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ION-DEPENDENT ACTIVATION OF AMP NUCLEOSIDASE FROM *AZOTOBACTER VINELANDII*

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Summary

The effect of divalent cations on the purified AMP nucleosidase (AMP phosphoribohydrolase, EC 3.2.2.4) from *Azotobacter vinelandii* was investigated. All alkaline earth metal-ATP complexes were essential activators of the enzyme, and free alkaline earths also activated the enzyme in an allosteric manner: the apparent K_a for ATP and n_H values (Hill interaction coefficient) decreased from 0.45 to 0.05 mM, and from 4 to 2, respectively, with increase in Mg^{2+} concentration. Transition metal-ATP complex also activated AMP nucleosidase, but a potent activation of the enzyme was followed by a progressive decrease in activity as the concentrations of transition metals increased. The enzyme fully activated in the presence of Mg^{2+} was inhibited by the higher concentrations of transition metals with the identical $I_{0.5}$ values when Mg^{2+} was present. These results suggest the presence of two classes of binding sites for divalent cations. One is the activating site for ATP-metal complex, which is suggested to be commonly occupied by alkaline earths and transition metals. The other sites are those for free metal binding, the sites for free alkaline earths and free transition metals are activating and inhibitory sites, respectively.

Introduction

AMP nucleosidase (AMP phosphoribohydrolase, EC 3.2.2.4), which is important to stabilize the adenylate energy charge [1] and in the conversion of adenine nucleotide to inosine or guanine nucleotide in *Azotobacter vinelandii* [2,3], had catalytic, regulatory and structural properties which are strikingly

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altered by several physiological ligands. The enzyme can be activated by ATP and certain cations, and inhibited by P_i and some nucleoside monophosphates [2,3]. Although activation by monovalent cations [4] or polyamines [5] has been studied in detail, much less is known about the mechanism and specificity of the activation by divalent cation. The present communication describes the effect of divalent cations on some of kinetic properties of AMP nucleosidase from *A. vinelandii*, alkaline earth metals and transition metals acted as potent activators of the enzyme. The effect of divalent and other cations including monovalent cations and polyamines as reported previously suggests that AMP nucleosidase had a broad specificity toward the activating cation-sites.

Materials and Methods

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

Enzyme and assay. AMP nucleosidase was purified from *A. vinelandii* by the method originally described by Yoshino [2] with a slight modification [4]. The enzyme activity was measured by the formation of adenine [2] or reducing sugar liberated [4].

Results

Fig. 1 shows the activation curves with respect to divalent cations including alkaline earth metals and transition metals in the presence of saturating concentration of ATP (2.5 mM). All divalent cations acted as activators of AMP nucleosidase to varying degrees. Since free metal concentration was almost negligible under the conditions where ATP is saturating, activating curves of

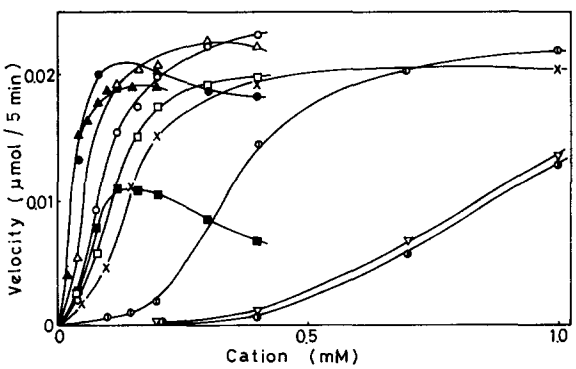

Fig. 1. Effect of concentrations of alkaline earth metals and transition metals on the activity of AMP nucleosidase from *A. vinelandii*. The reaction mixture contained 1 mM AMP, 2.5 mM ATP, 5 mM Tris-HCl buffer (pH 8.0), various concentrations of divalent cations and the enzyme in a final volume of 0.2 ml. The reaction was carried out at 37°C for 5 min and terminated by the addition of the reducing sugar reagent. Velocity is expressed as μmol reducing sugar formed under the assay conditions. ⊕, Mg^{2+}; X, Ca^{2+}; ▽, Sr^{2+}; ◑, Ba^{2+}; ○, Mn^{2+}; △, Co^{2+}; □, Ni^{2+}; ●, Cd^{2+}; ▲, Pb^{2+}; ■, Zn^{2+}.

