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Isolation, Purification, and Partial Characterization of an AMP Deaminase from *Saccharomyces cerevisiae* D

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Abstract An adenosine 5'-monophosphate deaminase (AMP aminohydrolase, EC 3.5.4.6) was purified to homogeneity from the cell-free extract of *Saccharomyces cerevisiae* D KCTC7248. The molecular mass of subunit was estimated to be 80 kDa on SDS-PAGE, and that of the holoenzyme was shown to be 240 kDa by gel filtration. The isoelectric point of the enzyme (AMP deaminase D) was determined to be 6.2. The AMP deaminase D was specific towards AMP with an apparent K_m value of 4.1 mM and a Hill coefficient, n_H, of 2.2. Both ATP and ADP were positive allosteric effectors of the AMP deaminase D: The apparent K_m was decreased to 1.6 mM and 3.3 mM in the presence of 0.1 mM ATP and ADP, respectively, lowering n_H to 1.0. Univalent cations like K^+, Na^+, and Li^+ activated the enzyme but some divalent cations such as Cu^{2+} and Cd^{2+} showed strong inhibitory effects. This enzyme displayed optimum activity at 30°C and pH 7.0. In addition, it was stable up to 45°C and over a wide pH range (pH 5.5–9.0). Amino acid sequences of its N-terminal region were analyzed to be ADYKMQMFADDA.

Key words: *Saccharomyces cerevisiae*, AMP deaminase, inosine 5'-monophosphate, baker's yeast

Monosodium glutamate (MSG) has been used in a variety of foods as a flavor enhancer. Recently, the use of MSG has been criticized by several reports because of its adverse reactions in human [1, 21]. In particular, its known function in the nervous system has seriously raised questions about its use as food additives. In these circumstances, many researchers have shown a growing interest in screening of new natural flavor-materials.

AMP deaminase catalyzes the irreversible aminohydrolysis of adenosine 5'-monophosphate (AMP) to equimolar amounts of IMP and ammonia. The enzyme has been found in a variety of eukaryotes including animals, plants, and yeast [5, 8, 10, 15, 17, 19, 20, 23]. IMP, the reaction product, is a flavor enhancer and has been partially substituted for MSG in some foodstuffs in low amounts with high efficiency. In addition, it has been found to possess the synergistic flavor enhancer property along with guanosine 5'-monophosphate [4]. Olmedo *et al.* [13] described a method to produce IMP using an immobilized AMP deaminase in a continuous packed bed reactor.

Microbial enzymes are currently receiving special attention because of their potential application in industry. An AMP deaminase originated from microbial sources has been purified and thoroughly studied [8, 9, 24]. However, data pertaining to AMP deaminase derived from microbial sources were restricted, until recently, only to the baker's yeast, *Saccharomyces cerevisiae*. There are many varieties within the species of *S. cerevisiae* based on physiological properties in addition to genetic analysis [2, 22]. Therefore, an investigation of AMP deaminase from other yeast strains has been meaningful not only in view of its biotechnological application but also for obtaining knowledge regarding the enzyme itself along with its metabolic function of AMP deaminase in yeast cells. For this reason, we screened a strain efficiently producing AMP deaminase out of various yeast strains. We also purified AMP deaminase from the isolated strain, and characterized its enzymatic properties to obtain basic data needed for application.

Materials and Methods

Microorganisms and Media

Yeasts used (Table 1) in this study were obtained from the Korean Collection for Type Cultures (KCTC). All liquid media were adjusted with 0.1 N NaOH (or HCl) to pH 7.0 prior to autoclaving. Yeast-malt extract (YM) contained 0.3% yeast extract, 0.3% malt extract, 0.5% peptone, and 1.0% glucose. Glucose-yeast extract (GYP) consisted of 4.0% glucose, 0.5% peptone, and 0.5% yeast extract.

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Table 1. AMP deaminase from various yeast strains.

