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AMP deaminases of rat small intestine

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Phosphocellulose column chromatography revealed the existence of two forms of AMP deaminase both in whole tissue and in the intestinal epithelium. AMP deaminase I, which eluted from the column as a first activity peak, exhibited hyperbolic, nonregulatory kinetics. The substrate half-saturation constants were determined to be 0.3 and 0.7 mM at pH 6.5 and 7.2, respectively, and did not change in the presence of ATP, GTP and P_i. AMP deaminase II, which eluted from the column as a second activity peak, was strongly activated by ATP and inhibited by GTP and P_i. The $S_{0.5}$ constants were 3.5 and 7.1 at pH 6.5 and 7.2, respectively. At pH 7.2 ATP (1 mM) $S_{0.5}$ decreased to 2.5 mM and caused the sigmoidicity to shift to hyperbolic. The ATP half-activation constant was increased 9-fold in the presence of GTP and was not affected by P_i. Mg^{2+} significantly altered the effects exerted by nucleotides. The $S_{0.5}$ value was lowered 10-fold in the presence of MgATP and 5-fold in the presence of MgATP, MgGTP and P_i. When MgATP was present, AMP deaminase II from rat small intestine was less susceptible to inhibition by GTP and P_i. A comparison of the kinetic properties of the enzyme, in particular the greater than 100% increase in V_{max} observed in the presence of $MgCl_2$ at low (1 mM) substrate concentration, indicates that MgATP is the true physiological activator. Guo*PP*[NH]*P* at low concentrations, in contrast to GTP, did not affect the enzyme and even activated it at concentrations above 0.2 mM. We postulate that AMP deaminase II may have a function similar to that of the rat liver enzyme. The significance of the existence of an additional, non-regulatory form of AMP deaminase in rat small intestine is discussed.

Introduction

AMP deaminase (EC 3.5.4.6), a regulatory and rate-limiting enzyme of AMP degradation, catalyses the deamination of 5'-AMP to IMP. It has been proposed that alteration in the regulatory properties of liver AMP deaminase may contribute to hyperuricaemia [1]. The mechanism would largely lie in a weaker susceptibility of the altered enzyme to the inhibitory effect of GTP. This in turn would cause a less restricted control of AMP catabolism, an enhanced flux of the adenine moiety through the adenine nucleotide pool and overproduction of uric acid. It has been shown [2,3] that a decrease in GTP concentration causes an enhanced production of allantoin in isolated rat hepatocytes.

The other tissue which might contribute to the plasma uric acid level is the small intestine. Experiments in vitro with intestinal segments [4] have shown that purine nucleotides, nucleosides and free bases are catabolized to uric acid by intestinal tissue. Furthermore, the vascular perfusion of isolated rat small intestine has also shown that uric

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Abbreviation: PMSF, phenylmethylsulfonyl fluoride.

acid is the primary metabolite recovered from the portal circulation [5], and studies by Sonoda and Tatibana [6] indicate that dietary adenine is readily incorporated into intestinal nucleotides.

Therefore, it seems interesting to compare the regulation of intestinal AMP deaminase with the enzyme from rat liver to find out whether deamination of AMP is controlled by a similar mechanism.

Materials and Methods

Purification of AMP deaminase

Male Wistar rats weighing 300–400 g were used. The animals were killed by decapitation and the small intestine was dissected, immediately flushed free of intestinal contents with cold water and weighed. The enzyme was prepared either from the whole intestinal wall or from the mucosal layer. If the entire intestine had to be used, the tube was cut into small (1–2 cm) pieces and homogenized in a Waring Blendor type homogenizer in 3 vol. of 0.4 M KCl buffered with 5 mM Tris-HCl buffer, pH 7.4. The homogenate was then diluted with an equal volume of 5 mM Tris-HCl, pH 7.4, stirred for 3 min and centrifuged at $18\,000 \times g$ for 30 min at 5°C.