Fig. 1 represent the effect of ATP-metal complex. The effect of alkaline earth metals was less than that observed with transition metals, the concentrations necessary for 50% activation of the enzyme activity, $A_{0.5}$ values for alkaline earth metals were relatively higher as compared with those for transition metals. Some transition metals showed a tendency to inhibit the enzyme at higher concentration, whereas none of alkaline earth metals exhibit any inhibitory effect. The maximal velocities in the presence of various metals were essentially identical except that yielded with Zn^{2+}, which is considered to have relatively lower K_i values, exhibiting apparently lower maximal velocity.

In order to examine the mechanism of activation by various metals, we studied the affinity of AMP nucleosidase for ATP in the presence of various alkaline earth metals (Fig. 2). Ca^{2+} was the most effective, followed by Mg^{2+}, Sr^{2+} and Ba^{2+} in that order. The addition of these cations decreases the apparent K_a values for ATP. We further examined the effect of free alkaline earth metals on the enzyme activity. Fig. 3 shows the rate-concentration curves with respect to ATP in the presence of various concentrations of Mg^{2+}. The rate-concentration plot was strongly sigmoidal under the conditions where Mg^{2+} added can exist as MgATP complex and free Mg^{2+} concentration was negligible when equimolar concentrations of $MgCl_2$ and ATP were used (Curve A in Fig. 3). Free and complex Mg^{2+} concentrations were calculated using the stability constant of 73 000 M^{-1} for MgATP. With increasing concentration of free Mg^{2+}, the apparent K_a values for ATP decreased from 0.45 to 0.05 mM with the decrease in an n_H value of 4 to 2. These results suggest that

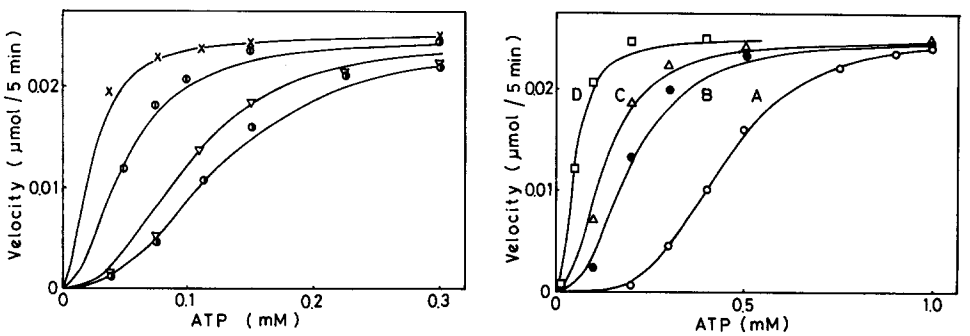

Fig. 2. Effect of ATP concentrations on the activity of AMP nucleosidase in the presence of various alkaline earth metals. Enzyme activity was determined at the varied concentrations of ATP, 5 mM Tris-HCl buffer (pH 8.0) and 1 mM AMP in the presence of 2.5 mM alkaline earth metals as chloride salts. Points are experimental data, and lines are theoretically drawn from Eqn. 1, using the following values of apparent K_a and n_H. X, Ca^{2+} (K_a = 0.025 mM, n_H = 2.0); ⊙, Mg^{2+} (K_a = 0.05 mM, n_H = 2.0); △, Sr^{2+} (K_a = 0.10 mM, n_H = 2.5); ●, Ba^{2+} (K_a = 0.13 mM, n_H = 2.5).

Fig. 3. Effect of ATP concentrations on the activity of AMP nucleosidase in the presence of various concentrations of Mg^{2+}. Enzyme activity was determined at the varied concentrations of $MgCl_2$ and ATP, 5 mM Tris-HCl buffer (pH 8.0) and 1 mM AMP. Points are experimental data and lines are theoretically drawn from Eqn. 1, using the values of apparent K_a and n_H indicated in parenthesis. Curve A, equimolar concentrations of $MgCl_2$ and ATP were used, which correspond to the concentrations of MgATP calculated according to the stability constant of 73 000 M^{-1} [4] (K_a = 0.44 mM, n_H = 4). Curve B, C and D, excess $MgCl_2$ was added to the reaction mixture containing equimolar concentrations of $MgCl_2$ and ATP at 0.5, 1 and 3 mM, respectively (B, K_a = 0.2 mM, n_H = 2.5; C, K_a = 0.13 mM, n_H = 2.2; D, K_a = 0.05 mM, n_H = 2.0).

