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EFFECTS OF MONOVALENT CATIONS ON AMP NUCLEOSIDASE FROM *AZOTOBACTER VINELANDII*

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Summary

The effect of monovalent cations on the purified AMP nucleosidase (AMP phosphoribohydrolase, EC 3.2.2.4) from *Azotobacter vinelandii* was investigated. All the monovalent cations were activators of the enzyme: Rb^+ and Cs^+ were the most effective, followed by K^+, Na^+, NH_4^+ and Li^+ in that order. The apparent K_a for MgATP and n_H values (Hill's interaction coefficient) decreased from 0.9 to 0.1 mM, and from 4 to 1, respectively, with the increase in K^+ concentration, suggesting that the cation effects are on MgATP binding rather than catalysis.

Gel filtration studies have revealed that the enzyme forms a non-dissociable enzyme species with a Stokes radius of 6.0—6.2 nm in the presence of saturating concentrations of monovalent cations, which can be distinguished from the 5.5-nm enzyme species showing temperature-dependent dissociation of the molecule in sulfate or phosphate. These results suggest that these ligands affect the association of the subunits through changes in the environment of the hydrophobic side chains of the enzyme molecules.

Introduction

Monovalent cations participate in biological processes in a variety of ways. One of the important function of these cations is as activators of several enzymes [1,2]. The mechanism of the activation, although not yet understood, is generally thought to involve an enzyme conformational transition induced or stabilized by the cation [3—5], a direct role of the cation in the orientation of the substrate during catalysis [6], or cation involvement in the mediation of the proper electronic effects for facilitation of the reaction [1,7].

AMP nucleosidase (AMP phosphoribohydrolase, EC 3.2.2.4), which is important to stabilize the adenylate energy charge [8] and in the conversion of adenine nucleotides to inosine or guanine nucleotides in *Azotobacter vinelandii* [9,10], has catalytic, regulatory and structural properties which are strikingly altered by several physiological ligands. The following study using AMP nucleosidase purified from *A. vinelandii* demonstrates that the kinetic and molecular properties are largely dependent on the presence of a monovalent cation, and the role of these cations in the regulation of AMP nucleosidase activity will be discussed from the molecular and physiological point of view.

Materials and Methods

Materials. AMP and ATP were products of Kyowa Hakko Co. (Tokyo, Japan). Crystalline bovine serum albumin and ovalbumin were purchased from Nutritional Biochem. Corp. and lactate dehydrogenase (EC 1.1.1.27), xanthine oxidase (EC 1.2.3.2), pyruvate kinase (EC 2.7.1.40) and fructose-1,6-biphosphate aldolase (EC 4.1.2.13) were obtained from Boehringer. Blue dextran 2000 and Sephacryl S-200 were products of Pharmacia. Other chemicals were reagent grade.

Enzyme and assay. AMP nucleosidase was purified by the method originally described by Yoshino [9]. In the modified method, aminohexyl-Sepharose 4B chromatography and gel filtration on Sephacryl S-200 were substituted for the protamine treatment and sucrose density gradient centrifugation, respectively. The preparation of the highest purification with a specific activity of approx. 2400 μmol/h per mg of protein was homogeneous as judged by its electrophoretic behaviour. The enzyme activity was measured by the formation of adenine [9] or reducing sugar [11] liberated.

The concentration of the MgATP complex in the reaction mixture was calculated using the stability constant of 73 000 M^{-1} as reported by O'Sullivan and Perrin [12]. All experiments were carried out at pH 8.0, where the phosphate groups of ATP and AMP are almost completely ionized and are therefore present as the ATP^{4-} and AMP^{2-}, respectively.

Gel filtration. Determination of the Stokes radius of the enzyme was performed by gel filtration on Sephacryl S-200. The column of Sephacryl S-200 (1 × 45 cm) was maintained at 2 or 37°C with a water jacket, and was equilibrated with (a) 0.2 M K_2SO_4 in 0.02 M Tris-HCl buffer and (b) 0.1 M KCl/0.02 M Tris-HCl or 0.1 M Tris-HCl buffer (pH 8.0). The column was calibrated with xanthine oxidase, pyruvate kinase, fructose-1,6-biphosphate aldolase, lactate dehydrogenase, bovine serum albumin and ovalbumin. Void volume (V_0) and internal gel volume (V_i) were determined from the elution volume of blue dextran and ammonium sulfate, respectively. Molecular sieve coefficient, σ was calculated from the equation $\sigma = (V_e - V_0)/V_i$.