Strain	Enzymatic activity (μmol mg^{-1})	Culture medium
Saccharomyces cerevisiae KCTC 7244	0.11	YM
S. cerevisiae KCTC 7904	0.09	YM
S. cerevisiae KCTC 7968	0.13	YM
S. cerevisiae KCTC 7905	0.11	YM
S. cerevisiae KCTC 7105	0.09	YM
S. cerevisiae KCTC 7108	0.09	YM
S. cerevisiae D KCTC 7248	0.20	SG
S. cerevisiae KCTC 7911 (baker's yeast)	0.07	YM
Shizosaccharomyces octosporus var. *octosporus* KCTC 7255	0.08	GPY

The following yeasts have specific activities of 0.02 to 0.07 μmol mg^{-1}: *S. cerevisiae* KCTC 7903, 7233, 7910, 7917, 7919, 7942, 7106, 7110, and 7112, *S. cerevisiae* D cf. *cerevisiae* KCTC 7906, 7913, 7915, and 7107, *S. chevalieri* KCTC 7237 and 7239, *S. ellipsoideus* KCTC 7243, *S. exiguus* KCTC 7169, *S. pastorianus* KCTC 7918, *Saccharomycodes ludwigii* KCTC 7126, *Shizosaccharomyces prombe* var. *prombe* KCTC 7522, *Sporobolomyces singularis* KCTC 7534, *Zygosaccharomyces florentinus* KCTC 7184, *Z. mrakii* KCTC 7183, *Z. rouxii* KCTC 7966 and 7191, and *Torulaspora delbrueckii* KCTC 7115.

Sabouraud's glucose (SG) was derived from Difco Lab. (Detroit, U.S.A.).

Screening of Yeasts for AMP Deaminase

The yeast cells from each strain were inoculated into 125-ml Erlenmeyer flasks containing 25 ml of liquid medium, and the flasks were incubated at 30°C for 2 days on a rotary shaker at 160 rpm. Yeast cells were harvested by low-speed centrifugation and washed once with an ice-chilled 50 mM imidazole buffer, pH 7.0. Yeast cells (100–150 mg, wet weight) were then resuspended in 1 ml of the buffer solution, mixed with glass beads (0.5 mm diameter), and disrupted by vortexing. The cell homogenate was centrifuged at 12,000 ×g for 30 min and the supernatant was used for enzyme assay.

Enzyme Assay

The deamination of AMP to IMP was measured using HPLC as described by Raffin and Thebault [14]. The incubation mixtures contained the following components in a final volume of 1 ml: 50 mM imidazole-HCl, pH 7.0, 0.1 mM KCl, 0.1 mM ATP, and 5 mM AMP and an appropriate amount of enzyme at the different stages of purification. After a 10 min incubation at 30°C, the reaction was terminated by adding 50 μl of 1 M HCl. For the HPLC analysis, sample solutions were neutralized by adding NaOH and an aliquot of sample was injected onto a reverse phase $C_{18}\mu$ Bondapak column (3.9 × 300 mm, flow rate 1.0 ml min^{-1}), and the percent conversion was determined by integration of the peaks at 248 nm. The solvent system was 0.1 M ammonium phosphate (pH 5.0) containing 5% methanol. One unit of the enzyme is defined as the amount of enzyme that catalyzes the formation of 1 μmol IMP per min.

Protein Determination

Protein concentrations were measured by Bradford's dye-binding method [3] using a protein assay kit (Bio-Rad Lab., Richmond, U.S.A.) with bovine serum albumin as the standard. For column fractions, the protein concentrations were determined by absorbance at 280 nm.

