THE EFFECT OF PURINE NUCLEOTIDES AND PHOSPHATE ON THE ACTIVITY OF AMP DEAMINASE IN A CYTOSOL EXTRACT OF RAT LIVER

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1. Introduction

AMP deaminase (EC 3.5.4.6) catalyses the hydrolytic deamination of AMP to IMP. This enzyme has been purified from several sources and has been extensively studied in extracts of brain [1–3] and muscle [4].

AMP deaminase purified from rat liver is known to be activated by ATP and inhibited by GTP [5].

Previous studies have also established that AMP deaminase present in a cytosol extract of rat liver is activated by ATP and analogues of ATP [6].

The purpose of the present paper is to describe further studies on AMP deaminase in a cytosol extract of rat liver, which were done in order to evaluate the possible physiological significance of this enzyme in liver.

2. Experimental

Experiments were carried out using a cytosol extract of rat liver prepared as described previously [6] in a medium consisting of 0.01 M 3-(*N*-morpholino)propanesulphonic acid, 0.15 M KCl, 1 mM EDTA and 0.1 mM-dithiothreitol, pH 7.0. Endogenous substrates were removed from the cytosol extract by gel-filtration on columns of Sephadex G-25 (coarse grade). The cytosol extract (10–20 mg protein/ml) was incubated with substrates at 37°C, pH 7.0, as described previously [6]. NH_3 was assayed by an enzymic method [7]. Phosphate was determined by a modification of the method of Berenblum and Chain [8].

3. Results

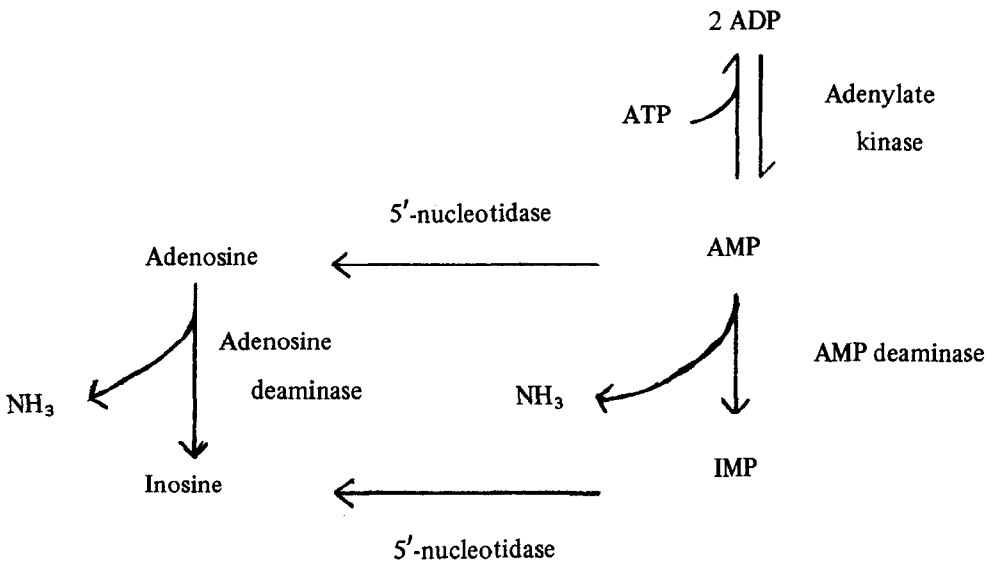
NH_3 may be produced from AMP added to a cytosol extract of rat liver both directly via AMP deaminase and indirectly by the combined actions of 5′-nucleotidase and adenosine deaminase (scheme 1). It was shown previously that the activity of 5′-nucleotidase in the cytosol extract was low in the absence of Mg^{2+} and was further inhibited by ATP [6]. Thus in the absence of Mg^{2+}, NH_3 production from AMP added to the cytosol extract may be considered to be due almost entirely to the action of AMP deaminase, especially in the presence of ATP.

It has also been demonstrated that in the presence of Mg^{2+}, the cytosol extract contained considerable adenylate kinase activity, but that in the absence of Mg^{2+} the activity of this enzyme was negligible [6].

Thus in order to avoid interference from other enzymic reactions, the properties of AMP deaminase were investigated in a cytosol extract in the absence of Mg^{2+}.