Results

Studies of AMP nucleosidase from *A. vinelandii* have shown that an allosteric interaction is involved [9,10]. When velocities were determined in the absence of alkali metals with varying levels of MgATP, a strongly sigmoid response

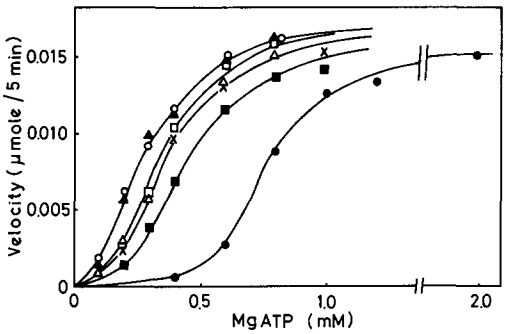

Fig. 1. MgATP saturation profiles in the absence and presence of various monovalent cations. Enzyme activity was determined at the varied concentrations of $MgCl_2$ and ATP, 4 mM Tris-HCl buffer (pH 8.0) and 0.5 mM AMP in the absence or presence of 7.5 mM monovalent cations. Equimolar concentrations of $MgCl_2$ and ATP were used to give the desired concentration of MgATP according to the relationship described under Materials and Methods. Velocity is expressed as μmol reducing sugar formed under the assay condition; ○, RbCl; ▲, CsCl; □, KCl; △, NaCl; X, NH_4Cl; ■, LiCl; ●, no addition.

curve typical of an allosteric enzyme was observed. In the presence of alkali metals such as Li^+, Na^+, K^+, Cs^+ or Rb^+, the velocity versus MgATP concentration curves became less sigmoid; however, the maximum velocity was not changed and the concentration of MgATP required to give half-maximal velocity was lowered (Fig. 1). Other monovalent cations including Tris and triethanolamine were effective to a lesser extent (data not shown). With increasing concentration of K^+, the apparent K_a values for MgATP decreased from 0.9 to 0.1 mM (Fig. 2) with the decrease in an n_H value of approximately 4 to 1. Theoretical curves calculated from the following equation yield a satisfactory fit for experimental activation curves in the absence and presence of K^+ (Fig. 2).

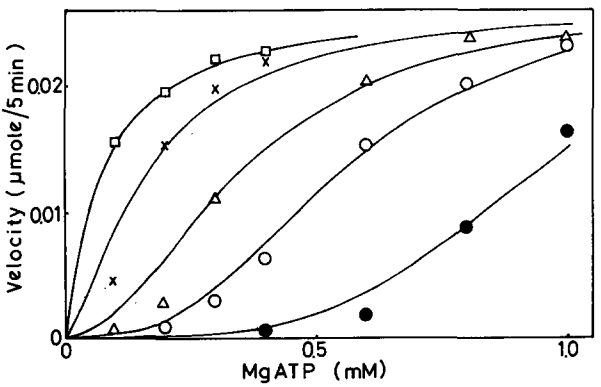

Fig. 2. Effect of MgATP concentrations on AMP nucleosidase in the absence and presence of K^+. Enzyme activity was determined at the varied concentrations of $MgCl_2$ and ATP, 4 mM Tris-HCl buffer (pH 8.0), and 0.5 mM AMP in the absence or presence of the fixed concentrations of K^+. Equimolar concentrations of $MgCl_2$ and ATP were used to give the desired concentrations of MgATP as described in Fig. 1. Velocity is expressed as μmol reducing sugar formed under the assay conditions. Points are experimental data, and lines are theoretically drawn from Eqn. 1, using the following values of apparent K_a and n_H: ●, no addition (K_a = 0.95 mM, n_H = 4.0); ○, 5 mM KCl (K_a = 0.56 mM, n_H = 2.8); △, 10 mM KCl (K_a = 0.36 mM, n_H = 2.0); X, 20 mM KCl (K_a = 0.169 mM, n_H = 1.8); □, 50 mM KCl (K_a = 0.075 mM, n_H = 1.0).

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

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. MgATP, an activator absolutely required for the AMP nucleosidase reaction, is known to be replaced by $MgPP_i$ [13]. The effect of K^+ concentration on $MgPP_i$ saturation curves was also examined: the addition of K^+ also decreases the apparent K_a for $MgPP_i$ and the Hill coefficient (data not shown). These findings suggest that alkali metals function as allosteric activators of the enzyme.

AMP nucleosidase is known to undergo association and dissociation in the presence of various ligands [14,15]. The association-dissociation or conformational changes in the presence of alkali metals were examined by application of gel filtration on Sephacryl S-200. Fig. 3 shows the elution patterns of AMP nucleosidase from Sephacryl S-200 column equilibrated with 0.2 M K_2SO_4 at 2°C and 37°C. The Stokes radius of the enzyme was calculated from molecular sieve coefficients to be 5.5 nm at 37°C, whereas the value decreased from 5.2 to 4.5 nm at 2°C with the decrease in protein concentration. The equilibrium