Purification of AMP Deaminase

AMP deaminase from *S. cerevisiae* D was purified using the cellulose phosphate adsorption chromatographic methods of Yoshino *et al.* [24] and Merkler *et al.* [8] with a slight modification. All purification steps were carried out at 4°C. Yeast cells weighing 365 g were suspended in 500 ml buffer A (20 mM potassium phosphate, pH 7.0, 0.3 M KCl, 0.1 mM dithiothreitol (DTT), 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 0.1 mM benzamidine, and 50 μg ml^{-1} NaN_3). The cell suspension was disrupted with a bead-beater (Biospec products, Inc.) filled with 0.5 mm glass beads. The resulting solution was diluted with an equal volume of buffer B (20 mM potassium phosphate, pH 7.0, 0.1 mM DTT, and 0.1 mM PMSF) and centrifuged for 2 h at 10,000 ×g to remove cellular debris. Cellulose phosphate (Sigma) equilibrated in buffer B containing 0.45 M KCl was added to the above cell-free extract and stirred for 12 h. After standing for 1 h, the cellulose phosphate was poured into a column (5 × 50 cm). The packed resin was washed with buffer B containing 0.45 M KCl until the eluent was colorless. AMP deaminase was eluted by washing with buffer B containing 1.5 M KCl. Active fractions were collected and concentrated by ultrafiltration with an Amicon PM 10 membrane. After changing the buffer with buffer B containing 0.9 M KCl, the enzyme solution was loaded on a column (2.8 × 23 cm) of Sephadex G-25 mixed with cellulose phosphate (1:1, wet vol.) equilibrated with buffer B containing 0.9 M KCl and eluted with the same buffer. AMP deaminase fractions were combined and concentrated using ultrafiltration. The above mentioned concentrate was mixed with cellulose phosphate equilibrated in buffer B containing 0.45 M KCl, and stirred for 12 h. After standing for 1 h, the cellulose phosphate was packed into a column (2.8 × 23 cm). The resin was washed with buffer B containing 0.45 M KCl and AMP deaminase was eluted with a linear gradient of 0.45–1.5 M KCl in buffer B. Active fractions were combined, concentrated, dialyzed with buffer B, and stored at -70°C.

Electrophoresis

SDS-PAGE using 8% acrylamide gel was performed according to Laemmli's method [7], and the gel was

stained with Coomassie Brilliant blue R250. Gel isoelectric focusing was done by Righetti's method [16]. The isoelectric point (pI) was estimated by comparing the migration position on the gel with other known proteins (Bio-Rad Lab, Hercules, U.S.A.).

Determination of Molecular Weight of the Enzyme

The molecular mass of the purified native enzyme was determined by gel filtration using fast protein liquid chromatography (FPLC, Pharmacia, Uppsala, Sweden) equipped with a Superose 12HR 10/30 column (Pharmacia) under flow rate of 0.4 ml min^{-1}. The 0.2-ml sample in 20 mM potassium phosphate, pH 7.0, containing 150 mM NaCl was applied to the column, which was calibrated with protein standards (Pharmacia).

N-Terminal Amino Acid Analysis

The purified enzyme was processed on 8% SDS-PAGE gel and electroblotted onto a polyvinylidene difluoride (PVDF) membrane (Bio-Rad Lab). The AMP deaminase band was cut out and its N-terminal amino acid sequence was analyzed by the Edman degradation procedure with Applied Biosystems model 476A Protein/Peptide sequencer (Applied Biosystems Inc., U.S.A.).

RESULTS

Screening of an AMP Deaminase-Producing Strain

Thirty-five yeast strains were screened for AMP deaminase activities and the results are shown in Table 1. Although AMP deaminase was found to be present in all samples of yeast strains examined, there was a large variation in their content. A high specific activity was found mainly in *S. cerevisiae*; in particular, *S. cerevisiae* D KCTC 7248, *S. cerevisiae* KCTC 7244, 7968, and 7905 produced a large amount of AMP deaminase in comparison with other yeast strains. Baker's yeast (*S. cerevisiae* KCTC 7911) from our collections showed a relatively low enzyme activity of 0.07 μmol min^{-1} mg^{-1}, which is similar to that

Table 2. Purification summary of AMP deaminase from *Saccharomyces cerevisiae* D.