For the preparation from intestinal mucosa, the intestine was cut into 10-cm pieces, opened lengthwise and the mucosal epithelium was carefully scraped using a blunt knife. The homogenate was prepared in the same way from the whole intestinal wall. Usually 30–35 g of the material were taken for one preparative run. The weight of the mucosal scrapings contributed up to about 50% of the total weight.

To the supernatant fraction of the homogenate 20 ml of wet phosphocellulose were added and the batch adsorption was carried out at 5°C for 30 min under gentle stirring; the phosphocellulose slurry was then washed three times with 0.25 M KCl and placed in a K 16/20 Pharmacia column.

The column was washed with 0.6 M KCl (adjusted to pH 7.0 with imidazole) and the AMP deaminase activity was eluted using a double gradient of KCl (0.6–0.7 M) and inorganic phosphate (0–0.1 M, pH 7.0, with KCl in the mixing vessel and KCl and phosphate in the reservoir) at a flow rate 30 ml per h. The fractions corresponding to the major portion of each activity peak were pooled in dialysing sacks, concentrated in poly(ethylene glycol) 20000 and followed by dialysis against 1.0 M KCl, buffered with 20 mM imidazole-HCl, pH 6.8. The specific activity of AMP deaminase preparations from rat small intestine purified in this way was in the range 10–14 and 16–20 μmol/min per mg of protein (assayed at 10.0 mM AMP) for forms I and II, respectively, the purification being 1000–2000-fold. The enzyme preparations were essentially free of adenosine deaminase, 5-nucleotidase and ATPase activities.

Kinetic studies

AMP deaminase activity was determined from the amount of ammonia produced by using the phenol-hypochlorite method [7] at 25°C. The reaction mixture contained, in 0.5 ml final volume, 50 mM sodium cacodylate buffer, pH 6.5 or 7.2, 150 mM KCl, enzyme, AMP and other compounds at the concentrations indicated in the tables and figures. The production of ammonia was linear during 5–15 min of incubation. For the evaluation of $s_{0.5}$, AMP deaminase activity was measured between 0.25 and 18.0 mM AMP and for the determination of $A_{0.5}$ activity was measured at a constant 1.0 mM AMP and between 0.01 and 5.0 mM ATP. Mg^{2+} was added to the incubation mixture in the form of $MgCl_2$ solution to give a final concentration of 0.8 mM in excess of total ATP and/or GTP concentrations. Under such conditions, at pH 7.2 92.3% of ATP was complexed with Mg^{2+}, the rest being in the form of ATP^{4-}, $KATP^{3-}$, $HATP^{3-}$ [8,26].

As suggested by Atkins [9] and Garfinkel and Fegley [10], the nonlinear regression method, which gives accurate and easy determination of kinetic parameters, was employed. Initial estimates for kinetic parameters (h and $S_{0.5}$) were computed from the data (0.1–0.9 of V_{max}) using a linear regression of the transformed Hill equation ($\log(v/V - v)$ on $\log s$). The experimental value of V_{max} was used for calculations at the initial stage. The final fit of experimental data to the kinetic function was performed using iterative (Gauss-Newton gradient method), nonlinear least-squares data fitting. An algorithm presented by Parker and Waud [11] was employed for this

purpose. To facilitate the calculations an overall computer program was written in BASIC. For the calculations of $A_{0.5}$ constants, the $v - v_0$ (v_0, velocity in the absence of activator) was introduced instead of v. The adequacy of a given fit to a set of data was judged by the following criteria: (i) whether the parameter values obtained were reasonable, (ii) whether the sum of squares of deviations between calculated and observed velocities was sufficiently small and (iii) whether the observed points were close to the computed curve [12].

Analytical measurements

Protein concentration int he extract was measured using the method of Gornall et al. [13], and in the purified enzyme preparations using the method of Bradford [14] with bovine serum albumin as a standard.

Reagents

AMP and phosphocellulose were supplied by Sigma (U.S.A.), GTP was from Boehringer Mannheim (F.R.G.), Guo*PP*[NH]*P* (guanylylimidodiphosphate) and PMSF were from ICN (U.S.A.), sodium cacodylate and 2-mercaptoethanol were from Loba Chem. (Austria), poly(ethylene glycol) 20000 was from Serva (F.R.G.) and Trasylol was from Bayer (F.R.G.). ATP and all other reagents were of the highest purity available and were purchased from POCH, Poland.

