHODS

Animals

Adult lizards (*Lacerta agilis*) were caught in their natural environment in the vicinity of Gdańsk in May or June. The animals were kept no longer than 30 days in a terrarium and fed their natural diet of grasshoppers, before being killed.

Purification procedure

Freshly excised livers were homogenized with 10 vol of 5 mM Tris–HCl buffer, pH 7.4 and 2 mM mercaptoethanol using a Potter–Elvehjem rough wall glass homogenizer. Homogenate was centrifuged for 40 min at 20,000 *g* and chromatographed on Sephadex G-25 in K 50/60 Pharmacia column, equilibrated with 1.0 M KCl containing 20 mM imidazole–HCl buffer, pH 6.8 and 1 mM mercaptoethanol. The whole protein eluate was diluted 4-fold with cold water and 10 ml of the Sigma phosphocellulose slurry was then added to it. Batch adsorption of the enzyme was performed at 4°C for 30 min under gentle stirring. Usually 60–90% of the AMP deaminase activity was bound to the resin under these conditions. Afterwards the phosphocellulose slurry was washed three times with 0.25 M KCl by repeated centrifugation and transferred into Pharmacia K 16/20 column. Additional washing of the material packed in the column was performed by using subsequently 100 ml of 0.25 M and 0.625 M KCl, both adjusted to pH 7.0 with 1.0 M K_2HPO_4 and containing 1.0 mM mercaptoethanol. The AMP deaminase activity was eluted using steps of 1.0 M and 2.0 M KCl, pH 7.0. The specific activities of the purified enzyme were in the range of 3.0–6.0 and 4.0–8.0 μmol per min and per mg of protein for the form I and II respectively.

AMP deaminase assay

Enzyme activity was measured from the amount of ammonia liberated, by using the phenol–hypochlorite method of Chaney and Marbach (1962). Microdiffusion was employed when the activity was measured in crude liver extracts (Seligson and Seligson, 1951). Incubation mixture contained 50 mM succinate/K^+ buffer, pH 6.5, 150 mM KCl, 0.05% albumin, substrate and various effectors at concentrations indicated. All assays were performed at 25°C.

Reagents

Phosphocellulose and all nucleotides were from Sigma Co., USA, Succinic acid was from BDH, England and G-25 was from Pharmacia Fine Chemicals, Sweden. All other chemicals were of analytical grade and were supplied by Polskie Odczynniki Chemiczne POCH, Poland.

RESULTS

The mean activity of AMP deaminase measured in the crude extracts from 6 lizard livers at 10 mM AMP was 1.96 ± 0.26 μmol per min and per g of fresh tissue. After the extract had been dialysed for 4 hr against 1.0 M KCl buffered with 20 mM imidazole–HCl, pH 6.8, the activity increased to the value of 5.47 ± 0.83 ($n = 5$) μmol $NH_3 \times min^{-1} \times g^{-1}$ of fresh liver weight. The isolation of the enzyme was performed by using standard procedure with phosphocellulose column chromatography (Spychała and Makarewicz, 1983). However, in the course of this

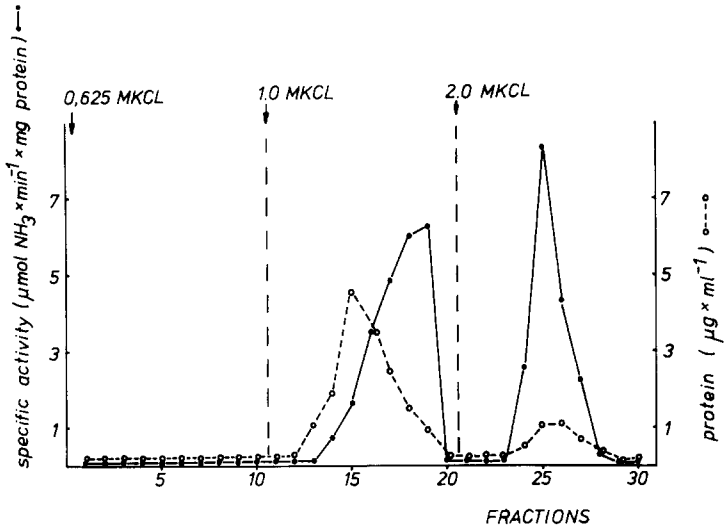

Fig. 1. Elution profile of lizard liver AMP deaminase from phosphocellulose column. Extract was submitted to gel filtration on Sephadex G-25 prior to phosphocellulose chromatography.

study it has been observed that a higher yield of AMP deaminase activity was achieved if the extraction of the tissue was performed with very diluted buffers. The recovery of the activity was even better if the extract had been submitted to chromatography on G-25 column in high ionic strength potassium chloride prior to phosphocellulose chromatography. For example, the purification procedure in which the G-25 chromatography was included usually yielded 60–90% of the starting AMP deaminase activity in the eluate from phosphocellulose, whereas the direct adsorption of the extract on phosphocellulose resin resulted in no more than 45% recovery of the enzyme activity.

