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AMP DEAMINASE FROM BAKER'S YEAST

PURIFICATION AND SOME REGULATORY PROPERTIES

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Summary

AMP deaminase (AMP aminohydrolase, EC 3.5.4.6) was found in extract of baker's yeast (*Saccharomyces cerevisiae*), and was purified to electrophoretic homogeneity using phosphocellulose adsorption chromatography and affinity elution by ATP. The enzyme shows cooperative binding of AMP (Hill coefficient, n_H, 1.7) with an $s_{0.5}$ value of 2.6 mM in the absence or presence of alkali metals. ATP acts as a positive effector, lowering n_H to 1.0 and $s_{0.5}$ to 0.02 mM. P_i inhibits the enzyme in an allosteric manner: $s_{0.5}$ and n_H values increase with increase in P_i concentration. In the physiological range of adenylate energy charge in yeast cells (0.5 to 0.9), the AMP deaminase activity increases sharply with decreasing energy charge, and the decrease in the size of adenylate pool causes a marked decrease in the rate of the deaminase reaction. AMP deaminase may act as a part of the system that protects against wide excursions of energy charge and adenylate pool size in yeast cells. These suggestions, based on the properties of the enzyme observed in vitro, are consistent with the results of experiments on baker's yeast in vivo reported by other workers.

Introduction

In higher eukaryotes such as mammals, birds and amphibians, AMP deaminase (AMP aminohydrolase, EC 3.5.4.6) catalyzes the hydrolytic removal of the 6-amino group of AMP to yield IMP. The enzyme has been intensively

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studied [1], and several lines of evidence indicate that the enzyme has a central role in the regulation of adenylate energy charge and total adenylate pool size [2—5] in addition to the interconversion of adenine, inosine and guanine nucleotides [6—9]. In lower eukaryotes including yeasts and fungi, however, little is known about the mechanism of the adenylate regulation and there is little information about AMP deaminase, although the stabilization of energy charge and the conversion of AMP to IMP have been demonstrated under certain conditions [10]. AMP deaminase is usually assumed to be absent from yeast, but this point does not appear to have been demonstrated experimentally [10]. In this paper we present a purification of AMP deaminase from baker's yeast which yields a homogeneous preparation as judged by its electrophoretic behavior, and examine some regulatory properties of the enzyme, focussing especially on those properties which might be relevant to adenylate regulation.

Materials and Methods

Materials. Various nucleotides, nucleosides and base were obtained from Sigma, Boehringer or Kyowa Hakko Co. (Tokyo). Phosphocellulose (Brown Co.) was purchased from Seikagaku Kogyo Co. (Tokyo). Commercial baker's yeast (*Saccharomyces cerevisiae*) was obtained locally.

Methods. The activity of AMP deaminase was determined essentially as described previously [3]: the reaction mixture of 0.2—4 ml contained 10 mM cacodylate buffer (pH 7.1), various concentrations of AMP and effectors, 0.02% bovine serum albumin, and the enzyme. The reaction was usually carried out at 37° C for 5 min. The amount of ammonia was determined by the phenol-hypochlorite reagent.

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was performed by the method of Weber and Osborn [11].

Protein concentrations were determined by the method of Lowry et al. [12].

For preparation of yeast extract, fresh baker's yeast in cake form was crumpled and blended with 2 vols. of 50 mM potassium phosphate buffer (pH 7.5) in a Vibrogen Cell Mill for 20 min. The cell debris was then removed by centrifugation at 100 000 $\times g$ for 1 h, and the supernatant was used as the crude extract.

Results

Deaminase activities in cell-free extracts of baker's yeast

Table I shows the effects of deaminase activity on AMP, ATP, adenosine and adenine in the cell-free extract of baker's yeast (*S. cerevisiae*). High AMP deaminating activity was detectable and the addition of ATP apparently activated the reaction: however, ATP itself was not deaminated. No activity of adenosine and adenine deaminases was observed in the extract. We have, thus, explored isolation and characterization of AMP deaminase from baker's yeast.

Purification of AMP deaminase

All procedures were performed at 0—4° C.

TABLE I

DEAMINASE ACTIVITY IN CRUDE EXTRACT OF BAKER'S YEAST

The reaction mixture consisted of the indicated concentrations of substrate and the additive, 10 mM cacodylate buffer (pH 7.1), 100 mM K^+, 10 mM Na^+, 0.02% bovine serum albumin, and the enzyme in a final volume of 0.20 ml. The reaction was initiated by the addition of 100 000 × *g* supernatant of baker's yeast extract.

