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ROLE OF CATIONS IN THE REGULATION OF BAKER'S YEAST AMP DEAMINASE

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Summary

The effect of polyamines and divalent cations including alkaline earth metals and transition metals on the AMP deaminase (AMP aminohydrolase EC 3.5.4.6) purified from baker's yeast was investigated. (1) Polyamines and alkaline earth metals activated the enzyme in the absence of ATP: these cations largely enhanced the maximal velocity without alteration of $S_{0.5}$ and n_H (Hill coefficient) values. However, transition metals acted as potent inhibitors, which decreased the maximal velocity of the enzyme in the absence of ATP. (2) All of the divalent cations showed an activation of the enzyme in the presence of ATP, followed by a progressive decrease in activity as the concentrations of transition metals increased. (3) The increase in the concentrations of polyamines or alkaline earth metals showed no more activating effect when the enzyme was fully activated by the addition of excess alkali metals in the absence of ATP, but divalent cation-activation was observed in the presence of ATP even if alkali metals were saturating.

These results suggest the presence of two types of binding sites for cations: 1, the sites for free cations and 2, those for ATP-metal complexes. The former sites include the activating sites for alkali metals, polyamines and free alkaline earth metals, and the inhibitory sites for free transition metals. The latter sites are the activating sites for ATP-metal complexes, which are suggested to be commonly occupied by alkaline earth metals and transition metals and to form an ATP bridge (E-ATP-M) complex.

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Introduction

Various cations participate in biological processes in a variety of ways. One of the important functions of these cations is as activators or inhibitors of enzymes [1,2]. We have studied the regulatory properties of AMP deaminase (AMP aminohydrolase EC 3.5.4.6) as well as AMP nucleosidase (AMP phosphoribohydrolase EC 3.2.2.4): the properties of these two enzymes are similar to each other with respect to the effects of monovalent cations, polyamines, ATP, P_i and adenylate energy charge [3—7]. The previous paper described the kinetic analysis of the effects of divalent cations on AMP nucleosidase from *A. vinelandii*, and the similarity in the properties of AMP nucleosidase and AMP deaminase was discussed [8]. However, the effects of these cations on yeast AMP deaminase were not analyzed in detail. In the present investigation, we studied the effects of alkaline earth metals and transition metals on baker's yeast AMP deaminase. Free alkaline earth metals and transition metals acted as activators and inhibitors of the enzyme, respectively, whereas ATP-metal complexes showed an activating effect of the enzyme. Possible coordination schemes for AMP deaminase-metal complexes are also presented.

Materials and Methods

Materials. AMP and ATP were products of Yamasa Co. (Tokyo, Japan). Polyamines were purchased from Sigma. Other chemicals were reagent grade.

Enzyme and assay. AMP deaminase was purified from commercial baker's yeast by the method of Yoshino et al. [5]. The enzyme activity was measured by the formation of ammonia [5].

Results

Studies of baker's yeast AMP deaminase have shown that an allosteric interaction is involved [5,9]. When velocities were determined in the absence of ATP with varying levels of AMP, a sigmoid saturation curve typical of an allosteric enzyme was obtained [5]. All polyamines and alkaline earth metals activated the enzyme to various extents, whereas transition metals such as Mn^{2+} and Zn^{2+} showed a potent inhibitory effect with a decrease in V (Fig. 1). The effect of polyamines and divalent cations as well as monovalent cations [5] was largely on the maximal velocity of the enzyme: the increase in the concentration of these cations did not affect $S_{0.5}$ values, the concentration of AMP required for half-maximal velocity which remained at 3.7 mM. Hill interaction coefficients were calculated as approx. 2 in the absence and presence of these cations. Theoretical saturation curves were computed in the absence and presence of these cations using V, apparent K_m and n_H values according to the following equation, and the best-fit curves were obtained (Fig. 1).

$$v = \frac{V \cdot [S]^n}{[S]^n + K_m^n} \quad (1)$$

where [S] is the concentration of AMP, K_m the concentration required for half-maximal activity and n the Hill coefficient.