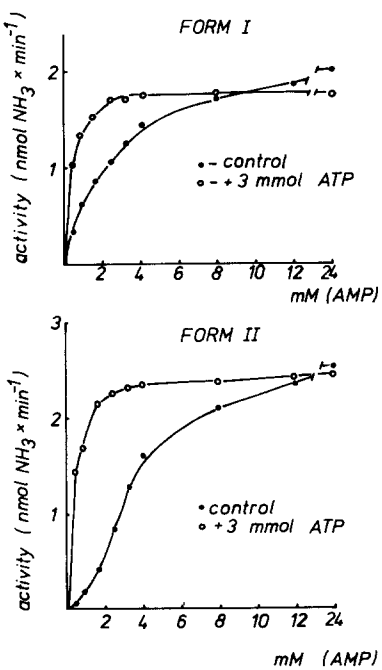

Fig. 2. Substrate saturation curves for lizard liver AMP deaminase I and II in the absence and in the presence of ATP. The V_{max} values were calculated by using the method of linearization of the Hill equation described by Endrenyi *et al.* (1975). The intersection point of the Hill plot with the abscissa at $\log (v/V - v) = 0$ allowed us to calculate the $S_{0.5}$ value.

Figure 1 presents a typical elution profile from the phosphocellulose column of the lizard liver AMP deaminase. Two peaks of the enzyme activity were obtained after subsequent elution with 1.0 and 2.0 M KCl. Further investigation of both enzyme forms revealed that they differ in their kinetic properties. In the absence of ATP form I exhibited hyperbolic kinetics while the form II displayed sigmoid shaped substrate saturation curve (Fig. 2). The $S_{0.5}$ values calculated for the form I and II were 2.5 ± 0.1 and 3.6 ± 0.4 mM ($n = 3$, $\pm$SD) respectively. These differences disappeared when 3.0 mM ATP was present in the incubation mixture. The effect of adenine and guanine nucleotides and orthophosphate on the activity of the lizard liver AMP deaminase I and II is presented in Table 1. All nucleotides investigated exhibited stimulatory effect on both forms of the enzyme, form II being influenced to a much higher extent. Inorganic phosphate inhibited both forms but concerted action of all effectors tested at their apparent physiological concentrations was acti-

Table 1. The effect of adenine and guanine nucleotides and orthophosphate on the activity of AMP deaminase I and II from lizard liver

Additions	Relative velocity	
	AMP deaminase I	AMP deaminase II
AMP 1 mM (control)	100	100
+ ATP (0.4 mM)	232	595
+ ADP (0.4 mM)	126	314
+ GTP (0.4 mM)	106	154
+ P_i (4.0 mM)	64	55
+ ATP (3.0 mM)		
ADP (0.4 mM)		
GTP (0.4 mM)		
P_i (4.0 mM)	159	573

vatory, forms I and II being stimulated 1.6 and 5.7-fold respectively.

DISCUSSION

In recent years, research in this laboratory has been continued on the premise that, if there are differences in the regulatory properties between the AMP deaminase isolated from the liver of ureo- and uricotelic species, comparative studies would provide more insight into the role of this enzyme in the purine nucleotides interconversion and breakdown (Spychała and Makarewicz, 1983; Spychała, 1984). It has been shown that in the chicken liver there are two molecular, kinetically distinct forms of AMP deaminase (Spychała and Makarewicz, 1983). Both these forms are activated by GTP whereas the enzyme from rat and turtle liver is rather inhibited by this compound (Spychała, 1984). The study performed on isolated chicken hepatocytes (Spychała and Van den Berghe, 1985, manuscript in preparation) also provides evidence that adenine nucleotides turnover and breakdown rates are regulated in a different manner than in rat liver.

Results presented in this paper show that also in the lizard liver two forms of AMP deaminase are present. These two forms resemble very much AMP deaminases isolated from chicken liver (Spychała and Makarewicz, 1983). They are activated by ATP and inhibited by inorganic phosphate as all vertebrate AMP deaminases from the liver are, but in contrast to the rat and turtle liver enzyme, the form II from the lizard liver is activated by GTP.

The results also strongly support the assumption that there is a correlation between regulatory properties of AMP deaminase and either ureo- or uricotelism. Although AMP deaminase seems to control the adenine nucleotide breakdown in all vertebrate species in the way demonstrated for rat liver (Van den Berghe *et al.*, 1980), in the uricotelic species a different mechanism of this regulation seems to be involved than in ureotelic animals.

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