Substrate	Concentration (mM)	Addition (mM)	Specific activity (μmol/min per mg protein)
AMP	5	—	0.060
	5	1 mM $MgCl_2$ + 1 mM ATP	0.100
ATP	5	—	0.004
Adenosine	4	—	0.002
Adenine	2	—	0.002

Crude extract. Fresh commercial baker's yeast (360 g, wet weight) was extracted as described in Materials and Methods.

First phosphocellulose chromatography. The supernatant was applied to a phosphocellulose column (5.0 × 20 cm) which had been equilibrated with 50 mM potassium phosphate buffer (pH 7.5). After washing with 0.1 M potassium phosphate buffer (pH 7.5), the enzyme was eluted with 0.2 M phosphate buffer, and diluted 2-fold with distilled water.

Second phosphocellulose chromatography. The enzyme solution was loaded on to a phosphocellulose column (2.5 × 20 cm) equilibrated with 0.1 M phosphate buffer. After washing, the enzyme was eluted with 5 mM ATP in the same buffer.

Third phosphocellulose chromatography. The pooled solution was diluted 2-fold with cold distilled water and applied to a third phosphocellulose column (2.0 × 15 cm), which had been equilibrated with 50 mM potassium phosphate buffer (pH 7.5). After washing with the same buffer and further with 0.1 M potassium phosphate buffer, 0.15 M NaCl, a linear gradient of 0—0.6 mM ATP in the latter buffer (200 ml per chamber) was then applied to the column. The fractions containing higher activity were pooled and concentrated in a DIAFLO ultrafilter and a Collodion bag. A summary of the purification is given in Table II.

TABLE II

SUMMARY OF PURIFICATION

Fraction	Total volume (ml)	Total protein (mg)	Specific activity (μmol/min per mg protein)	-fold
Crude extract *	685	26 715	0.08	1
P-cellulose I	200	600	2.0	25
P-cellulose II	40	48	16.1	201
P-cellulose III	2	0.3	343.3	4291

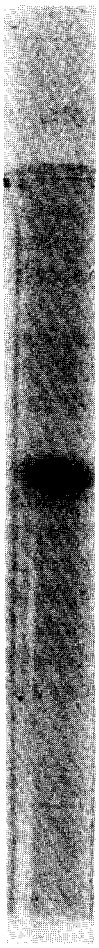
* 360 g commercial baker's yeast were used.

Homogeneity

Homogeneity was checked by electrophoresis on polyacrylamide gel in the presence of sodium dodecyl sulfate. Electrophoresis of 10 μg of the enzyme after the third phosphocellulose chromatography gave a single band (Fig. 1) with a specific activity of about 400 μmol/min per mg.

Effect of pH

The effect of pH on the activity of AMP deaminase was determined at 20 mM AMP in the presence of 100 mM alkali metals. A typical bell-shaped curve was obtained, with an optimum between pH 7.0 and pH 7.1 (Fig. 2), similar to the chicken and human erythrocyte enzymes [13,14].



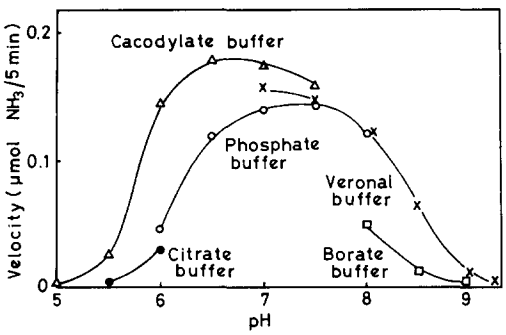


Fig. 1. Sodium dodecyl sulfate gel electrophoresis of baker's yeast AMP deaminase. A 10-μg sample, prior to electrophoresis, was incubated for 3 min in boiling water in 0.01 M sodium phosphate buffer (pH 7.0) containing 1% sodium dodecyl sulfate and 0.5% 2-mercaptoethanol, and analyzed in the system of Weber and Osborn [11].

Fig. 2. Effect of pH on the rate of reaction. The reaction mixture contained 20 mM AMP, 100 mM alkali metals (K^+ and Na^+), 0.02% bovine serum albumin, 10 mM buffer and the enzyme in a final volume of 1 ml.

TABLE III

SUBSTRATE SPECIFICITY OF BAKER'S YEAST AMP DEAMINASE

Assays were carried out in the presence of the indicated concentrations of the substrate, 1 mM ATP, 1 mM $MgCl_2$, 100 mM K^+, 20 mM Na^+, 10 mM cacodylate buffer (pH 7.1). 0.02% bovine serum albumin and the enzyme in a final volume of 0.2 ml.

