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# REGULATORY PROPERTIES OF RAT HEART AMP DEAMINASE

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## Summary

The kinetic and regulatory properties of purified rat heart AMP deaminase were investigated. In the presence of 100 mM KCl, the enzyme exhibited a slightly sigmoid-shaped plot of reaction rate, vs. substrate concentration, which shifted to a more hyperbolic form when ATP, ADP or GTP were added. ATP was the most potent activator of the enzyme, whereas GTP at low (less than 0.25 mM) concentrations increased the enzyme activity. The activation effect was negligible at higher concentrations of GTP. The calculated value of $K_{0.5}$ of approx. 3 mM for unactivated enzyme decreased to approx. 0.6 mM and 1.1 mM when 0.5 mM ATP or 1.5 mM ADP were present in the incubation mixture, respectively. The theoretical model (Monod, J., Wyman, J. and Changeux, J.P. (1965) J. Mol. Biol. 12, 88—118) gave a partial explanation of these results.

## Introduction

AMP deaminase (AMP aminohydrolase, EC 3.5.4.6) is a regulatory enzyme which exists in multiple molecular forms in different rat tissues [1—4]. On the basis of chromatographic, immunological and kinetic properties, three parental forms (type A, B and C) of the enzyme have been detected. Type A is the only form found in skeletal muscle and type C in heart muscle, whereas type B is found in the kidney, liver and testis. In other tissues, such as brain, spleen and lung, in addition to types B and C, heterogenic hybrid forms of the enzyme were also detected [5].

Regulatory properties of rat skeletal muscle AMP deaminase have been examined and it was shown that the enzyme was strongly activated by $K^+$, whereas inorganic phosphate was an inhibitor of the enzyme activity [6—8]. Some nucleotides, such as ATP, ADP and GTP, take part in the regulation of

this enzyme's activity. The extent of this regulation depends on the salt composition of the reaction mixture [8] and on the temperature at which the reaction is carried out [9]. In contrast to the enzyme from skeletal muscle, the kinetics and regulatory properties of AMP deaminase isolated from heart are much less characterised.

The studies of Burger and Lowenstein [10] with rat heart muscle extracts indicated that the activity of the enzyme, which seems to play an important role in adenine metabolism in the heart, is regulated by ATP and GTP. Ogasawara et l. [2,4] reported a strong activation by $Na^+$ of the activity of the enzyme purified from rat heart. The experiments of Chung and Bridger [11] on isolated AMP deaminase from rabbit heart muscle showed a sigmoid-shaped kinetic plot (reaction rate vs. substrate concentration) of AMP deamination in the presence of relatively high concentration of $Na^+$. We found that, even in the presence of 100 mM $K^+$, the kinetic plot of adenylate deamination in bovine heart was also sigmoid-shaped. We reported [12] that ATP, but not ADP, was the most potent activator of AMP deaminase from this source. These results are in contrast to those of Chung and Bridger [11], who reported that much higher concentrations of ATP than ADP are required to produce a demonstrable activating effect.

The influence of ATP, ADP and GTP on the kinetic properties of AMP deaminase purified from rat hearts in the presence of $K^+$ (the main physiological ion of heart tissue) was examined in the work reported in the present paper.

## Materials and Methods

*Isolation of the enzyme.* AMP deaminase from rat hearts was prepared by the procedure of Smiley et al. [13]. The enzyme was adsorbed on a phosphocellulose column and eluted with 1 M KCl. The activity peak was pooled and concentrated by ultrafiltration. The enzyme thereby obtained was purified about 680-fold and the spec. act. was 7.3 $\mu$mol $NH_3$ liberated by mg protein/min at 6 mM substrate concentration, 30°C and optimal pH 6.5.

*Enzyme assay.* The incubation medium in the final volume of 0.5 ml contained 0.1 M succinate-KOH buffer (pH 6.5), 100 mM KCl and different concentrations of AMP and effectors (ATP, ADP and GTP).