Results

Elution profiles of rat small intestine AMP deaminase from the phosphocellulose column show that two forms of the enzyme are present both in the whole tissue and in the intestinal epithelium (Fig. 1). Preliminary experiments showed that the two forms were also separable by using the step-elution procedure, form I being eluted between 0.6 and 0.7 M KCl and form II between 0.7 and 1.0 M KCl. However, the specific activities of the resulting preparations were significiantly lower, being 5- and 3-fold lower for forms I and II, respectively, when compared with the preparations obtained by our double-gradient procedure (see Materials and Methods for details). The proportion of the amount of activity present in forms I and II varied from

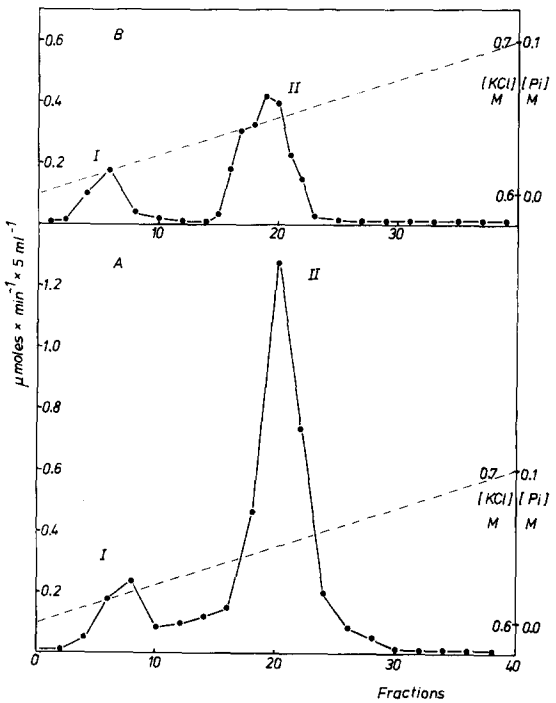

Fig. 1. Elution profiles of AMP deaminase from phosphocellulose column using: A, the whole small intestine; B, the scrapings of the mucosa as starting material.

preparation to preparation; however, this variation was not affected if proteinase inhibitors (PMSF, 1.0 mM and Trasylol, 10000 U/ml) were included in the homogenization medium. Preliminary results also revealed that the extraction of the enzyme activity was most effective if 0.3–0.4 M KCl was present. Low ionic strength buffers (5 mM Tris-HCl), successfully used during preparation of AMP deaminase from the liver and kidney, and phosphate buffer (0.089 M) with 0.18 M KCl, commonly used for extraction of muscle enzyme, appeared to be much less effective as far as total activity and the efficiency of the binding of the enzyme with the phosphocellulose resin were concerned.

Table I presents kinetic parameters of the purified AMP deaminase preparations from rat small intestine, measured at pH 7.2. At pH 6.5 the values of $S_{0.5}$ in the absence of effectors were 50% lower, being 0.33 ± 0.04 ($n = 4$) and 3.46 ± 0.50 ($n = 3$) for forms I and II, respectively. The two enzyme forms represent entirely different kinetics:

TABLE I

KINETIC PARAMETERS OF AMP DEAMINASE I AND II FROM RAT SMALL INTESTINE IN THE PRESENCE OF VARIOUS COMBINATIONS OF ATP, GTP, P_i AND $MgCl_2$

The values represent means from two experiments or means ± S.D. from 3–5 experiments, each experiment performed with a different enzyme preparation. Effectors were present at the following concentrations; ATP, 1.0 mM; GTP, 0.2 mM; P_i, 4 mM.