Purification step	Total protein (mg)	Total activity (units)	Specific activity (units mg^{-1})	Purification fold	Recovery (%)
Cell extract	3700	860	0.23	1	100
Batchwise cellulose phosphate	110	500	4.6	20	58
Cellulose phosphate/Sephadex G-25	14	230	16	70	27
Batchwise cellulose phosphate-KCl	1.2	150	125	540	17

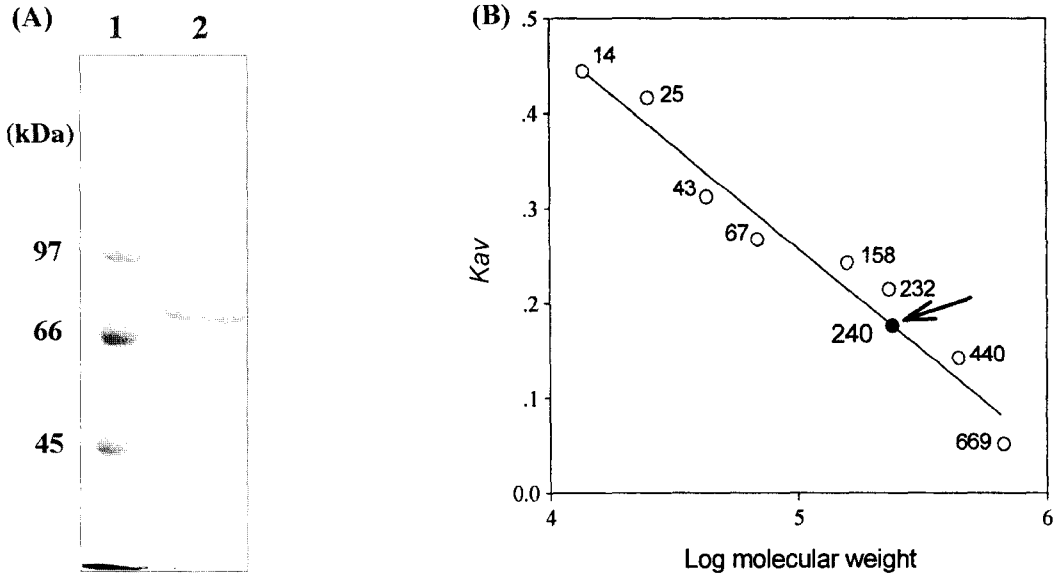

Fig. 1. Molecular weight determination of AMP deaminase using SDS-PAGE and gel filtration chromatography.
The purified enzyme was electrophoresed on an 8% SDS-polyacrylamide gel (A). Lane 1: the molecular mass marker proteins containing phosphorylase b (97 kDa), bovine serum albumin (66 kDa), and ovalbumin (45 kDa). Lane 2: purified AMP deaminase stained with Coomassie Brilliant blue R-250. Gel filtration (B) was performed with FPLC using a Superose 12HR 10/30 column. Molecular marker proteins (○) include ribonuclease (14 kDa), chymotrypsinogen (25 kDa), ovalbumin (43 kDa), albumin (67 kDa), aldolase (158 kDa), catalase (232 kDa), ferritin (440 kDa), and thyroglobulin (669 kDa). AMP deaminase D (●) is indicated by an arrow.

reported by Yoshino *et al.* [24]. Since, *S. cerevisiae* D was a yeast strain that produced the enzyme most abundantly, it was selected as an effective AMP deaminase producer in this work in which its enzyme was purified and characterized.

Purification and Properties of AMP Deaminase D

AMP deaminase D was purified approximately 540-fold and a 17% yield from a cell-free extract of *S. cerevisiae* D by a series of procedures with extraction, batchwise cellulose phosphate, cellulose phosphate/Sephadex G-25, and batchwise cellulose phosphate-KCl as described in Table 2. The final enzyme preparation presented a specific activity of 125 U mg^{-1} of protein.