After equilibration of the medium at 30°C, 10 $\mu$l of the appropriately diluted solution of enzyme (2.8 $\mu$g protein) was added to start the reaction. The incubation was carried out for 10 min and the initial velocity of the reaction was determined from the mean concentration of ammonia liberated in three parallel incubations. Ammonia was estimated by the phenol-hypochlorite method [14].

In the range between 1—10 $\mu$g protein, the rate of AMP deamination was proportional to the protein concentration in the incubation medium.

*Calculation of kinetic parameters.* In the absence of nucleotides, the kinetic curve of AMP deamination was sigmoid-shaped; to calculate the maximum velocity of the reaction ($V$) the method of Endrenyi et al. [15], based on linearization of the Hill equation, was used. In the case of sigmoidal kinetic

curves, i.e. when $n \neq 1$, the $K'$ parameter in the Hill equation:

$$\log[v_0/(V - v_0)] = n \log[S] - \log K' \quad (1)$$

differs from the $K_{0.5}$ value. On plotting $\log[v_0/(V - v_0)]$ vs. $\log[S]$, the concentration of the substrate giving the half-maximum velocity of the reaction ($K_{0.5}$) equals that at which $\log[v_0/(V - v_0)] = 0$. The method of linear regression was used for calculation of the cooperativity coefficient ($n_H$) and the $K_{0.5}$ value.

In the case when the reaction exhibited hyperbolic kinetics, the statistical method of Wilkinson [16], based on non-linear regression analysis of the Michaelis-Menten equation, was used to calculate the ligand concentration giving half-maximum velocity under a particular set of conditions ($K_{0.5}$ or $A_{0.5}$) and maximum velocity of the reaction ($V_{max}$ or $V$).

The experimentally obtained data were interpreted in terms of the concerted transition theory of Monod et al. [17], assuming a perfect 'K-system'. According to this model, an allosteric protein made of $n$ protomers can exist at least in two conformational state, R and T. State R displays high affinity for the substrate and activator, whereas state T has high affinity for the inhibitor. Assuming an exclusive ligand binding (i.e. $c = K_R/K_T = 0$) to one of these two states, the saturation function: $\overline{Y}_s = v_0/V$ for the substrate may be determined by the equation:

$$\overline{Y}_s = \frac{(1 + \alpha)^{n-1}}{L' + (1 + \alpha)^n} \quad (2)$$

where

$$L' = L(1 + \beta)^n/(1 + \gamma)^n , \qquad \alpha = [S]/K_{R(S)} , \qquad \beta = [I]/K_{T(I)}$$

and

$$\gamma = [A]/K_{R(A)} .$$

Equation (2) may be rearranged to a linear form [18]:

$$\log(\alpha/\overline{Y}_s - \alpha - 1) = \log L' - (n - 1) \log(1 + \alpha) \quad (3)$$

from which it can be seen that the plot of $\log(\alpha/\overline{Y}_s - \alpha - 1)$ vs. $\log(1 + \alpha)$ gives a straight line with a slope $-(n - 1)$, intercepting the abscissa at $\log L'/(n - 1)$ and the ordinate at $\log L'$.

*Reagents.* AMP, ATP, ADP and GTP ($Na^+$ salts) were purchased from Sigma Chemicals (St. Louis, MO, U.S.A.); cellulose phosphate was from Whatman (Maidstone, England). All other chemicals were from Polskie Odcynniki Chemizcne (Gliwice, Poland).

## Results

In the presence of 100 mM KCl in the incubation medium, when no ATP, ADP or GTP was added, the kinetic curve showed a sigmoid-shaped profile (Fig. 1), which indicated that cooperative interaction was promoted by AMP. The values of the cooperativity coefficient ($n_H$) and the substrate concentration