Substrate	Concentration (mM)	Relative velocity *
AMP	20	1.00
dAMP	20	0.07
ATP	2	0.01
Adenosine	10	0
Adenine	5	0

* Enzyme activity for AMP as the substrate was expressed as an arbitrary value of 1.00.

Substrate specificity

The substrate specificity of baker's yeast enzyme is given in Table III. Like the mammalian enzyme, the baker's yeast AMP deaminase catalyzed the deamination of AMP as the preferred substrate, and had no activity toward ATP, adenosine and adenine. dAMP was hydrolyzed at 7% or less of the rate observed for AMP at the maximum rate of deamination.

Kinetic properties

Baker's yeast AMP deaminase was activated by monovalent cations and ATP. Fig. 3 shows that the enzyme requires monovalent cations for optimum activity. The effect of monovalent cations was largely on the maximal velocity of the enzyme: the increase in the concentration of monovalent cations did not affect $s_{0.5}$ values, the concentration of AMP required for half-maximal velocity which remained at 2.6 mM. Hill's interaction coefficients were calculated as 1.7

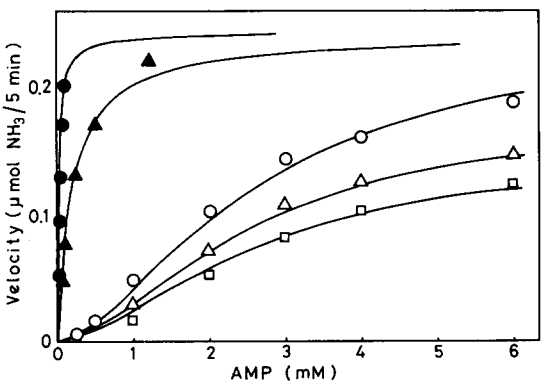


Fig. 3. Rate of the reaction catalyzed by baker's yeast AMP deaminase as a function of substrate concentration. The reaction mixture contained 10 mM cacodylate buffer (pH 7.1), 20 mM Na^+, additives, various concentrations of AMP and KCl, and the enzyme in a final volume of 4 ml. The reaction was carried out at 37°C for 5 min. Points are experimental data, and lines are theoretically drawn from Eqn. 1, using the following values of apparent K_m and n_H: □, no addition (K_m = 2.6 mM, n_H = 1.7); △, 10 mM KCl (K_m = 2.6 mM, n_H = 1.7); ○, 50 mM KCl (K_m = 2.6 mM, n_H = 1.7); ▲, 50 mM KCl plus 2 mM ATP (K_m = 0.2 mM, n_H = 1.0); ●, 50 mM KCl plus 2 mM ATP and $MgCl_2$ (K_m = 0.02 mM, n_H = 1.0).

in the absence and presence of monovalent cations. In contrast, the effect of ATP was largely on the affinity of the enzyme for AMP: $s_{0.5}$ values were 0.2 and 0.02 mM in the presence of 2 mM ATP and MgATP, respectively, and Hill coefficients decrease to 1.0, suggesting that activation of AMP deaminase by ATP requires Mg^{2+} for optimal activity. Theoretical saturation curves were computed in the absence and presence of K^+ and ATP using V, apparent K_m and n_H values according to the following equation, and the best-fit curves were obtained (Fig. 3).

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

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

The effect of Mg^{2+} alone on the enzyme was similar to that of mono-valent cations: Mg^{2+} affect the maximal velocity of the enzyme without alteration of $s_{0.5}$ and n_H values.

The effect of P_i on the enzyme activity was studied kinetically. With increasing concentrations of P_i, the AMP saturation curves became more sigmoidal in shape, and the $s_{0.5}$ values raised without alteration of the maximum velocity (Fig. 4). The cooperative effect of AMP, represented in the form of Hill coefficient, was such that this coefficient increased from 1.7 to 2.0 as these ligands increased from 0 to 10 mM. These results suggest that P_i may be an allosteric inhibitor of the enzyme.