SDS-PAGE of the purified enzyme revealed a single protein band (Fig. 1A). The molecular mass of the purified AMP deaminase D was calculated to be 80 kDa on the basis of its mobility relative to those of standard calibration proteins. Gel filtration by FPLC of the enzyme indicated that the molecular mass of the native AMP deaminase D was approximately 240 kDa (Fig. 1B). These results suggest that AMP deaminase D might be composed of three identical polypeptides. Isoelectric focusing of the protein gave a single band with an isoelectric point of 6.2 (data not shown).

N-Terminal Amino Acid Analysis

Amino acid sequencing using the Edman degradation method revealed that the N-terminal of AMP deaminase D starts with the following sequence: Ala-Asp-Tyr-Lys-Met-Gln-Met-Phe-Ala-Asp-Asp-Ala. When this N-terminal sequence was compared with other protein sequences in the SWISSPROT database using the FASTA program, the N-terminal sequence matched well with a sequence of baker's yeast AMP deaminase. It was found that the N-terminal amino acid, alanine, made a match with Ala166 in baker's yeast enzyme and nine amino acids were identical among twelve amino acids determined (Table 3).

Substrate Specificity and Kinetic Properties

AMP deaminase D activity towards various nucleotides was examined at 5 mM concentration (Table 4). The enzyme catalyzed the deamination of AMP as a preferred substrate, and the addition of ATP was found to activate the reaction. However, ATP by itself and other nucleotide analogues such as adenosine, adenine, 8-bromoadenine, cAMP, and dAMP were not deaminated. This substrate specificity of this enzyme was similar to those of the AMP deaminase from baker's yeast and animals.

The enzyme activity against AMP concentration was determined. In the absence of any effectors, the AMP saturation curve for AMP deaminase D was sigmoidal with an apparent K_m of 4.1 mM and a V_{max} of 149 μmol min^{-1} mg^{-1} of protein, which indicated that a substrate cooperative effect was present. Hill's interaction coefficient was calculated to be 2.2. In the presence of ATP and ADP, the substrate saturation curve became a typical hyperbolic curve. The apparent K_m values were calculated to be 1.6 and 3.3 mM in 0.1 mM ATP and ADP, respectively, with

Table 4. Substrate specificity of *Saccharomyces cerevisiae* D AMP deaminase.

Substrate (5 mM)	Additive	Relative activity
AMP	0.1 mM ATP	100
AMP	-	76
cAMP	-	0
ATP	-	0
ADP	-	0
Adenosine	-	0
Adenine	-	0
8-Bromoadenine	-	0

The reaction mixture consisted of 5 mM of the indicated substrate, the additive, 50 mM imidazole-HCl buffer, 100 mM KCl, and the enzyme at pH 7.0.

Table 5. Kinetic constants for *Saccharomyces cerevisiae* D AMP deaminase.

Allosteric activator	K_m (mM)	V_{max} (μmol min^{-1} mg^{-1})	n_H^*
None	4.1	150	2.2
0.1 mM ATP	1.6	112	1.0
0.1 mM ADP	3.3	144	1.0

*Hill coefficient.
Kinetic experiments were performed at 30°C by adding the enzyme to the reaction mixture containing 50 mM imidazole-HCl and 100 mM KCl at pH 7.0 in the absence or presence of allosteric activators.

Table 3. Amino acid sequence comparison of the N-terminal region of AMP deaminases from *Saccharomyces cerevisiae* D and baker's yeast.

Organism	Sequence								Source
	161	166	171	176	181	186	191	196	
Baker's yeast	QVRTS	ASYKM	GMLAD	DASQQ	FLDDP	SSELI	DLYSK	VAECR	M30449 (EMBL)
Saccharomyces cerevisiae D		ADYKM	QMFAD	DA- - -	- - - - -	- - - - -	- - - - -	- - - - -	This study
		* * * *	* * *	* *					

*Asterisks indicate the identical amino acids of AMP deaminases from *S. cerevisiae* D and baker's yeast. Underlined amino acids are N-terminal amino acids analyzed from the purified AMP deaminase of baker's yeast.