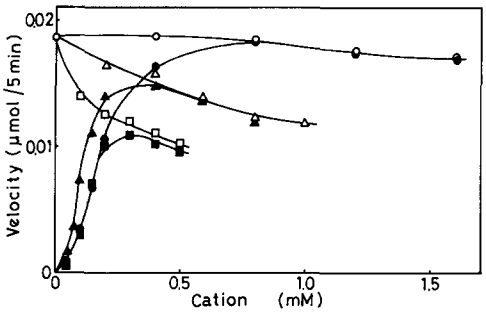

Fig. 4. Effect of concentrations of transition metals on the activity of AMP nucleosidase in the absence and presence of $MgCl_2$. Enzyme activity was determined at the varied concentrations of Mn^{2+}, Co^{2+} and Ni^{2+}, 0.2 mM, 5 mM Tris-HCl buffer (pH 8.0) and 1 mM AMP in the absence or presence of 1.5 mM $MgCl_2$. ○, ●, Mn^{2+}; △, ▲, Co^{2+}; □, ■, Ni^{2+}. Open symbols show the values in the presence of 1.5 mM $MgCl_2$ and closed symbols in the absence of Mg^{2+}.

excess Mg^{2+} and other divalent cations act as allosteric activators of the enzyme, as demonstrated in the effect of monovalent cations [4]. Theoretical curves calculated from the following equation yield a satisfactory fit for experimental activation curves in the presence of various concentration of Mg^{2+}.

$$\frac{v}{V} = \frac{[A]^n}{[A]^n + K_a^n} \tag{1}$$

where [A] is the concentration of ATP, K_a the concentration required for 50% activation and n the Hill coefficient. K_a and n were determined from Hill plots.

The effect of transition metals such as Mn^{2+}, Co^{2+} and Ni^{2+} was investigated. A potent activation of the enzyme was followed by a progressive decrease in activity as the concentration of transition metal increased under the conditions where ATP concentration was limited (0.2 mM) (Fig. 4). Co^{2+}- or Ni^{2+}-inhibition of the enzyme was observed at the metal concentrations above 0.2—0.3 mM. Transition metals do exist essentially as ATP-metal complex below 0.2 mM metals in these conditions, and free metal cations can exist above 0.2 mM according to the calculation of free and complex forms of these metals using the log stability constants of 4.71, 4.54 and 4.88 for Co-, Ni- and Mn-ATP complexes, respectively [6], suggesting that ATP-transition metal complexes as well as ATP-alkaline earth complexes are essential activators of the enzyme, whereas free transition metals can act as inhibitors. AMP nucleosidase was also inhibited by the higher concentrations of these transition metals in the presence of 1.5 mM Mg^{2+}, which fully activated the enzyme with no inhibitory effect. The $I_{0.5}$ values for these metals appear to be identical in the absence and presence of Mg^{2+}. These data also confirm the above conclusion.

Discussion

Kinetic studies of AMP nucleosidase reaction have been extensively carried out. The enzyme can be activated by ATP and certain cations including Mn^{2+}, monovalent cations and polyamines and inhibited by P_i, some nucleoside monophosphates and flavodoxin [2—5,7]. Although Ca^{2+}, Mn^{2+} and Co^{2+} of

the divalent cations were demonstrated to replace Mg^{2+} to varying degrees by Hurwitz et al. [8], kinetic studies on the action of divalent cations have not been performed. As shown in this paper, divalent cations including alkaline earth metals and transition metals activate AMP nucleosidase, these cations are absolutely required for the enzyme catalysis as ATP-metal complex, and furthermore, excess alkaline earth metals activate AMP nucleosidase by decreasing K_a and n_H values for ATP in an allosteric manner. The activating effect of free alkaline earth metals was observed only when concentration of ATP-metal complex was not saturated; AMP nucleosidase cannot be activated by free cations in the presence of saturating levels of MgATP (Fig. 3). AMP nucleosidase is, thus, activated by free alkaline earth metals as well as monovalent cations with the identical mechanism, which includes the decrease in K_a and n_H values for ATP-metal complex. The addition of these monovalent and divalent cations activates the enzyme by changing the kinetics from sigmoidal to nearly hyperbolic with respect to MgATP as reported previously [2]. On the other hand, transition metals differ from alkaline earth metals with respect to their lower K_a values and inhibitory effect at higher concentrations. The fact that the complexing ability of transition metals with ATP is greater than that of alkaline earths can account for the difference in the K_a values of the enzyme between two groups of metals. It should be noted that the inhibition of the enzyme by transition metals can be due to the action of free transition metals, the enzyme was not or inhibited little by these metals in the presence of relatively higher concentration of ATP, that is, under the conditions where concentrations of free metals are negligible (Fig. 1). However, significant inhibition was observed under the conditions where free transition metals can exist when ATP concentration is lowered (Fig. 3).

