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Multiple forms of AMP deaminase in rat brain

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SUMMARY

Rat brain contained four types of AMP deaminases (AMP aminohydrolase, EC 3.5.4.6) which were separated by phosphocellulose chromatography. They were designated as Types I, II, III and IV, respectively, in the order of their elution from the column. These four AMP deaminases have similar molecular weights and all seem to be allosteric enzymes. They are different in apparent K_m values for AMP in the absence or presence of activators.

AMP deaminase (AMP aminohydrolase, EC 3.5.4.6) catalyzes the conversion of AMP to IMP and ammonia. It is found in a variety of animal tissues. The kinetic properties vary widely depending on the source and purification of the enzyme. The enzyme from rabbit muscle[1,2], erythrocyte[3] and calf brain[4-6] has been studied extensively. In general, the enzyme is activated by alkali metal ions and ATP, and inhibited by GTP.

Recently, Birnbaum *et al.*[7] reported that AMP deaminase from calf muscle was highly purified and resolved into two major forms by chromatography on phosphocellulose, and the forms differ from each other with respect to their activation by alkali metal ions. The results reported in this paper suggest that rat brain contains four AMP deaminases, I, II, III and IV, which are similar in molecular sizes. They are kinetically distinct from each other and readily separated on phosphocellulose columns.

The cerebrum (cerebral hemisphere dissected free of cerebellum and brain stem) of adult Wistar rats weighing 180–200 g were used throughout.

AMP deaminase activity was measured colorimetrically by the following two methods. *Assay 1.* Typical reaction mixtures contained 5 mM AMP, 5 mM ATP, 20 mM potassium phosphate, pH 6.9, 100 mM NaCl, 0.02% 2-mercaptoethanol, and enzyme to a

final volume of 0.25 ml. The reaction was allowed to proceed at 37°C for 5 or 10 min, and was stopped by addition of 0.5 ml of phenol reagent. The amount of ammonia was determined directly by the phenol-hypochlorite method[8,9]. *Assay 2.* This assay was used in the kinetic experiments. The reaction mixtures contained 0.2 M Tris-acetate, pH 6.9, 0.02% 2-mercaptoethanol, 0.05% bovine serum albumin, various concentrations of AMP and effectors, and enzyme to a final volume of 0.25 ml. All nucleotides were Tris salts. Incubation was carried out for 5, 10 and 15 min at 37°C, and the reaction was stopped by the addition of 1 ml of 5% trichloroacetic acid. Ammonia was diffused and trapped by the method of Seligson and Seligson[10]. The diffused ammonia was allowed to react with phenol-hypochlorite reagent[8], and the resulting color was read at 630 nm.

When the 10 000 × *g* supernatant of rat brain extracts was chromatographed on a phosphocellulose column, using a NaCl gradient for elution, four peaks of AMP deaminase activity were obtained (Fig. 1). They were designated as I, II, III and IV, in the order of their elution from the column. Under the conditions of this experiment, Type I was eluted at 0.3 M NaCl, Type II at 0.5 M, Type III at 0.65 M and Type IV at 0.8 M. Further elution with 2.0 M NaCl revealed no other activity peak.

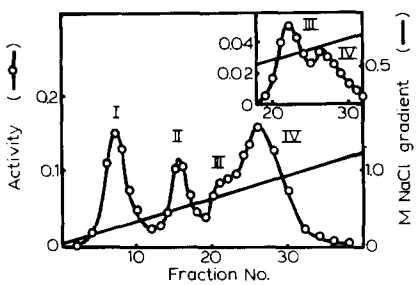


Fig. 1. Phosphocellulose chromatography of AMP deaminase from an extract of rat brain. Rat brain (11 g) was blended for 5 min with 5 vol. of cold 0.02 M potassium phosphate, pH 6.9, contained 0.1% 2-mercaptoethanol (Buffer A) in a Waring Blender at high speed (18 000 rev./min). After centrifugation at 10 000 × *g*, the supernatant solution was saved and 2 M NaCl was added to a final concentration of 0.1 M. The solution was applied to a phosphocellulose column (2.5 cm × 10 cm) which had been equilibrated with 0.1 M NaCl in Buffer A. The column was washed with 100 ml of 0.1 M NaCl in Buffer A. Elution was carried out with a linear NaCl gradient, from 0.1 to 1.2 M NaCl in Buffer A. The reservoir and mixing bottle each contained 200 ml of the appropriate solutions. Fractions of about 10 ml each were collected and assayed for enzyme activity (*Assay 1*). Activity was expressed as μmoles ammonia formed per min per ml of the fraction. Insert: a rechromatography of Fractions 20–23.