Hill coefficients that decreased to 1.0 when these activators approached saturation. These results indicate that both ATP and ADP are allosteric activators of AMP deaminase D, with ATP being more effective than ADP. The kinetic parameters are summarized in Table 5.

Effect of pH and Temperature

AMP deaminase D activity was measured in the pH range of 4.5-10.5. Five different buffers of 100 mM ionic strength used were citrate-NaOH (pH 4.5-6.5), imidazole-HCl (pH 6.2-7.6), KH_2PO_4-NaOH (pH 7.0-8.0), borate-NaOH (pH 8.0-10.0), and glycine-NaOH (pH 9.0-10.5) buffers. The pH activity profile has a bell shape with an enzyme optimum pH of 7.0 (Fig. 2A). It corresponds to the usual pH optima of mammalian and the baker's yeast AMP deaminase [24]. In order to determine the pH stability, AMP deaminase D was incubated against various buffers for 2 h at room temperature and the remaining activity was measured at pH 7.0. The enzyme was stable at the pH range of 5.5-9.0, but about 90% of the enzyme activity was lost at the lower and higher pH values of 4.5 and 10.0 respectively (Fig. 2A).

AMP deaminase D activity was measured at various temperatures ranging from 15-70°C. As shown in Fig. 2B, the enzyme displayed the highest activity at 30°C which is similar to those of other AMP deaminases. To test thermal stability, the activity of AMP deaminase D was measured after incubation at various temperatures for 20 min. No loss of activity was observed when the enzyme was incubated up to 45°C, but the activity was drastically reduced above 50°C (Fig. 2B).

Effect of Alkali Metal Ions and Various Chemicals

It has been reported that several AMP deaminases from different sources were activated by monovalent ions [18]. Therefore, to determine how the presence of these ions in the incubation mixture would affect the enzyme activity was an interesting task. It was clear that the enzyme activity was stimulated by some monovalent ions. Among the ions tested, Li^+ and Na^+ were the most effective and K^+ was relatively effective (Fig. 3).

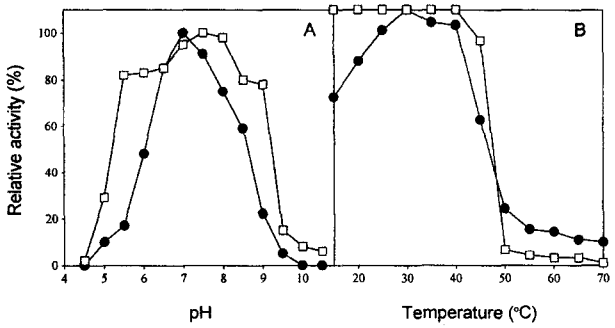

Fig. 2. Effects of pH (A) and temperature (B) on the AMP deaminase activity and stability.

The enzyme was assayed at different pHs (●) and temperatures (●). For the pH stability (□), the enzyme was preincubated in various pH buffers for 2 h and the remaining activity was measured at pH 7.0. For the thermal stability (□), the enzyme was preincubated at various temperatures for 20 min, and the remaining activity was measured at 30°C for 10 min.

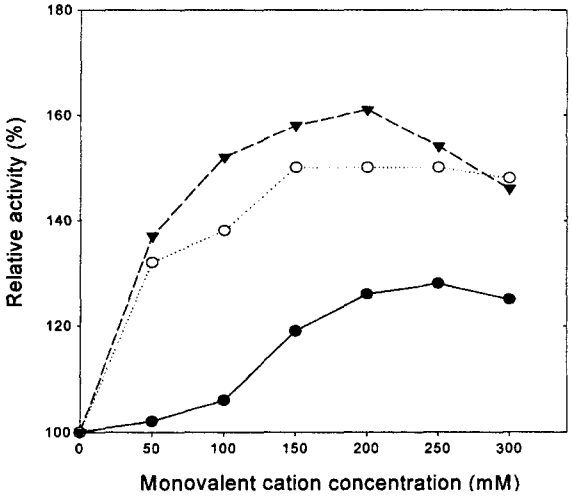

Fig. 3. Effect of univalent cations on the AMP deaminase activation.