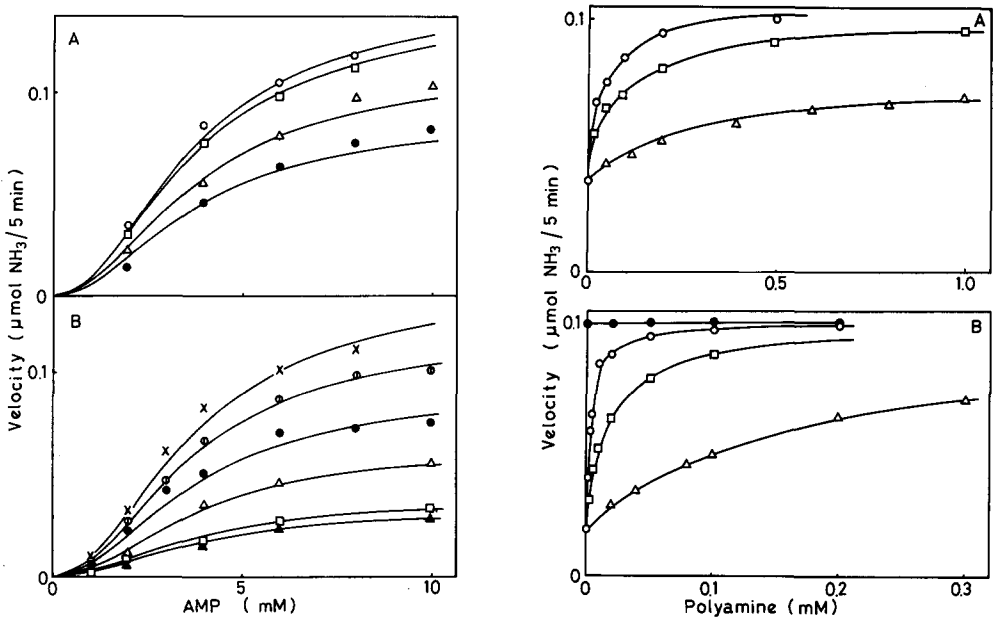


Fig. 1. Rate of the reaction catalyzed by AMP deaminase as a function of substrate concentration in the absence and presence of polyamine (A) or divalent cations (B). The reaction mixture contained 10 mM cacodylate buffer (pH 7.1), 30 mM Na^+, various concentrations of AMP, polyamines (A) or divalent metals (B), and the enzyme in a final volume of 1 ml. The reaction was carried out at 37°C for 5 min. Points are experimental data, and lines are theoretically drawn from Eqn. 1, using the n value of 2.0 and the apparent K_m of 3.7 mM. Each metal cation was added as chloride salt. A: ●, no addition; ○, 1 mM spermine; □, 1 mM spermidine; △, 1 mM putrescine. B: ●, no addition; X, 2 mM Ca^{2+}; ⊕, 2 mM Mg^{2+}; □, 2 mM Mn^{2+}; △, 5 μM Zn^{2+}; ▲, 7.5 μM Zn^{2+}.

Fig. 2. Effect of polyamine concentrations on AMP deaminase in the absence (A) and presence of ATP (B). (A) The assay mixture was similar to that in Fig. 1 except that AMP was held constant at 10 mM, and that the concentration of polyamines was varied. (B) The assay mixture was similar to that in (A) except that AMP was held constant at 0.5 mM, and that 1 mM ATP was included. ○, spermine; □, spermidine; △, putrescine; ●, spermine in the presence of 0.1 M KCl.

The effect of increasing concentrations of polyamines on the enzyme activity was examined in the absence and presence of ATP. Fig. 2 indicated a powerful activating effect of polyamines, particularly spermine and spermidine, on the enzyme activity: the $A_{0.5}$ values, the concentrations necessary for 50% activation of the enzyme activity, for spermidine were 25 and 3.5 μM in the absence and presence of ATP, respectively.