In conclusion, the kinetic properties of AMP nucleosidase can be accounted for by the assumption that the enzyme has two classes of binding sites for divalent cations, one is the activating site for ATP-metal complex, which is suggested to be commonly occupied by ATP-metal complex of alkaline earth metals and transition metals. The other sites are those for free metal cations; the site for free alkaline earth metals and free transition metals are activating and inhibitory sites, respectively.

Several lines of evidence have indicated that AMP nucleosidase is important in the stabilization of adenylate energy charge [1], adenylate pool size and conversion of adenine nucleotide to inosine or guanine nucleotide in *A. vinelandii* and probably in other prokaryotic cells [2,3,9,10]. Adenylate regulation in eukaryotes apparently occurs by AMP deaminase (EC 3.5.4.6) which also degrades AMP to form IMP and ammonia [11]. AMP nucleosidase in prokaryotes and AMP deaminase in eukaryotes are similar in their kinetic properties, both enzymes are inhibited by P_i and some nucleotides [2,3,12], are activated by ATP [2,3,13], and show similar responses to the adenylate energy charge [1,11,14]. In connection with the present paper, noteworthy is the finding on the effects of various cations on the enzymes. All alkali metal cations activated AMP nucleosidase [4] and AMP deaminase to a same extent [4,12,13], and alkaline earth metals and transition metals also activated and inhibited AMP deaminase [13] as well as AMP nucleosidase. Several regulatory properties of AMP nucleosidase and AMP deaminase, which suggest a common

TABLE I

EFFECT OF VARIOUS CATIONS ON THE ACTIVITY OF AMP NUCLEOSIDASE AND AMP DEAMINASE

Cations	AMP nucleosidase (EC 3.2.2.4)	AMP deaminase (EC 3.5.4.6)
Monovalent cations	All alkali metal cations activated the enzyme to a same extent [4,12,17]	
Divalent cations		
Alkaline earth metals	Activation [this paper]	Activation [13]
Transition metals	Activation at lower concentration (ATP-metal complex).	
	Inhibition at higher concentration (free metal cation) [this paper]	Inhibition [13]
Polyamines	Activation [5]	Activation [33]

metabolic role for these enzymes in prokaryotes and eukaryotes, respectively, are summarized in Table I, and may suggest the characteristics of the binding sites for these cations including mono- and divalent cations and polyamines. As noted from tables of monovalent cation-requiring enzymes compiled by Evans and Sorger [15] and Suelter [16], most of these enzymes represent relatively strict specificity toward activating monovalent cations. (1) These enzymes activated by K^+, are also usually activated by NH_4^+ and Rb^+ but are activated little by Na^+ and not at all by Li^+. (2) A few enzymes activated by Na^+ and also activated by Li^+ or Cs^+, but they are activated much less by K^+, NH_4^+ and Rb^+ [15]. However, Smiley et al. [17] claimed that AMP deaminase is a unique enzyme activated equally efficiently by K^+ and Na^+, and that these properties appear to be correlated with the radius of the unhydrated ion. AMP nucleosidase appears to belong to the enzyme of this group [4]. These enzymes show broad specificity for activating cations, monovalent cations of both K^+-NH_4^+ and Na^+-Li^+ groups are equally efficient and alkaline earth metals and transition metals also affect the enzyme activity. Enzymes belonging to this group are glycerol-3-phosphate dehydrogenase (EC 1.1.1.8) [18], glucose 6-phosphate dehydrogenase (EC 1.1.1.49) [19—21], tetrahydrofolate dehydrogenase (EC 1.5.1.3) [22,23], RNA polymerase (EC 2.7.7.6) [24,25], DNA polymerase (EC 2.7.7.6) [26], UDP glucose 4-epimerase (EC 5.1.3.2) [27,28], aminoacyl-tRNA synthetase (EC 6.1.1.1.-21) [29—32] and AMP deaminase [11,33], which are known to be also activated by some divalent cations and polyamines. These enzymes may have unique regulatory sites with a broad specificity toward the several cations including mono- and divalent cations and polyamines. The analysis of the regulatory mechanism of AMP nucleosidase and AMP deaminase activity may contribute toward understanding of the mechanism of the interaction of the above enzymes with these cations.

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