Figure 1 shows that the rate of deamination of AMP in the absence of ATP or ADP was low, but that the addition of 1 mM ATP or 2 mM ADP caused a considerable enhancement of this rate. The concomitant rate of phosphate production was negligible, indicating that the production of NH_3 was due to the action of AMP deaminase. Half-maximal stimulation of the rate of NH_3 production from 2 mM AMP was achieved at an ATP concentration of 0.25 mM. In the case of ADP, no value for half-maximal activation was obtained since the rate of NH_3 production from 2 mM AMP in the presence of ADP continued to increase as the concentration

Scheme 1
Some reactions of AMP in the cytosol extract of rat liver



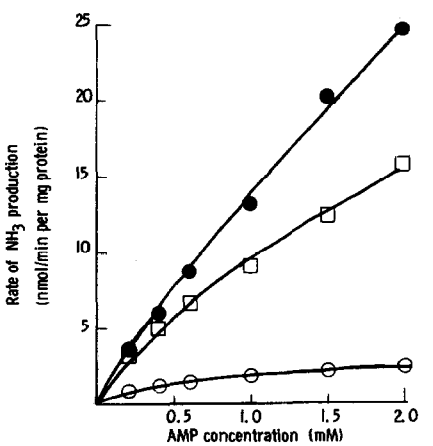


Fig. 1. The deamination of AMP in the presence and absence of ATP or ADP. Cytosol extract was incubated with shaking at 37°C, pH 7.0. The reaction was started by the addition to the extract of adenine nucleotides as appropriate. The rate of NH_3 production was linear with time over the first 5 min of the incubation. The rates of NH_3 production from 1 mM ATP or 2 mM ADP in the absence of AMP were negligible. (○) AMP only, (□) + 2 mM ADP, (●) + 1 mM ATP.

of ADP was increased. It was however found that 1 mM ADP caused a 3-fold stimulation of the rate of NH_3 production from 2 mM AMP. The rates of NH_3 production from ATP or ADP in the absence of AMP were negligible. Phosphate and GTP were found to be inhibitors of ATP or ADP-activated AMP deaminase, but were without effect in the absence of activators (table 1). Reaction velocity data presented in the form of Dixon plots (figs 2 and 3) shows that both phosphate and GTP were competitive with respect to ATP. The apparent K_i for phosphate was 0.75 mM and that for GTP was 0.05 mM.

The rate of NH_3 production from 2 mM AMP by ATP-activated AMP deaminase in the presence of a number of compounds was also investigated. None of the following at a concentration of 5 mM was found to affect the rate of NH_3 production under these conditions: L-alanine, L-leucine, L-glutamate, L-aspartate, L-glutamine, ornithine, citrulline, *N*-acetyl glutamate, creatine phosphate. In view of the finding that AMP deaminase activity in the cytosol extract was affected by ATP, ADP, GTP and phosphate, it was of interest to investigate the activity of the enzyme in the simultaneous

Table 1
The effect of phosphate or GTP on the rate of deamination of AMP in the presence and absence of activators

Additions	Rates of NH_3 production (nmol/min/mg protein)			
	AMP	AMP + ADP	AMP + ATP	AMP + ADP + ATP
None	2.95 ± 0.75 (7)	11.9 ± 0.80 (6)	22.8 ± 4.1 (11)	22.9 ± 0.6 (3)
Phosphate (5 mM)	2.50 ± 0.30 (3)	4.1 ± 0.90 (3)	10.4 ± 2.2 (6)	11.9 ± 0.4 (4)
GTP (0.5 mM)	2.70 ± 0.25 (3)	3.8 ± 0.40 (3)	15.5 ± 2.8 (3)	17.7 ± 0.5 (3)

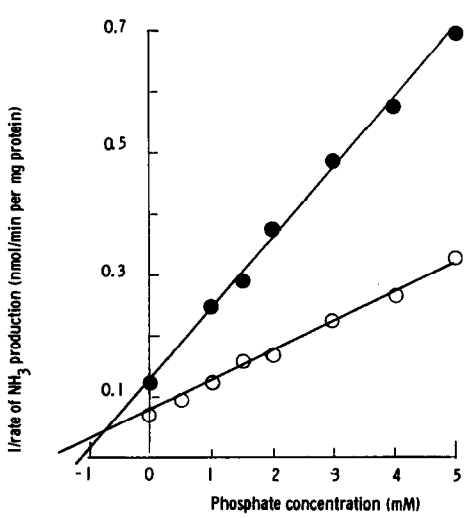
The reaction was started by the addition to the cytosol extract of adenine nucleotides and phosphate or GTP as appropriate. All adenine nucleotides were present at a concentration of 2 mM. The values quoted are the means ± SE with the number of separate preparations given in brackets. Each determination was performed in duplicate. When AMP, ADP and ATP were present simultaneously with 5 mM phosphate and 0.5 mM GTP, the rate of NH_3 production was 7.4 nmol/min/mg protein. (SE of 3 preparations.)