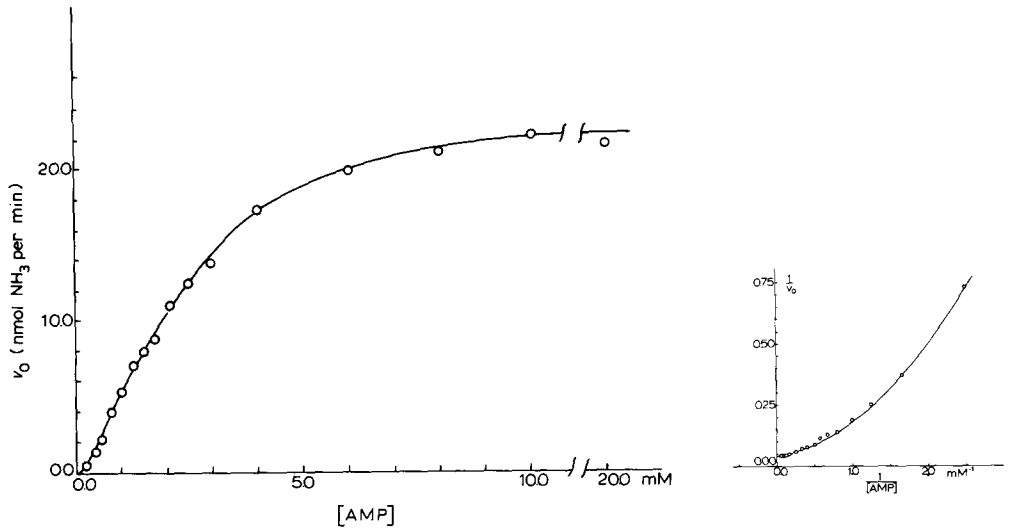


Fig. 1. Effect of AMP concentration on the initial velocity of the reaction catalysed by rat heart muscle AMP deaminase. The reaction mixture contained 0.1 M succinate-KOH buffer, 100 mM KCl, pH 6.5. Inset: double reciprocal plot of the data presented on Fig. 1. $v_0$ is the velocity of the reaction with no effector [ATP, ADP or GTP] added.

required to reach half-maximum velocity ($K_{0.5}$), calculated from the Hill equation, were 1.30 (S.D. = ±0.05) and 3.04 mM (S.D. = ±0.28), respectively.

When ATP or ADP was present in the reaction medium, the sigmoid-shaped curve of the Michaelis plot was transformed to hyperbolic, as concluded from the straight-line dependence observed on Lineweaver-Burk plots (Fig. 2a and b). Table I presents the calculated values of $K_{0.5}$ parameter for the reaction catalysed by rat heart AMP deaminase at several different concentrations of these activators. Both ATP and ADP activated the enzyme, influencing its cooperative response with respect to the substrate. As may be seen from Fig. 2a, 0.05 mM ATP was sufficient to change the kinetic curve of the enzyme reaction from a sigmoidal to a hyperbolic form. The cooperativity coefficient decreased from 1.3 to 1.08 causing, in consequence, a decrease of $K_{0.5}$ to about 1 mM (Table I). When the concentration of ATP was raised to 0.25 mM, $K_{0.5}$ reached about 0.6 mM and did not change with further increase of concentration of ATP. The calculated values of the Hill coefficient were 1.03 at 0.125 mM and 0.96 at 0.5 mM ATP concentration.

When the same concentration (i.e. 0.5 mM) of ADP was used, the kinetic curve of AMP deamination by rat heart enzyme was also hyperbolic (Fig. 2b), but the activation effect was much lower than previously observed in the presence of ATP (Table I). The $K_{0.5}$ value of 1.2 did not change with further increase of ADP concentration (Table I), and the cooperativity coefficient was unchanged, being about 1 at all ADP concentrations tested.

In the presence of GTP, the reaction catalysed by rat heart AMP deaminase exhibited unexpected kinetics. In the presence of 0.05 mM GTP, a double reciprocal plot showed a straight-line relationship (Fig. 3), which signifies a hyperbolic response with respect to adenylic acid. However, this response was