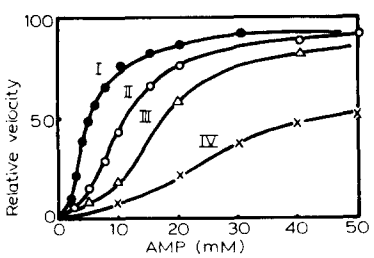
To elucidate characteristic and kinetic differences, each type was purified from 250 g of frozen brain using repeated phosphocellulose chromatography, DEAE-cellulose chromatogaphy, ammonium sulfate fractionation and Sepharose 6 B gel filtration[11]. Compared with the 10 000 × *g* supernatant the specific activities of the purified preparations which could be obtained with these methods were increased 100-fold for Types I and II, 350-fold for Type III and 850-fold for Type IV. The purified fractions did

not contain either adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) or nucleotidase (5′-ribonucleotide phosphohydrolase, EC 3.1.3.5), indicating that phosphohydrolysis of AMP, followed by deamination of adenosine could be ruled out. Indeed, the product formed during or at the end of catalysis was only IMP. Neither adenosine nor inosine was detectable by means of cellulose thin-layer chromatography. When purified Types I, II, III or IV were applied individually to phosphocellulose columns, each chromatographed as a single peak at the same elution position as that for the original fraction. These patterns were not changed at various purification steps. When two or three types were mixed and stored overnight at 4°C, the mixtures yielded only the peaks corresponding to the parental types by phosphocellulose column chromatography.

Examinations of molecular size showed that the four types of enzyme were similar in their molecular weights. This was confirmed by gel filtration and centrifugation in a sucrose gradient. The enzymes I, II, III and IV were eluted from Sepharose 6 B at the same position indicating no appreciable difference in molecular sizes. Molecular weights of about 200 000 were obtained from the elution position of the enzyme activities. The four types were also subjected to sedimentation velocity analysis in sucrose density gradient and showed s values of about 10.

Effects of AMP concentrations on the activity are quite different in the four types. Plots of enzyme activity against AMP concentration in the absence of ATP and alkali metal ions yield strongly sigmoidal curves (Fig. 2). The apparent K_m values for AMP were 5, 11, 17, and 42 mM for Types I, II, III and IV, respectively. The data for saturation curves in Fig. 2 have been replotted in terms of $\log(v/V-v)$ with respect to $\log[\text{AMP}]$. The Hill coefficients thus obtained were 2.7, 2.0, 1.9 and 1.7 for Types I, II, III and IV, respectively.

In the presence of 5 mM ATP and 100 mM NaCl, however, the AMP saturation curves become almost hyperbolic (Fig. 3). The K_m values for AMP were 0.8, 2.4, 3.8 and



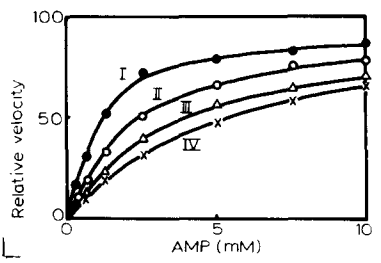


Fig. 2. Effect of AMP concentration on the activity of AMP deaminases. Enzyme activity was determined as described for *Assay 2*. The enzyme used was purified and dialyzed against 0.1 M Tris–acetate, pH 6.9, contained 0.1% 2-mercaptoethanol. Reaction mixtures contained 0.2 M Tris-acetate, pH 6.9, 0.02% 2-mercaptoethanol, 0.05% bovine serum albumin, various concentrations of AMP and enzyme in a final volume of 0.25 ml. Reaction rates are expressed as percentages of the maximum velocities.

Fig. 3. Effect of AMP concentration on the activity of AMP deaminases in the presence of ATP and NaCl. Conditions are described in the legend to Fig. 2. Reaction mixtures also contained 5 mM ATP and 100 mM NaCl.