The enzyme reaction was carried out in 50 mM imidazole-HCl buffer, with 5 mM AMP substrate at pH 7.0 at different univalent cation (●, K^+; ○, Na^+; ▲, Li^+) concentrations.

Since most nucleotide-requiring proteins bind a metal-ATP complex, effects of divalent metal ions were determined in the presence of ATP. As shown in Table 6, almost complete inhibition was observed with 1 mM Cu^{2+} and Cd^{2+}, while moderate inhibition was seen with 5 mM of Co^{2+}, Mn^{2+}, and Zn^{2+}. Activity was unaffected by $CaCl_2$

Table 6. Effect of divalent metal ions and EDTA on AMP deaminase activity.

Metal	Residual activity (%)	
	1 mM	5 mM
None	100	100
$RbCl_2$	96	96
$CoCl_2$	80	56
$MnCl_2$	80	56
$BaCl_2$	96	92
$ZnCl_2$	89	20
$CuCl_2$	2	0
$MgCl_2$	98	93
$CaCl_2$	99	96
$CdCl_2$	15	1
EDTA	95	91

Enzyme was kept at 30°C for 30 min with various metal ions, and for 15 min with EDTa. Residual activity of the enzyme was determined by the addition of the treated enzymes to the reaction mixture of 5 mM AMP, 50 mM imidazole-HCl buffer, 100 mM KCl, and 0.1 mM ATP at 30°C for 10 min.

Table 7. Effect of detergents on AMP deaminase activity.

Detergent	Concn. (%; w/v)	Residual activity (%)
SDS	1	0
Tween 20	1	97
Tween 40	1	100
Tween 60	1	100
Tween 80	1	96
Triton X-100	1	96
Taurocholic acid	1	0

Enzyme was kept at 30°C for 15 min with detergents. Residual activity of the enzyme was determined by the addition of the treated enzymes to the reaction mixture of 5 mM AMP, 50 mM imidazole-HCl buffer, 100 mM KCl, and 0.1 mM ATP at 30°C for 10 min.

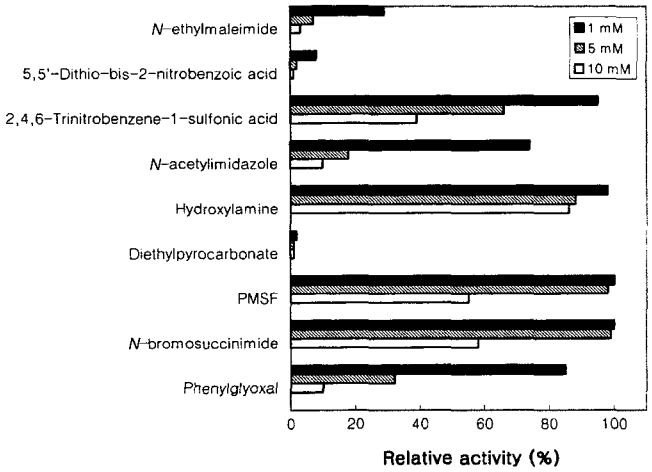

Fig. 4. Effect of chemical modifiers on AMP deaminase activity.
The enzyme was preincubated with various chemical modifiers at room temperature for 30 min.

and $MgCl_2$. Furthermore, EDTA has no effect on the enzyme activity, suggesting that these two metal ions are not necessary for the enzyme activity.