Response to energy charge

It has been well established by Atkinson [15] that the balance among concentrations of adenine nucleotides, rather than absolute concentrations, is a major regulatory factor in many enzymatic reactions. AMP deaminases from several sources have been shown to response to changes in the adenylate energy charge ratio [2,5,16]. Therefore, it was considerable interest to determine if

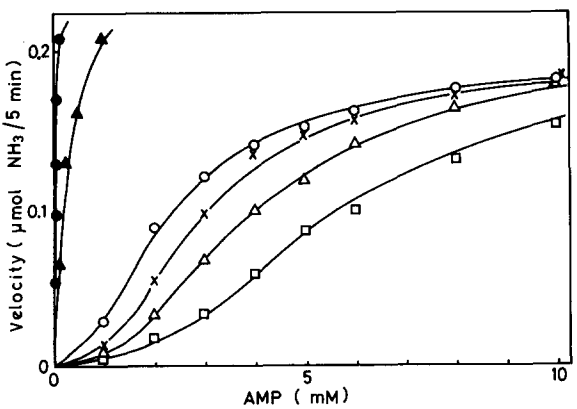


Fig. 4. Effect of AMP concentration on velocity of AMP deaminase in the presence of P_i. The reaction mixture contained 100 mM KCl, 20 mM Na^+, 10 mM cacodylate buffer (pH 7.1), various concentration of AMP and P_i, and the enzyme in a final volume of 1 ml. ○, no addition; ×, 2 mM P_i; △, 5 mM P_i; □, 10 mM P_i; ▲, 2 mM ATP; ●, 2 mM ATP and $MgCl_2$.

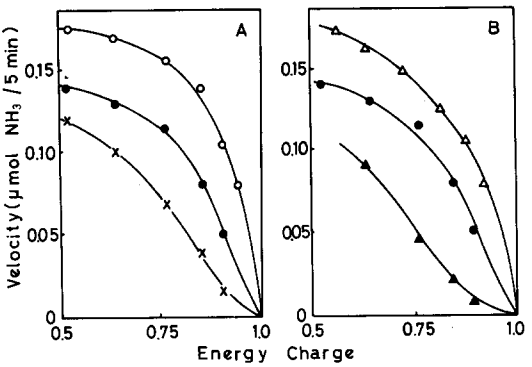


Fig. 5. Response to baker's yeast AMP deaminase to variation in the adenylate energy charge. (A) Effect of P_i concentration. The total adenine nucleotide concentration was held constant at 2 mM and the concentration of P_i was varied from 0 to 10 mM. The assay mixture contained 10 mM cacodylate buffer (pH 7.1), 100 mM KCl, 4 mM Mg^{2+}, 2 mM adenine nucleotides, various concentration of P_i and the enzyme in a final volume of 1 ml. ○, no addition; ●, 5 mM P_i; X, 10 mM P_i. (B) Effect of the variation in the total adenine nucleotide concentration. The assay mixture was similar to that in (A) except that total adenine nucleotide concentration was varied from 1 to 3 mM, and that P_i was held constant at 5 mM. The relative amount of ATP, ADP and AMP at different energy charge values were calculated on the basis of the equilibrium constant of the adenulate kinase reaction according to Chapman and Atkinson [2]. Total adenine nucleotides: △, 3 mM; ●, 2 mM; ▲, 1 mM.

the yeast enzyme showed a similar response to alterations in the energy charge ratio and the total adenylate pool size.

When the sum of the adenine nucleotides was kept constant, AMP deaminase responded to variation in the energy charge as shown in Fig. 5A. The shape of the energy charge response curve, which resemble that of rat liver or Ehrlich ascites tumor AMP deaminase [2,16], illustrates that, although the enzyme is activated by ATP, the reaction velocity proceeds much more slowly at physiological energy charge (0.9) because the concentration of substrate AMP is limiting. We further examined the effect of P_i, the inhibitor of AMP deaminase, on the activity response to varying energy charge under the conditions, which are close to the levels of adenine nucleotides normally found in yeast [17]: the enzyme responded more effectively to the variation in the energy charge in the presence of higher concentration of P_i (Fig. 5A). Fig. 5B shows the response of the enzyme to variation in the energy charge at different adenylate pool size in the presence of 5 mM P_i. With the decrease in the adenylate pool size, the enzyme activity proceeded much more slowly, although the enzyme responded to the variation in the energy charge. This effect presumably serves to prevent excessive depletion of the adenine nucleotide pool with the decrease in energy charge.

Discussion

This paper describes a method to purify AMP deaminase from baker's yeast yielding an enzyme having specific activity of about 400 μmole per mg protein. The overall purification was 4000- to 5000-fold over the crude extract. The major purification was achieved by using adsorption to phosphocellulose column and the affinity elution by ATP. The purified preparation was homogeneous as judged by polyacrylamide gel electrophoresis in the presence of

sodium dodecyl sulfate. However, the specific activity of the best fractions were 550 μmol per mg, which is considerably less than that expected when comparisons are made with other preparations such as rat and rabbit liver (specific activity of 2000) [2,18], rat liver (1000) [18], or chicken erythrocyte (1000) [19]. We suspect that the protein may have been obtained without its full complement of the cofactor such as Zn^{2+}, as discussed in the case of the human erythrocyte enzyme [14].