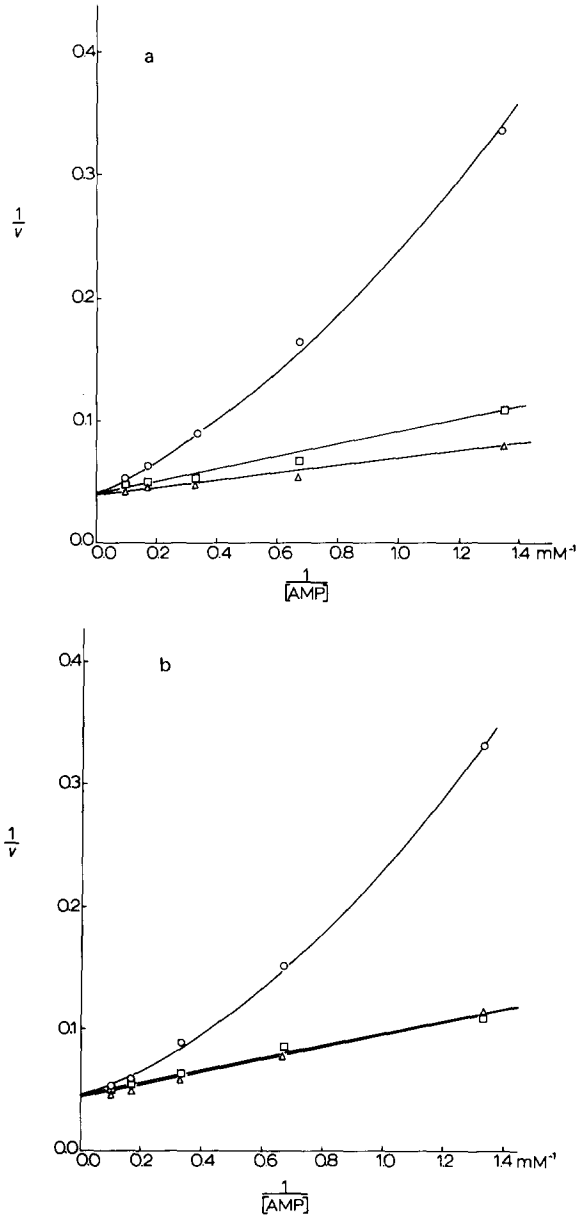


Fig. 2. Influence of AMP concentration on the rate of AMP deamination in the absence ○———○ and a, in the presence of 0.05 mM □———□ and 0.5 mM △———△ ATP or b, in the presence of 0.05 mM □—·—□ and 1.5 mM △———△ ADP.

not observed when 0.5 mM GTP was added to the incubation medium; the plot showed little curvature under these conditions. The values of $n_H$ calculated from the Hill equation were 1.13 at 0.5 mM GTP, 1.06 at 0.125 mM GTP and approx. 1 at lower concentration of this nucleotide.

Another interesting feature seen from Fig. 3 is the lower rate of AMP

TABLE I

EFFECT OF THE CONCENTRATION OF ATP, ADP AND GTP ON $K_{0.5}$ AND $V$ VALUES FOR THE REACTION CATALYSED BY RAT HEART ADENYLATE DEAMINASE

The values in the brackets represent S.E. or *S.D.

| Effectors added (mM) | $K_{0.5}$ (mM) | $V$ (nmol/min) |
|---|---|---|
| ATP | | |
| 0.05 | 1.00 (0.18) | 23.62 (1.12) |
| 0.125 | 0.71 (0.04) | 22.90 (0.30) |
| 0.25 | 0.64 (0.03) | 24.23 (0.27) |
| 0.50 | 0.61 (0.11) | 24.67 (0.95) |
| ADP | | |
| 0.50 | 1.24 (0.02) | 23.76 (0.09) |
| 0.75 | 1.21 (0.08) | 22.74 (0.42) |
| 1.00 | 1.18 (0.08) | 21.69 (0.40) |
| 1.50 | 1.12 (0.14) | 21.71 (0.75) |
| GTP | | |
| 0.05 | 2.63 (0.11) | 23.19 (0.44) |
| 0.125 | 2.14 (0.26) | 21.90 (0.93) |
| 0.25 | 2.11 (0.31) | 21.74 (1.13) |
| 0.50 | 2.73 (0.32) | 22.53 (1.09) |
| None | | |
| — | 3.04 *(0.28) | 22.67 *(1.54) |