AMP deaminase	$MgCl_2$	Effector	$S_{0.5}$ (mM)	Hill coefficient
I	–	–	0.75 ± 0.27	1.1 ± 0.1
	+	–	0.96	1.2
	–	ATP	0.67	1.0
	+	ATP	0.97	0.8
	–	ATP, GTP, P_i	0.78	0.8
	+	ATP, GTP, P_i	0.60	0.9
II	–	–	7.12 ± 0.62	2.2 ± 0.2
	+	–	6.44 ± 0.52	2.3 ± 0.2
	–	ATP	2.50 ± 0.42	1.0 ± 0.1
	+	ATP	0.61 ± 0.20	1.0 ± 0.1
	–	ATP, GTP	4.22 ± 0.52	1.0 ± 0.1
	+	ATP, GTP	0.71 ± 0.18	1.0 ± 0.1
	–	ATP, P_i	2.90 ± 0.32	1.0 ± 0.1
	+	ATP, P_i	0.61 ± 0.15	1.0 ± 0.1
	–	ATP, GTP, P_i	5.00 ± 0.38	1.2 ± 0.3
	+	ATP, GTP, P_i	1.21 ± 0.31	1.0 ± 0.1

the substrate saturation curve for form I is hyperbolic whereas for form II it is sigmoidal, with a Hill coefficient above 2 (Fig. 2). The data presented in Fig. 2 also show that the two enzyme forms differ significantly in their susceptibility to activation by ATP and inhibition by GTP and P_i, form I being rather insensitive to these effectors (see also Table I). On the other hand, ATP appeared to be a powerful activator of form II. It caused the sigmoidicity to shift to hyperbolic. The $S_{0.5}$ value decreased to 2.5 mM in the presence of 1.0 mM ATP. The concomitant presence of ATP and GTP caused a shift of the $S_{0.5}$ value to 4.2 mM, and further addition of inorganic phosphate raised it to 5.0 mM. P_i alone did not affect the apparent half-saturation constant significantly.

The addition of Mg^{2+} to the incubation mixture with AMP deaminase II brought about further, significant enhancement of activation by ATP, although $MgCl_2$ alone (0.1–20.0 mM) did not affect enzyme activity. As is clear from Fig. 2A and B, activation by 1.0 mM ATP in the presence of Mg^{2+} at lower substrate concentrations was several times stronger than in the absence of bivalent cation. This important difference is further supported in Fig. 3, where at low, 1.0 mM, substrate concentration over 2-fold higher V_{max} in the presence of Mg^{2+} is shown. This difference seems to diminish as the concentration of AMP increases (Fig. 2).

Furthermore, in the presence of MgATP the enzyme was less susceptible to the inhibitory effect of GTP and inorganic phosphate. The $S_{0.5}$ value decreased 10-fold in the presence of MgATP alone, it remained the same if either GTP or P_i were added and when all the effectors were present simultaneously it was only twice as high as in the presence of MgATP alone (Table I).

However, further studies on the activation by various concentrations of ATP in the absence and in the presence of Mg^{2+} of AMP deaminase II from rat small intestine at constant and low substrate concentration revealed that MgATP, in contrast to ATP, exerted a biphasic effect. At lower activator concentrations (10–100 μM) the enzyme appeared to be more sensitive to ATP than to MgATP (Fig. 3), whereas at concentrations above 0.2 mM the activity of AMP deaminase II became more influenced by MgATP. This behaviour meant that the $A_{0.5}$ constant was significantly lower for ATP than for MgATP (Table II). It is also noticeable that in the absence of Mg^{2+} both inhibitors (GTP, P_i) decreased maximum velocity (by about

TABLE II

THE ATP $A_{0.5}$ VALUES (mM) FOR AMP DEAMINASE II FROM RAT SMALL INTESTINE IN THE PRESENCE OF VARIOUS COMBINATIONS OF Mg^{2+}, GTP AND P_i

AMP deaminase activity was measured at 1.0 mM AMP and 0.01–5.0 mM ATP. GTP was 0.2 mM, P_i was 4.0 mM and the concentrations of $MgCl_2$ as indicated in Materials and Methods. The values (mM) represent means from two or three experiments, each performed with different enzyme preparations.