The effects of various detergents on AMP deaminase D activity were compared and the results are shown in Table 7. The enzyme was strongly inhibited by some anionic detergents such as SDS and taurocholic acid, but others were not inhibitory.

Also, an investigation was made to obtain the outcome of a range of relatively group-specific potential inhibitors of the enzyme by determining the residual activity after incubation with the compounds. Incubation of the enzyme with 1 mM diethylpyrocarbonate, 5,5'-dithio-bis-2-nitrobenzoic acid, and *N*-ethylmaleimide led to inhibitions of 99%, 92%, and 71%, respectively (Fig. 4). These results suggest that the involvement of histidine and cysteine residues at or near the active sites of the AMP deaminase D was present, as shown in rabbit skeletal-muscle enzyme [15] and in human uterine smooth muscle [11].

DISCUSSION

The purpose of this study was to screen a strain producing AMP deaminase, which is a natural flavor-material producer, and to investigate its enzymatic properties. AMP deaminase activities were detected in all 35 yeast strains tested, although there was a large variation in their content. This is the first report showing a wide distribution of AMP deaminase enzyme in most of the representative yeast strains. Among them, *S. cerevisiae* D KCTC7248, a nonpathogenic microorganism [6], was selected to be an AMP deaminase producer in our research, based mainly on the highest specific activity in the cell-free extract in which it produced about 3-fold higher specific activity than the baker's yeast (*S. cerevisiae* KCTC7911) did (Table 1).

The molecular mass of the AMP deaminase D from *S. cerevisiae* D was estimated to be 80 kDa on SDS-PAGE, and the holoenzyme molecular mass was shown to be 240 kDa by gel filtration chromatography, which suggests that it is composed of three identical subunits. Various molecular masses ranging from 160 kDa to 330 kDa for several AMP deaminases have been reported [20]. All AMP deaminases from mammalian sources have been shown to be homotetramers [20], with an exception of the native AMP deaminase protein present in rat tissues with a composition of different types of subunits [12]. In the case of baker's yeast, the molecular mass of the native AMP deaminase was estimated to be 360 kDa, composed of four identical molecular subunits (86 kDa) [8]. Therefore, AMP deaminase D seems to be different, in terms of its molecular structure, from other known AMP deaminase including the baker's yeast enzyme.

The AMP deaminase gene from baker's yeast was cloned and sequenced by Meyer *et al.* [9]. According to the sequence data, it was known previously that the purified baker's yeast AMP deaminase was a processed protein. The N-terminal of the purified enzyme started at Tyr-193, which signifies that the upstream 192 amino acids were not present [8]. Interestingly, the N-terminal part of our purified AMP deaminase matched with the Ala-166 in baker's yeast AMP deaminase gene, which suggests that the AMP deaminase D had processed at a different position with the baker's yeast enzyme. In addition, three amino acids, Asp-167, Gln-171, and Phe-173, in the aligned sequence were different between the two enzymes.

The substrate saturation plot for AMP deaminase D is sigmoidal. In the presence of 0.1 mM ATP, an allosteric activator, the enzyme exhibits normal saturation kinetics. The kinetic properties of AMP deaminase D differ significantly from those of the baker's yeast enzyme. In the presence of 0.1 mM ATP, the apparent K_m of 1.6 mM AMP for this enzyme is greater than the K_m of 0.3 mM for the baker's yeast AMP deaminase [8], which implies that the AMP deaminase D has a much lower substrate affinity.

This experiment demonstrated that the *S. cerevisiae* D strain produced a relatively large number of AMP deaminase among the representative 35 yeast strains tested. In addition, it differed in some parts of the primary sequence and in its subunit structure from the AMP deaminase from baker's yeast, in spite of the fact that some properties such as pH, temperature, and substrate specificity were found to be similar. As a result, a comparison based on the gene sequence of the AMP deaminase D is necessary to further understand the detailed structural and biochemical properties of this unique enzyme.

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