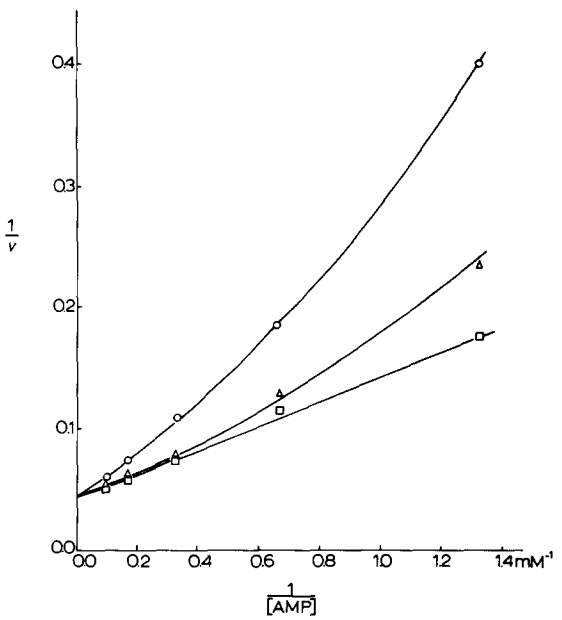


Fig. 3. Influence of AMP concentration on the rate of AMP deamination in the absence ○———○ and in the presence of 0.05 mM □———□ and 0.5 mM △———△ GTP.

deamination (especially at low substrate concentration) at 0.5 mM GTP than at 0.05 mM GTP. The constant $K_{0.5}$ (2.6 mM at 0.05 mM GTP) decreased to 2.1 mM at 0.125 mM GTP and did not change when the concentration of this effector was raised to 0.25 mM. However a further increase of GTP concentration caused an increase of $K_{0.5}$ to 2.7 mM, i.e. to a value somewhat lower than that in the absence of nucleotides (Table I). When the kinetic data obtained for the reaction catalysed by rat heart AMP deaminase in the presence of GTP were presented in the form of a Cornish-Bowden plot [19], a set of parallel lines for different concentrations of the substrate was obtained (Fig. 4). The same data, if presented in the form of a Dixon plot, gave a set of lines intersecting above the abscissa. These data are representative of competitive inhibition affected by GTP. The value of inhibition constant ($K_i$) calculated from the Dixon plot was 0.72 mM (S.D. = ±0.09).

It is of interest that the maximum velocity of the reaction ($V$) was practically the same in the presence of various nucleotide concentrations (Table I).

The dependences of the increase of the enzyme activity on ATP and ADP concentrations, at several concentrations of the substrate, are presented in Figs. 5 and 6. A set of hyperbolic curves was obtained when the increase in enzyme activity was plotted against ATP and ADP concentrations. This is in contrast with arc-shaped curves obtained for different GTP concentrations (Fig. 7). It may also be seen that the activation by both ATP and ADP was dependent on substrate concentration and was greater at low concentrations of AMP (Figs. 5 and 6). The values of $A_{0.5}$ (concentration of activator required to produce 50% activation) and $V$ (the maximal activated velocity of the reaction obtainable at a fixed substrate concentration) calculated for the dependences represented by Figs. 5 and 6 are shown in Table II. It may be concluded from these data that the values of $A_{0.5}$ did not change significantly with the substrate concentration for either nucleotide and were about 4 times higher for

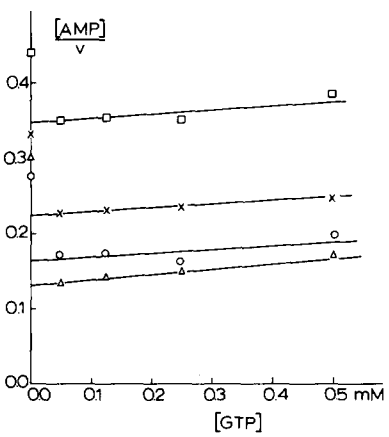


Fig. 4. Cornish-Bowden plots for the reaction catalysed by rat heart AMP deaminase in the presence of GTP at four different AMP concentrations: △———△, 0.75 mM; ○———○, 1.5 mM; X———X, 3.0 mM and □———□, 6.0 mM. The straight lines were fitted by the method of linear regression.