	$+Mg^{2+}$	$-Mg^{2+}$
Control	0.26	0.08
+ GTP	0.53	0.70
+ P_i	0.30	0.08
+ GTP, P_i	0.84	1.20

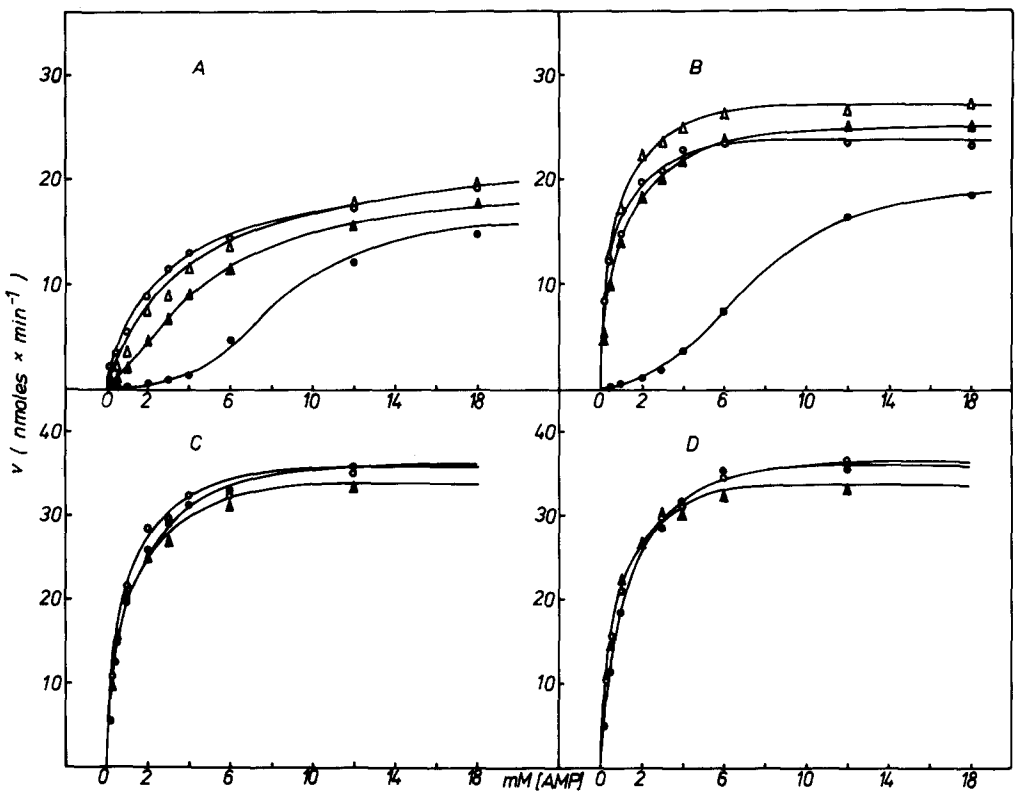

Fig. 2. The substrate saturation curves of AMP deaminases I (C, D) and II (A, B) from rat small intestine in the absence (A, C) and in the presence (B, D) of Mg^{2+} and in the presence of 1.0 mM ATP (○), 1.0 mM ATP and 0.2 mM GTP (△) or 1.0 mM ATP, 0.2 mM GTP and 4.0 mM P_i (▲) and in the absence of effectors (●).