The effect of increasing concentrations of alkaline earth metals was also investigated in the absence of ATP. All of the free alkaline earth metals were activators of the enzyme in the absence of ATP, although the effect was less than that with the alkali metals (Fig. 3A).

Polyamines, alkali metals and free alkaline earth metals seem to affect the enzyme in similar fashion: these ligands enhance the V of the enzyme. Furthermore, the increase in the concentrations of these ligands showed no more activating effect when the enzyme was fully activated by the addition of 0.1 M KCl (Figs. 2B and 3A): in contrast, no more activation of the enzyme was observed by the addition of K^+ in the presence of 0.1 mM spermine (data not

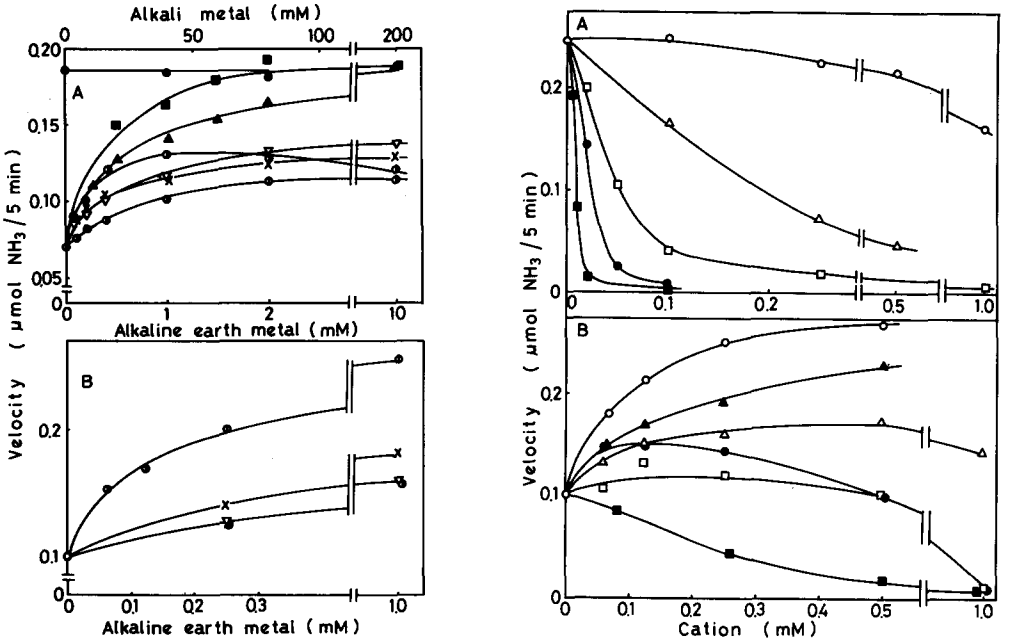


Fig. 3. Effect of alkaline earth metals and monovalent cations on AMP deaminase in the absence (A) and presence of ATP (B). (A) The reaction mixture was similar to that in Fig. 2A except that alkaline earth metals and alkali metals were substituted for polyamines. (B) Enzyme activity was determined at 10 mM cacodylate buffer (pH 7.1), 0.1 mM AMP, 1 mM ATP, 50 mM KCl, the indicated concentration of alkaline earth metals, and the enzyme in a final volume of 4 ml. Each metal cation was added as chloride salt. ⊕, Mg^{2+}; ●, Ba^{2+}; X, Ca^{2+}; ▽, Sr^{2+}; ■, K^+; ▲, Na^+; ●, Ca^{2+} in the presence of 0.1 M KCl.