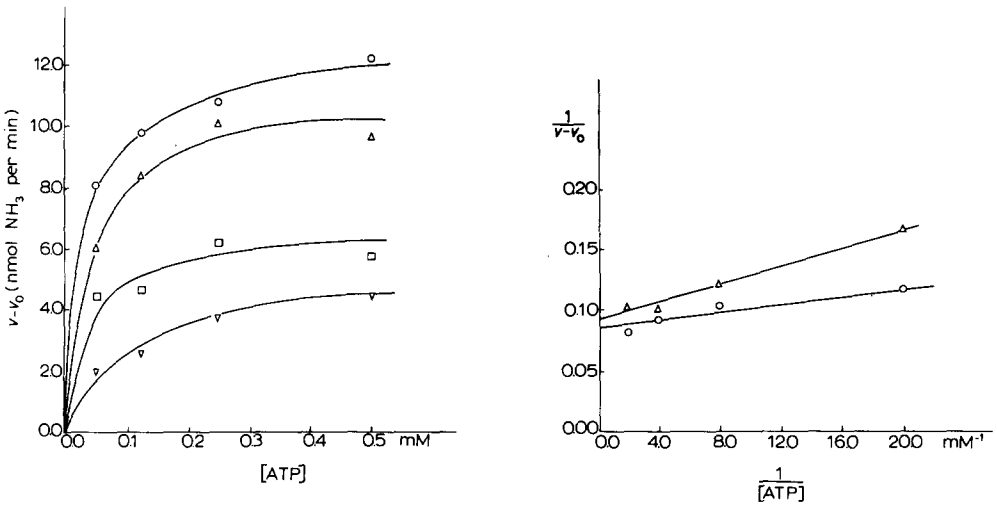


Fig. 5. a, The effect of AMP upon the activity of AMP deaminase as a function of ATP concentration for four different substrate concentrations: △———△, 0.75 mM; ○———○, 1.5 mM; □———□, 6.0 mM and ▽———▽, 10.0 mM. b, The double reciprocal plots of the data presented in a for 0.75 mM △———△ and 1.5 mM ○———○ AMP. $v$ represents the velocity of the reaction in the presence of ATP and $v_0$ is the velocity with no effector added.

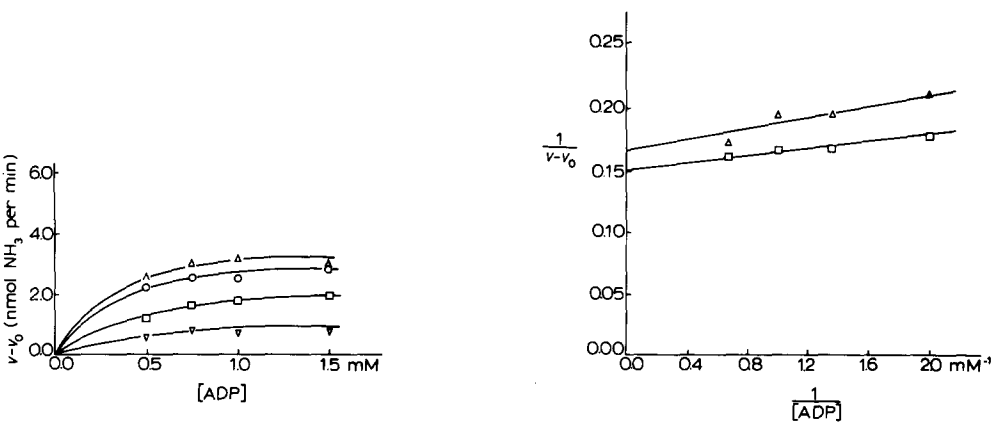


Fig. 6. a, The effect of AMP upon the activity of AMP deaminase as a function of ADP concentration for four different substrate concentrations: △———△, 0.75 mM; ○———○, 1.5 mM; □———□, 6.0 mM and ▽———▽, 10.0 mM. b, Double reciprocal plots of the data presented in a for 0.75 mM △———△ and 1.5 mM □———□ AMP. $v$ represents the velocity of the reaction in the presence of ADP and $v_0$ is the velocity with no effector added.