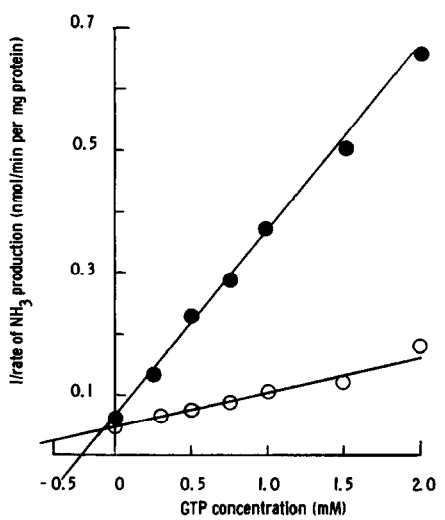
presence of these effectors at concentrations similar to those which may be found in the liver. Such concentrations of ATP, ADP, AMP, and phosphate were calculated from values for the levels of these compounds found in the perfused liver [9,10]. The concentration of GTP in the liver was taken to be 0.3 mM (Dr C. I. Pogson, personal communication).

Figure 4 shows the dependence of the rate of NH_3 production on AMP concentration in the presence of physiological concentrations of ATP, ADP, GTP and phosphate. It may be seen that the rate of NH_3 production from 0.4 mM AMP (the physiological concentration) under these conditions was 7.2 nmol/min/mg protein. Assuming a value of 158 mg of cytosol protein/g wet wt liver [6], this rate may be expressed as 1.14 μmol/min/g wet wt liver.

4. Discussion

The results presented show that AMP deaminase in





Figs 2 and 3. Dixon plots of reaction velocity data for the deamination of AMP in the presence of ATP as a function of the concentration of phosphate (fig.2) or GTP (fig.3). The reaction was started by the addition to the cytosol extract of AMP, ATP and phosphate or GTP. The AMP concentration was 2 mM. When GTP was present in the incubation, a correction was made for the inhibition of glutamate dehydrogenase by GTP during the NH_3 assays. (○) + 1 mM ATP, (●) + 0.25 mM ATP.

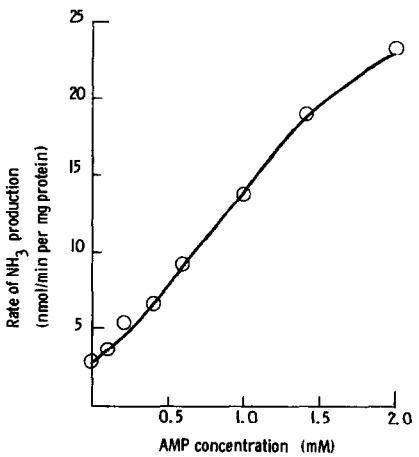


Fig.4. The deamination of AMP in the presence of physiological concentrations of activators and inhibitors. The reaction was started by the addition to the cytosol extract at 37°C, pH 7.0, of varying concentrations of AMP in the presence of 3 mM ATP, 1 mM ADP, 4 mM phosphate and 0.3 mM GTP.

a cytosol extract of rat liver is strongly activated by ATP and to a lesser extent by ADP. GTP and phosphate were found to be inhibitors of ATP- or ADP-activated AMP deaminase. Both GTP and phosphate were competitive with respect to ATP. The effects of ATP, ADP and GTP on AMP deaminase in the cytosol extract are in agreement with the findings of Smith and Kizer [5] for the isolated rat liver enzyme. It is noteworthy that rat liver AMP deaminase is activated by ATP since the enzyme from muscle is inhibited by this nucleotide [4]. The metabolic significance of this difference awaits clarification.

Appreciable AMP deaminase activity was demonstrated in the presence of physiological concentrations of substrate and effectors. The rate of NH_3 production via AMP deaminase under these conditions may be calculated to be 1.14 μmol/min/g wet wt liver. By comparison, the rate of urea synthesis from L-alanine in isolated liver cells has been reported to be 1.06 μmol/min/g wet wt liver [11]. If the properties of AMP deaminase in the liver in vivo are similar to those of the enzyme in the cytosol extract, it is possible to infer that AMP deaminase may be an important ammoniogenic enzyme in liver.

Faupel et al. [12] have demonstrated that the level of AMP in the normoxic, freeze-clamped liver is very low (approximately 15–30 nmol/g wet wt) but that during anoxia, a considerable increase in the level of AMP occurs. These findings would be compatible with a high AMP deaminase activity in liver. Under normal metabolic conditions, a low level of AMP would be maintained as a result of AMP deaminase activity. During anoxia however, the level of ATP in the liver falls while that of phosphate rises. As a result the activity of AMP deaminase would be expected to decrease, thus accounting for the increase in the AMP level seen under these conditions.

It is however recognised that in order to determine the activity of AMP deaminase in vivo, it is necessary to obtain information concerning the rate of turnover of the 6-amino group of AMP.

Acknowledgement

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