Fig. 4. Effect of transition metals on AMP deaminase in the absence (A) and presence of ATP (B). (A) The reaction mixture was similar to that in the legend to Fig. 2A except that transition metals were substituted for polyamines. (B) The reaction mixture was similar to that in Fig. 3B except that transition metals were substituted for alkaline earth metals. Each metal cation was added as chloride salt. ○, Mn^{2+}; △, Co^{2+}; □, Ni^{2+}; ●, Cd^{2+}; ■, Zn^{2+}; ▲, Fe^{2+}.

shown). These results suggest that polyamines, alkali metals and free alkaline earth metals can interact with the enzyme at the identical sites.

When ATP was added, Mg^{2+} showed a more potent activating effect than the other alkaline earth metals did (Fig. 3B). It should be noted that these activating effects were observed in the presence of 50 mM K^+.

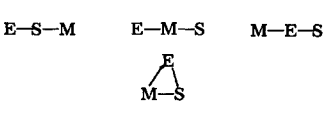
Free transition metals exhibited an inhibitory effect on the enzyme in the absence of ATP: Zn^{2+} was the most effective, followed by Cd^{2+}, Ni^{2+}, Co^{2+} and Mn^{2+} in that order (Fig. 4A). If ATP was included, a potent activation of the enzyme was followed by a progressive decrease in activity as the concentration of transition metals increased (Fig. 4B), suggesting that ATP-transition metal complexes as well as ATP-alkaline earth metal complexes can act as activators of the enzyme, whereas free transition metals are inhibitors. It is noteworthy that the activating effect of ATP-transition metal complexes as well as ATP-alkaline earth metal complexes is observed in the presence of excess alkali metal. Zn^{2+} showed only an inhibitory effect in the absence and presence of ATP. The mechanism was not examined in detail; however, a small portion of Zn^{2+} remained as a free metal ion, although a large portion of Zn^{2+} exists as ATP-metal complexes in the presence of ATP. Zn^{2+} is considered to have lower

K_i and relatively higher K_a values for its free metal ion and for ATP-metal complex form, respectively, although other transition metals may have lower K_a values for its ATP-metal complexes. This assumption can account for only an apparent inhibition of the enzyme by Zn^{2+} in the presence of ATP.

Discussion

Extensive studies on the kinetic properties of AMP deaminase isolated from various animal tissues have revealed that the enzyme can be activated by ATP and monovalent cations and inhibited by P_i [10]. Some divalent cations display unique regulatory effects on AMP deaminase: Zn^{2+} acts as a potent inhibitor [11], which might regulate the purine nucleotide cycle, whereas alkaline earth metals activate the yeast AMP deaminase [9]. However, studies on the specificity and kinetics of the action of divalent cations have not been performed, and it remains obscure as to whether or not divalent and monovalent cations interact with the enzyme at the identical sites. As shown in this paper, polyamines and free alkaline earth metals activated AMP deaminase by enhancement of the maximal velocity without alteration of K_m for AMP, while free transition metals inhibited the enzyme noncompetitively. Furthermore, no more activation of the enzyme by the increase in alkaline earth metals and polyamines was observed when the enzyme was fully activated by excess alkali metals in the absence of ATP (see, Figs. 2 and 3). These results suggest that polyamines, alkali metals and free alkaline earth metals can interact with the enzyme at identical sites. The inhibitory sites for free transition metals are also distinguishable from the substrate-binding sites and the sites for free alkaline earth metals or alkali metals because of the noncompetitive nature of transition metal-inhibition and of incomplete reversal by the addition of alkali metals or alkaline earth metals of the inhibition. On the other hand, all of the alkaline earth metals and transition metals except Zn^{2+} activate the enzyme in the presence of ATP even if the alkali metals are saturating, suggesting that these metal-ATP complexes can bind to the specific sites of the enzyme. These sites are clearly different from the sites for free alkaline earths.