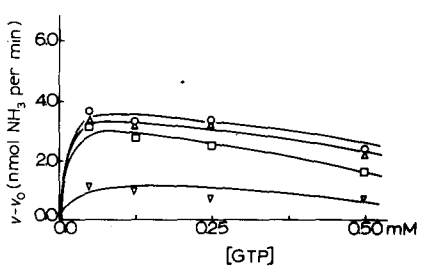


Fig. 7. The effect of AMP upon the activity of AMP deaminase as a function of GTP concentration at four different substrate concentrations: △———△, 0.75 mM; ○———○, 1.5 mM; □———□, 6.0 mM and ▽———▽ 10.0 mM. $v$ represents the velocity with GTP and $v_0$ is the velocity with no effector added.

TABLE III

RELATIVE VELOCITIES OF THE REACTION CATALYSED BY RAT HEART AMP DEAMINASE IN THE PRESENCE OF DIFFERENT CONCENTRATIONS OF ATP AND GTP. ADENYLATE CONCENTRATION WAS 1.5 mM. $v_0$ IS THE REACTION RATE IN THE ABSENCE OF BOTH ATP AND GTP

| ATP (mM) | $v/v_0$ | GTP (mM) | $v/v_0$ |
|---|---|---|---|
| | | 0.0125 | 1.4 |
| | | 0.025 | 1.6 |
| 0.05 | 2.4 | 0.05 | 1.6 |
| 0.125 | 2.6 | 0.125 | 1.7 |
| 0.25 | 2.8 | 0.25 | 1.7 |
| 0.50 | 3.0 | 0.50 | 1.4 |
| 6.25 | 2.9 | 6.25 | 1.1 |

TABLE II

EFFECT OF SUBSTRATE CONCENTRATION ON $A_{0.5}$ (THE VALUE OF ACTIVATOR CONCENTRATION REQUIRED TO PRODUCE 50% ACTIVATION), $V$ (THE VALUE OF MAXIMAL ACTIVATED VELOCITY OF THE REACTION OBTAINABLE AT A FIXED SUBSTRATE CONCENTRATION) AND $v$ (THE VALUE OF EXPERIMENTAL INITIAL VELOCITY OF THE REACTION AT THE GIVEN SUBSTRATE CONCENTRATION AND 0.5 mM ATP OR 1.5 mM ADP)

The values in the brackets represent S.E. or *S.D.

| AMP (mM) | ATP | | | ADP | | |
|---|---|---|---|---|---|---|
| | $A_{0.5}$ (mM) | $V$ (nmol/min) | $v$ (nmol/min) | $A_{0.5}$ (mM) | $V$ (nmol/min) | $v$ (nmol/min) |
| 0.75 | 0.037 (0.014) | 12.68 (0.82) | 12.68 *(0.12) | 0.095 (0.050) | 8.96 (0.37) | 9.15 *(0.34) |
| 1.50 | 0.026 (0.008) | 18.38 (0.72) | 18.40 *(0.32) | 0.109 (0.060) | 12.44 (1.06) | 11.59 *(0.37) |
| 3.00 | 0.026 (0.013) | 21.42 (0.96) | 21.38 *(0.24) | 0.133 (0.070) | 17.08 (0.44) | 16.20 *(0.59) |

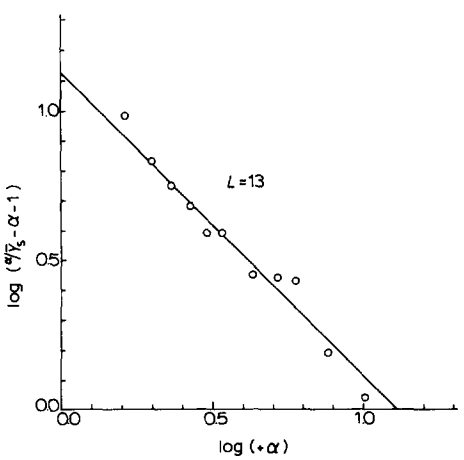


Fig. 8. Linearization of the simplified saturation function. The data of Fig. 1 were plotted according to Eqn. 3. The straight line was fitted by the method of linear regression.