AMP deaminase, which is widely distributed in a variety of animals [1,20], was purified from various sources, and they behave as allosteric enzymes [1]. Extensive studies on AMP deaminase in rat tissues have revealed the presence of three parental isozymes: A (skeletal muscle type), B (liver) and C (heart muscle) [21]. They differed from one another with respect to their kinetic, molecular and other properties [18,21]. The kinetic properties of the baker's yeast enzyme differed from those of AMP deaminases from higher animals in that the activation by alkali metals was largely on the maximal velocity of the enzyme without alteration of $s_{0.5}$ and n_H values. However, other regulatory properties of the yeast enzyme resemble those of the rat liver [2] or Ehrlich ascites tumor enzyme [16]. For example, these enzymes show nearly identical responses to the variation in the energy charge [2,5,16]: the response of the baker's yeast enzyme to P_i is very similar to that of the ascites tumor enzyme reported by Chapman et al. [16].

Several lines of evidence have indicated that AMP deaminase is important in the stabilization of adenylate energy charge [2—5], the control of adenylate pool size [6—9], and as a regulatory enzyme in the purine nucleotide cycle in various mammalian tissues [22,23]. However, little is known about AMP deaminase in lower eukaryotes such as yeast. Ball and Atkinson [17] have reported that baker's yeast cells, when grown aerobically on glucose and allowed to pass stationary phase, maintain a normal range (0.8 to 0.9) of adenylate energy charge with decrease in adenylate pool size. AMP-degrading enzyme(s) might be responsible for this phenomenon, but the mechanism has not been studied in detail. Recent observation by Burridge et al. on the formation of a large amount of hypoxanthine and its derivatives (inosine, IMP) from adenine was believed to be due to the combined action of adenine phosphoribosyl transferase (EC 2.4.2.7), 5′-nucleotidase (EC 3.1.3.5), adenosine deaminase (EC 3.5.4.4) and purine nucleoside phosphorylase (EC 2.4.2.1), on the basis of the lack of adenine deaminase (EC 3.5.4.2) and the usual assumption that AMP deaminase is absent from yeast [10]. However, the lack of AMP deaminase has not been demonstrated experimentally in yeast and seems to be based on physiological and genetical studies of *Schizosaccharomyces pombe* [24]. Our findings on the occurrence and regulatory properties of AMP deaminase in baker's yeast can account for the stabilization of adenylate energy charge with the decrease in adenylate pool size during prolonged starvation. AMP deaminase and related enzymes can also account for the conversion of AMP to IMP, inosine and hypoxanthine in yeast cells incubated with adenine: AMP synthesized from adenine is deaminated to IMP by AMP deaminase and further dephosphorylated to inosine and finally hypoxanthine. The results described here indicate that the yeast AMP deaminase resemble the enzyme from rat liver or Ehrlich ascites tumor with respect to some regulatory properties, especially

responses to the energy charge: the energy charge and adenylate pool size are suggested to be regulated through the control of AMP deaminase activity in yeast cells as well as rat liver or tumor cells.

The common observation that the total adenylate pool size varies and/or changes rapidly with metabolic perturbations [25] indicates that most, if not all organisms have AMP-degrading enzymes which play a role in regulating the adenylate pool size. AMP deaminase may be responsible for the adenylate regulation in eukaryotes such as mammals, birds and yeasts, but the enzyme has not been detected in prokaryotes. Adenylate regulation in prokaryotes apparently occurs by AMP nucleosidase (EC 3.2.2.4) which also degraded AMP to form adenine and ribose 5-phosphate [26—29]. A notable exception appears to be the marine bacterium *Benekea natriegens* which has been reported to contain AMP deaminase activity but not AMP nucleosidase [30]. AMP deaminase in eukaryotes and AMP nucleosidase in prokaryotes are similar in that both enzymes are inhibited by P_i [2,31,32], are activated by ATP [2,27,32] and polyamines [33,34], and show similar responses to the adenylate energy charge [2,26]. These observations, together with the observed regulation of adenylate pools in many organisms, suggest a common metabolic role for these enzymes. A common feature of all regulatory mechanisms is the degradation of AMP, which decreases the adenylate pool size with the stabilization of the adenylate energy charge in both eukaryotes and prokaryotes.

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