5.2 mM for Types I, II, III and IV, respectively. The enzymes do not show an absolute dependence on either ATP or alkali metal ions. The maximum velocities in the absence and presence of ATP and NaCl were the same in all four enzymes. This means that the four types of AMP deaminase are allosteric enzymes and belong to the K system according to Monod *et al.*[12].

Because of the importance of this enzyme in adenine to guanine nucleotides conversion[13], studies of the activation and inhibition of the enzymes were carried out. Effects of Na^+ and K^+ as well as ATP on the purified deaminases from rat brain are summarized in Table I(A). In the presence of 0.8 mM AMP, Na^+ and K^+ stimulated activities of all four types at 50 mM, however, their activating effects are much less than those obtained in the presence of 5 mM ATP. The effect of different alkali metal ions on the rate of reaction was also tested at 100 mM in the presence of 0.8 mM AMP and it was observed that in all four types of enzyme not only Na^+ and K^+ but also Li^+ and Rb^+ could stimulate the reaction to similar extents. However, no activation was observed in the presence of Cs^+. At high concentrations of alkali metal ions (0.2–1.0 M), the activities of all types of enzyme were inhibited.

TABLE I

ACTIVATION AND INHIBITION OF THE FOUR TYPES OF AMP DEAMINASE

Additions		*Relative velocity*			
		I	*II*	*III*	*IV*
(A)*	None	0	0	0	0
	Na^+, 50 mM	34	30	8	21
	K^+, 50 mM	20	9	16	6
	ATP, 2.5 mM	83	84	83	96
	ATP, 2.5 mM + Na^+, 50 mM	100	100	100	100
	ATP, 2.5 mM + K^+, 50 mM	80	94	76	85
(B)**	None	100	100	100	100
	GTP, 0.05 mM	30	28	77	80
	GTP, 0.2 mM	17	39	42	64

*Reaction mixtures contained 0.8 mM AMP and alkali metal ions or ATP as indicated. All other procedures were as described in the legend to Fig. 2.
**Reaction mixtures contained 2 mM AMP and the Tris salt of GTP as indicated. All assays were performed as described in the legend to Fig. 2.

It has been reported that GTP inhibited the activity of AMP deaminase from various tissues of the rat[13]. The effect of GTP on the purified enzymes from rat brain was studied and is shown in Table I(B). At a substrate concentration of 2 mM, GTP inhibited the enzyme activities in all four types, however the rates of inhibition were more predominant in Types I and II than in Types III and IV.

With the assumption that the four types would be compartmentalized differently and be under different metabolic regulations, the subcellular distribution of the four enzymes was tested according to Eichberg *et al.*[14]. The localization of AMP deaminase in

the nuclear crude mitochondrial, microsomal and soluble fractions after differential centrifugation showed that 39% of the AMP deaminase activity of the original homogenate appeared in the crude mitochondrial fraction and 41% in the cytoplasmic fraction. After sucrose density gradient fractionation of the crude mitochondrial fraction, 41% and 48% of the activity appeared in the synaptosomal and mitochondrial fractions, respectively. The synaptosomal, mitochondrial and cytoplasmic fractions were analyzed for the types of enzyme, using chromatography on phosphocellulose. Both the cytoplasmic and particulate fractions contained all four species of enzyme. However, in the cytoplasmic fraction the content of Type I was much less than that obtained from whole homogenate, while in the particulate fractions the content of Type I was highest, indicating that Type I is mainly associated with particulate fractions.

Although AMP deaminase has been shown to have a wide occurrence, the physiological role of this enzyme is not clear yet. Setlow *et al.*[13] showed that AMP deaminase may be involved in the regulation of the relative purine nucleotide level in the cell. Recently, Lowenstein and Tornheim[15] proposed metabolic functions for the purine nucleotide cycle which consists of the reactions catalyzed by AMP deaminase, adenylosuccinate synthetase (IMP: L-aspartate ligase (GDP), EC 6.3.4.4) and adenylosuccinase (adenylosuccinate AMP-lyase, EC 4.3.2.2). We have demonstrated the occurrence of four different AMP deaminases in rat brain. The existence of four enzymes in the same tissue makes it interesting to speculate that AMP deaminase may have a more diverse function than mere involvement in purine nucleotide metabolism.

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