ADP than for ATP. $V$ increased substantially with AMP concentration and, interestingly, corresponded to the values of the velocity of the reaction ($V$) obtained from experiments in the presence of 0.5 mM ATP or 1.5 mM ADP (Table II).

Table III presents the values of relative velocity of the reaction catalysed by rat heart muscle AMP deaminase in the presence ($v$) and in the absence ($v_0$) of ATP or GTP at 1.5 mM concentration of the substrate and different concentrations of the effectors. The extent of activation did not change when the ATP concentration was raised from 0.25 to 6.25 mM. Over the same concentration range, the activating effect of GTP diminished substantially.

Fig. 8 is a graphical illustration of the dependence described by Eqn. 3. From the slope of the line (—1) and from its intercept with the ordinate, the value of $n \approx 2$, describing the number of protomers, and the value of allosteric constant $L \approx 13$ were obtained.

## Discussion

The hyperbolic saturation curve obtained in the presence of 0.5 mM ATP (Fig. 2a) suggests that at this concentration of the activator the equilibrium of the allosteric transition is completely shifted towards the R conformation. Taking into account the fact that maximally activated velocities of the reaction at a fixed substrate concentration ($V$) do not differ from initial velocities ($v$) obtained from experiments (Table II), as well as the lack of stronger activation of the enzyme in the presence of high concentrations of ATP (Table III) we can assume that $c = K_R/K_T = 0$, i.e. that an exclusive ligand binding to the state R of the enzyme protein is taking place [20].

On the other hand Table I shows that the calculated values of maximum velocities of the enzyme reaction ($V$) are practically unaffected by all nucleotides tested, which suggests the validity of the assumption of a perfect 'K-system' for the enzyme kinetics.

The relatively low value of the allosteric constant $L$, which was calculated from the data obtained in the absence of nucleotides, indicates that although the majority of the enzyme molecules are not accessible to the substrate in the T conformation, nevertheless the number of enzyme molecules in the R conformation must also be significant in the absence of effector nucleotides. This fact may also explain the weak sigmoidicity of the curve of Michaelis depencence of Fig. 1. From the value —1 of the slope of the Horn-Börning plot one may also conclude that the enzyme exists in a dimeric form in these conditions.

As may be seen from Figs. 3 and 7, AMP deaminase activity was influenced differently by GTP than by ATP and ADP. The rate of AMP deamination is increased at all concentrations of GTP, but the activation effect falls with the rise of this nucleotide concentration from 0.05 to 0.5 mM (Fig. 7). This phenomenon of GTP action may be caused by the fact that at higher concentration GTP may act as an active-site-directed inhibitor of the enzyme [21]. The weak competition between GTP and AMP (Fig. 4) may be a possible explanation for the rise in $K_{0.5}$ and $n_H$ values found at 0.5 mM GTP and which was not coupled with the change in $V$ value (Table I). This behaviour with GTP is very interesting in view of the fact that it takes place in the physiological concentration

range of the nucleotide. At very high concentrations of GTP (6 mM), activation did not occur and the relative velocity ($v/v_0$) of the reaction at 1.5 mM AMP was about 1 (Table III). These values agree with the experiments of Burger and Lowenstein [10], performed by using the so-called 'excluded protein fraction' of rat heart, which specifically deaminates adenosine monophosphate.

Unlike Chung and Bridger [11], we have not found ADP to be a very strong activator of the enzyme activity; the effect exerted by ATP was much stronger.

Deamination of adenine compounds in the heart is known to play an important role in the regulation of heart metabolism. Adenosine monophosphate deaminase, one of the enzymes utilising the pool of adenine compounds, takes part in the extremely subtle interplay of enzyme systems producing and degrading adenine nucleotides [22]. The regulatory properties of heart muscle AMP deaminase may be important for a better understanding of heart physiology.

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