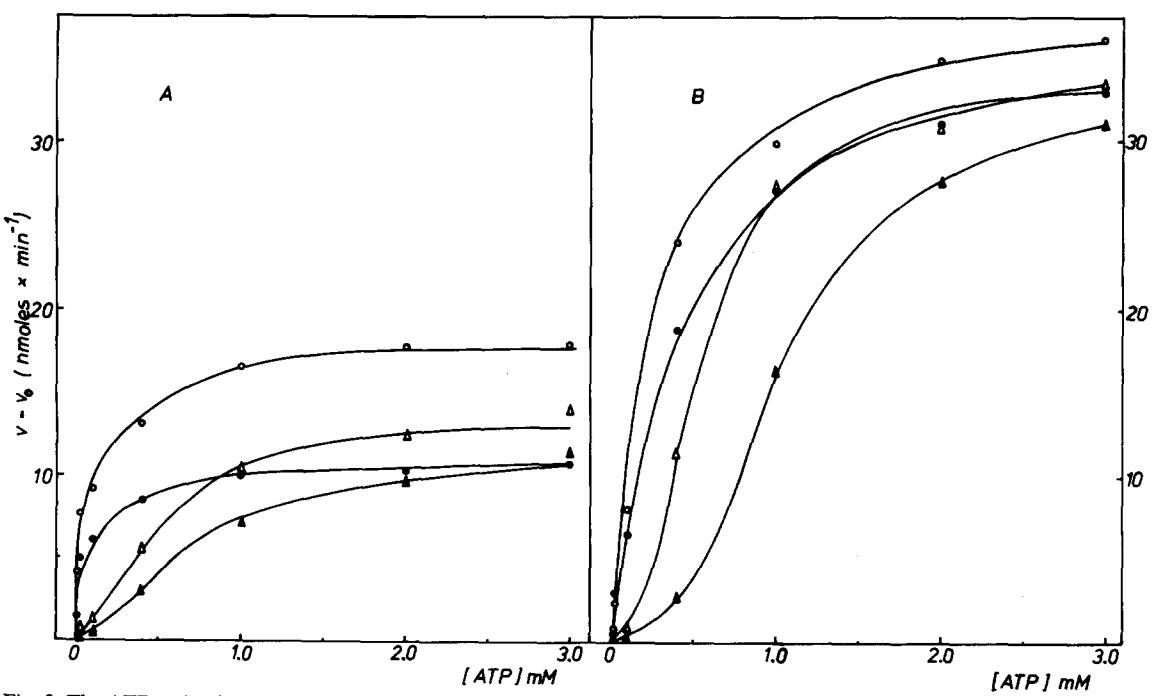

Fig. 3. The ATP-activation curves of AMP deaminase II from rat small intestine in the absence (A) and in the presence (B) of Mg^{2+}. ○, ATP only; ATP in the presence of: (△) 0.2 mM GTP, (●) 4.0 mM P_i and (▲) 0.2 mM GTP and 4.0 mM P_i.

30–40%), whereas in the presence of Mg^{2+} they did not.

The data presented in Fig. 4 show that both GTP and MgGTP are strong inhibitors of AMP deaminase from rat small intestine. However, like the activation by ATP in the absence of Mg^{2+}, the inhibitory effect of GTP at low concentrations was stronger than in the presence of Mg^{2+}. On the other hand, both Guo*PP*[NH]*P* and MgGuo*PP*[NH]*P* were without effect at lower concentrations but surprisingly activated at concentrations above 200 μM. This is rather unexpected, since it is believed that Guo*PP*[NH]*P* mimics the effects of GTP provided that hydrolysis is not involved [15,16].

Furthermore, the data presented in Fig. 5 show that, in contrast to GTP, Guo*PP*[NH]*P* did not affect significantly the activation exerted by ATP, even in the presence of inorganic phosphate. The

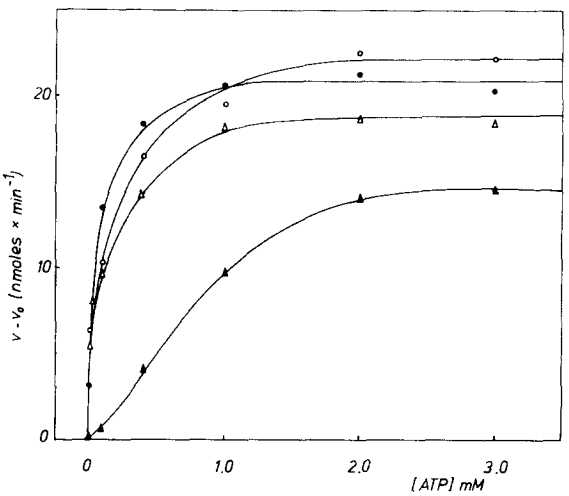

Fig. 5. Comparison of the effect of 0.2 mM GTP and 0.4 mM Guo*PP*[NH]*P* on the activation exerted by ATP in the absence and in the presence of 4.0 mM P_i. ○, ATP only; ATP in the presence of: Guo*PP*[NH]*P* (●), Gu*PP*[NH]*P* and P_i (△), GTP and P_i (▲).