Metal-activated enzymes require the addition of metal ions for activity and are distinguishable from metalloenzymes which retain stoichiometric, tightly bound, functional metal ions upon purification [1]. For metal-activated enzymes, which form 1 : 1 : 1 complexes of enzyme, metal, and substrate, four coordination schemes are presented [1]:



These are substrate bridge complex, the metal bridge complexes (simple and cyclic), and the enzyme bridge complex. The activating effect of alkaline earth metals, particularly Ca^{2+} in the presence of ATP excludes the possibility of formation of metal bridge complex with AMP deaminase: Ca^{2+}, which forms an eclipsed pyrophosphate chelate complex, is an activator of E-S-M enzymes but a potent inhibitor of most E-M-S enzymes [1]. It is obvious that one group of

cations (M_1) such as alkali metals, alkaline earth metals and polyamines and the other group transition metals (M_2) bind to the activating and inhibitory sites of the enzyme in the absence of ATP, respectively. Two enzyme bridge complexes are formed under these conditions: M_1-E-S and M_2-E-S. When ATP was included, all of the alkaline earth metals and transition metals activated the enzyme even in the presence of K^+, suggesting the formation of an E-ATP-M complex.

On the basis of the results presented here, reasonable coordination schemes for AMP deaminase are as shown:

(I) ATP absent

$$\begin{array}{c} M_1 \\ | \\ M_2\text{—E—AMP} \end{array}$$

(II) ATP present

$$\begin{array}{c} M_1 \\ | \\ M_2\text{—E—AMP} \\ | \\ M_2\text{—ATP} \\ (M_1) \end{array}$$

where M_1 designates alkali metals, alkaline earth metals or polyamines, and M_2 is transition metals. Alkaline earth metals can be substituted for transition metals in the formation of an ATP-metal complex (Case II). The kinetic properties of yeast AMP deaminase can, thus, be accounted for by the assumption that the enzyme has two types of binding site for these cations: 1, the sites for free metal cations and 2, those for AMP-metal complexes. The former sites include the activating sites for free alkaline earth metals, alkali metals or polyamines, and the inhibitory sites for free transition metals. The latter sites are the activating sites for ATP-metal complexes, which are suggested to be commonly occupied by alkaline earth metals and transition metals and to form ATP-bridge (E-ATP-M) complex.

As noted from the tables of metal-activated enzymes compiled by Mildvan [1], most, but not all, kinase reactions form enzyme-nucleotide-metal (E-S-M) complexes, except for kinases and other phosphotransferases which utilize pyruvate or phospho*enol*pyruvate [1]. These and other enzymes which catalyze reactions of phospho*enol*pyruvate form enzyme-metal-substrate bridge (E-M-S) complexes as for carboxylating enzymes. The role for divalent metals in the reaction of E-M-S and E-S-M complexes has been extensively investigated. On the other hand, the role of metals in M-E-S complexes is presumed to be structural, to stabilize a catalytically active conformation, as has been suggested for monovalent cations [12]. The role of alkaline earth metals, alkali metals and polyamines in the AMP deaminase reaction appears to stabilize an allosterically active conformation of the enzyme. A complex of similar type M-E-S was observed in glutamine synthetase (EC 6.3.1.2) [13,14]. A purely
M
structural role for divalent cations in glutamine synthetase from *E. coli* is presented: preincubation with Ca^{2+}, Mg^{2+}, or Mn^{2+} but not Co^{2+}, slowly reactivates the inactivated enzyme which has been treated by ethylenediaminetetraacetic acid, whereas Mg^{2+}, Mn^{2+}, or Co^{2+} but not Ca^{2+}, are necessary for maximal activity in the enzyme assay. Kinetic and some physico-chemical studies have presented the above coordination scheme for glutamine synthetase. This

paper clearly demonstrates two probable coordination schemes for AMP deaminase, and kinetic analyses have shown that free metal binding sites of the enzyme can be discriminated from the ATP-metal binding sites. However, the kinetic techniques fail to provide an unequivocal diagnosis of the coordination scheme [1]: confirmatory evidence must await further examination of physicochemical techniques including binding studies, electron paramagnetic resonance or NMR studies.

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