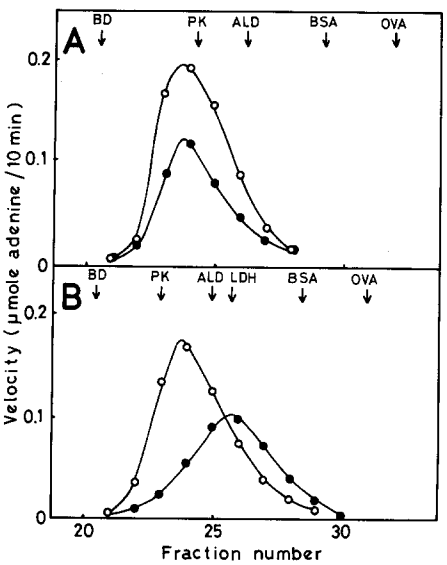

Fig. 3. Gel filtration of AMP nucleosidase in 0.2 M K_2SO_4 at 37°C and 2°C. (A) AMP nucleosidase of 6 (●) and 30 μg (○) was applied to a 1 × 45 cm column of Sephacryl S-200 equilibrated with 0.2 M K_2SO_4 in 0.02 M Tris-HCl buffer (pH 8.0) at 37°C. Elution was performed with the same buffer, and fractions of 20 drops each were collected. Samples were analyzed for the enzyme activity. (B) The same sample of the enzyme was filtered through the same column as that in (A), except that the temperature of the column was maintained at 2°C. Enzyme activity was determined with aliquots of 0.02 (○) or 0.06 ml (●) from each tube. Velocity is expressed as formation of adenine under the assay conditions. The positions of elution for standard proteins were shown with arrows. Abbreviations used: BD, blue dextran; PK, pyruvate kinase; ALD, fructose-1,6-biphosphate aldolase; LDH, lactate dehydrogenase; BSA, bovine serum albumin; OVA, ovalbumin.

between the dissociated and associated forms of the enzyme as demonstrated previously [15] was suggested to be shifted toward the latter at higher temperature or at higher enzyme concentration in the presence of 0.2 M K_2SO_4; however, the enzyme tends to be dissociated at 2° C since the Stokes radius of the completely dissociated enzyme is 4.5 nm in low concentration of sulfate [14,15]. The Stokes radius of the enzyme was shown to be 6.0—6.2 nm at 2° C or 37° C in the absence of sulfate when the Sephacryl S-200 gel filtration column was equilibrated with 0.1 M Tris-HCl buffer or 0.1 M KCl (data not shown). Alkali metal-induced form of the enzyme in the absence of sulfate or phosphate can be distinguished from the associated form in these anions by its larger Stokes radius and no dissociation of the enzyme.

Discussion

Kinetic studies of AMP nucleosidase reaction have been extensively carried out. However, kinetic parameters, especially K_a for ATP vary widely depending on the conditions employed [10,16,17]. As shown in this paper, monovalent cations activate AMP nucleosidase by decreasing K_a for MgATP: the value was estimated to be approx. 0.1 mM, which is in good agreement with the data previously reported [16,17], when the enzyme was fully activated by the addition of 50 mM or higher concentration of alkali metals. These results suggest that the cation may induce or stabilize enzyme conformations which have higher affinity for MgATP.

Alteration of K_m or K_a is observed for some enzymes activated by monovalent cations and in V for others [3,5]: for example, alkali metals activate AMP deaminase (EC 3.5.4.6) [18,19] and tryptophanase (EC 4.1.99.1) [20] by decreasing K_m for their substrate, but increase the V for pyruvate kinase [7] and aspartokinases (EC 2.7.2.4) [21]. A general mechanism which could account for these observations is a conformational change in the protein altering binding in the former and catalysis in the latter cases. Direct evidence of such conformational change is available in several cases: ultraviolet difference spectra are induced by KCl for aspartokinase [22,23] and pyruvate kinase [24], and enzyme subunits are associated by alkali metals for AMP deaminase [25]. As demonstrated in this paper, the conformation induced by the addition of activating monovalent cations in the absence of sulfate or phosphate was somewhat different from the associated form obtained in the presence of these anions by its larger Stokes radius and no dissociation of the enzyme molecule. The possibility of ligand-induced changes in the environment of the hydrophobic side chains of proteins is attractive in view of the demonstration of low temperature dissociation of the enzyme under certain conditions: this dissociation indicates that apolar interactions, which are significantly weakened at low temperature [26,27], are important in the subunit association of the enzyme in the presence of sulfate or phosphate, but these interactions are not responsible for the subunit association in the presence of alkali metals without sulfate or phosphate.

The available information regarding the role of monovalent cations in enzyme catalysis leaves open the question of whether the cation serves a specific function of the enzyme active site or a more general function related

to conformational aspects of the protein. The data described above seem to support the more general function.

AMP nucleosidase may be responsible for the adenylate energy charge [8], adenylate pool size and conversion of adenine nucleotides to inosine and guanine nucleotides in *A. vinelandii* [9,10] and probably also in other prokaryotic cells [28]. Adenylate regulation in eukaryotes apparently occurs by AMP deaminase which also degrades AMP to form IMP and ammonia [18,19]. AMP nucleosidase in prokaryotes and AMP deaminase in eukaryotes are similar in that both enzymes are inhibited by P_i [10,18,19,30], are activated by ATP [10,17,18,29] and by polyamines [31,32], and show similar responses to the adenylate energy charge [8,29]. The present results together with these regulatory properties of the enzyme suggest a common metabolic role for AMP nucleosidase and AMP deaminase in prokaryotes and eukaryotes, respectively.

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