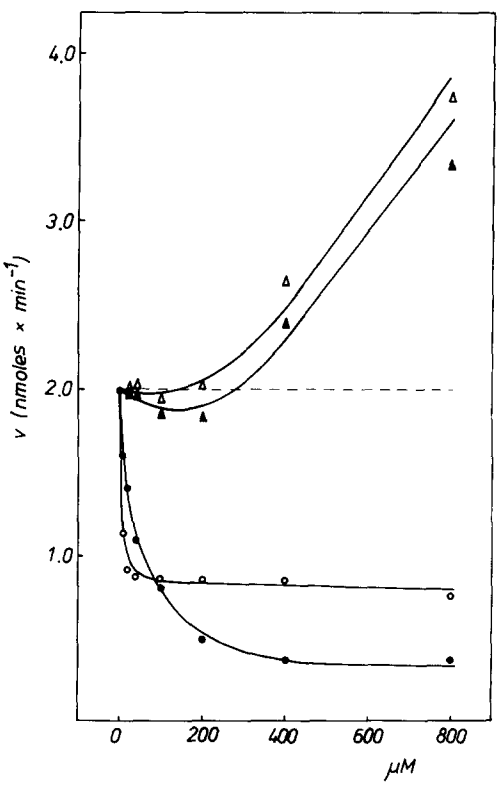

Fig. 4. Comparison of the effect of GTP (○, ●) and Guo*PP*[NH]*P* (△, ▲) on the activity of AMP deaminase II from rat small intestine in the absence (○, △) and in the presence (●, ▲) of Mg^{2+}.

half-activation constant for ATP increased in the presence of 0.4 mM Guo*PP*[NH]*P* only 2.5-fold (up to the value of 0.2 mM) and in the presence of both the GTP analogue and P_i it remained unchanged (0.1 mM) whereas, as was shown previously for 0.2 mM GTP, the $A_{0.5}$ values were 0.7 and 1.2 mM in the presence of GTP alone and both GTP and P_i, respectively (Table II).

Discussion

The experiments presented in this paper show that two molecular forms of AMP deaminase exist in rat intestinal epithelium. Recently it has been reported that two forms of this enzyme are also present in the liver of uricotelic animals [17–19]. Jackson et al. reported [20] that in rat liver during hepatocarcinogenesis a second form of AMP deaminase appears and Ogasawara et al. [21] reported that in the rat liver, a shift from multiple forms of AMP deaminase toward one adult form occurs during ontogenesis. The occurrence of an additional form of AMP deaminase, which exhibits hyperbolic kinetics and which is not susceptible to regulation by ATP, GTP or P_i, might be of great importance to some tissues. In the adult chicken liver this may be connected with uricote-

lism, in the hepatomas and perhaps in the developing liver with the increased proliferation rate of hepatocytes and hence with the increased turnover rate of purine nucleotides, and in skeletal muscle the existence of only single, hyperbolic-type AMP deaminase may reflect the intensive metabolism of purine nucleotides, particularly during muscle work [22]. Intestinal epithelium cells also display a very high proliferation rate and accordingly one should expect a high rate of purine nucleotide metabolism.

From the kinetic point of view the two forms of AMP deaminase from rat small intestine may be regarded as a muscle-type (form I) and as a liver-type (form II). In the absence of Mg^{2+} form II appears to be regulated in essentially the same way as the rat liver AMP deaminase [2]. Both enzymes are strongly activated by ATP and inhibited by GTP. However, up to now, the regulatory properties of these two enzymes have not been studied in the presence of $MgCl_2$. The conversion of free ATP into Mg-ATP complex significantly changes the regulatory effect exerted by this nucleotide. It is most clearly visible at low (but still above physiological level) AMP concentrations (Figs. 2 and 3). First of all, MgATP appears to be a much more powerful activator of form II. This conclusion is based on the following: (i) the addition of Mg^{2+} causes a marked increase of enzyme affinity toward substrate ($S_{0.5}$ decreases 10-fold) in the presence of ATP, (ii) V_{max} at low, 1.0 mM, AMP and at a saturating concentration of ATP is increased by over 100% in the presence of Mg^{2+}, (iii) in the presence of 1.0 mM ATP (Fig. 2) substrate saturation is attained at 4 mM AMP when Mg^{2+} is present and is not reached even at 18.0 mM AMP in the absence of the bivalent cation. It is also noteworthy that the regulatory properties of AMP deaminase II in the absence and in the presence of Mg^{2+} resemble those of the enzyme from human platelets [23].

Although Mg^{2+} influences in a positive way the activity of AMP deaminase II from rat small intestine in the presence of ATP, as presented in Figs. 2 and 3, the enzyme seems to display a higher affinity for free ATP than for MgATP. This may be concluded from a lower $A_{0.5}$ value for ATP in the absence of Mg^{2+} (Table II). This apparent discrepancy may be explained by the fact that at low concentrations (10–40 μM) ATP binds to a site which has higher affinity for the free nucleotide, whereas at higher concentrations it binds to the other site common for both Mg-complexed and uncomplexed nucleotides and which in turn has higher affinity for MgATP. It is possible that AMP also either binds or affects the binding of free ATP to the latter site, since at high AMP concentrations the difference in maximal velocity seems to disappear (Fig. 2). It is difficult to assume that the regulation of AMP deaminase II by 10–100 μM ATP^{4-} has any physiological significance and therefore the only reason for the existence of a high-affinity site which binds only free ATP seems to be that it is in fact a site of action of another, still undiscovered adenine nucleotide-like compound which operates in the cell in micromolar concentrations.

GTP was postulated to be a very important cellular regulator of AMP deaminase from rat liver [2]. It has also been shown that the effect of this compound on the enzyme from the liver of urico- and ureotelic species is different [18]. In the present study the evidence is presented that GTP strongly inhibits AMP deaminase II from rat small intestine and even at a low (0.2 mM) concentration decreases the activation by ATP, particularly in the presence of inorganic phosphate. Surprisingly, Guo*PP*[NH]*P*, the nonhydrolysable analogue of GTP, was found to exert different effects. At present we cannot provide any reasonable explanation for these differences, as only a small difference in the structure of the β and γ phosphate groups exists between GTP and Guo*PP*[NH]*P*. In other studies in which Guo*PP*[NH]*P* was employed as a nonhydrolysable analogue of GTP, it exerted quite similar effects to those of GTP [15,16]. Another possibility is that inhibition by GTP involves its hydrolysis; however, this assumption needs thorough investigation.

Small intestine AMP deaminase II is similar in its regulatory properties to the rat liver enzyme, thus it is possible that both enzymes have similar functions. It has been proved recently [3] that in rat liver the reaction catalysed by AMP deaminase is rate-limiting in the degradation of adenine nucleotides. A model of uricaemia has been developed in which rapid phosphorylation of fructose

caused depletion of ATP and concomitantly of the total adenine nucleotides, AMP deaminase being primarily involved [24]. It is also known that in the rat small intestine all the enzymes of fructose metabolism are present [25]. Therefore, it is assumed that if the adenine nucleotide pool became depleted in rat small intestine under conditions similar to those in the liver, AMP deaminase might play a similar function in this process and hence the small intestine might cooperate with the liver in developing overproduction of uric acid under certain circumstances.

On the other hand, Sonoda and Tatibana [6] revealed that dietary adenine is readily incorporated into intestinal nucleotides, both acid-soluble and insoluble. This gain supports the assumption that a high adenine content in the diet accompanied by factors lowering the adenine nucleotide concentration and in this way enhancing adenine turnover may cause uric acid overproduction by the intestine. To prove this assumption, further metabolic experiments are required.

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