Biosci. Biotechnol. Biochem., **72** (10), 2580–2588, 2008

Gene Cloning of α-Methylserine Aldolase from *Variovorax paradoxus* and Purification and Characterization of the Recombinant Enzyme

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Received April 23, 2008; Accepted June 16, 2008; Online Publication, October 7, 2008
[doi:10.1271/bbb.80274]

The α-methylserine aldolase gene from *Variovorax paradoxus* strains AJ110406, NBRC15149, and NBRC15150 was cloned and expressed in *Escherichia coli*. Formaldehyde release activity from α-methyl-L-serine was detected in the cell-free extract of *E.coli* expressing the gene from three strains. The recombinant enzyme from *V. paradoxus* NBRC15150 was purified. The V_{max} and K_m of the enzyme for the formaldehyde release reaction from α-methyl-L-serine were 1.89 μmol min^{-1} mg^{-1} and 1.2 mM respectively. The enzyme was also capable of catalyzing the synthesis of α-methyl-L-serine and α-ethyl-L-serine from L-alanine and L-2-aminobutyric acid respectively, accompanied by hydroxymethyl transfer from formaldehyde. The purified enzyme also catalyzed alanine racemization. It contained 1 mole of pyridoxal 5′-phosphate per mol of the enzyme subunit, and exhibited a specific spectral peak at 429 nm. With L-alanine and L-2-aminobutyric acid as substrates, the specific peak, assumed to be a result of the formation of a quinonoid intermediate, increased at 498 nm and 500 nm respectively.

Key words: α-methylserine aldolase; α-methyl-L-serine; pyridoxal 5′-phosphate; *Variovorax paradoxus*

α-Methylserine hydroxymethyltransferase (EC 2.1.2.7) can catalyze stereospecific hydroxymethyl transfer *via* tetrahydrofolate between α-methyl-L-serine and D-alanine with pyridoxal 5′-phosphate (PLP) as a cofactor.[1–4)]

In contrast, α-methylserine aldolase, a novel enzyme found by our group, is capable of catalyzing interconversion between α-methyl-L-serine and L-alanine by stereospecific hydroxymethyl transfer with PLP as the cofactor, and α-methyl-L-serine and α-ethyl-L-serine can be produced effectively by this enzyme without using tetrahydrofolate.[5)]

Stereospecific transfer of hydroxylmethyl group to α-amino acid appears to be one of the most effective methods of α-alkyl-L-serine production, but there has been few reports on these enzymes.[1–5)] In recent years, there has been growing interest in α-alkyl-α-amino acids and peptides containing these residues.[6–8)] These peptides might act as enzyme inhibitors or antagonists because they provide restricted conformational freedom and resistance against hydrolysis by proteolytic enzymes.

This article describes the cloning of the α-methylserine aldolase gene from three strains of *Variovorax paradoxus*, and the purification and characterization of the recombinant enzyme from *V. paradoxus* NBRC15150.

Materials and Methods

Materials. α-Methyl-L-serine and α-methyl-D-serine were purchased from Acros Organics (Geel, Belgium). α-Methyl-DL-serine, (*S*)-2-amino-1-propanol, (*R*)-2-amino-1-propanol, (*S*)-α-hydroxymethyltyrosine, and (*R*)-α-hydroxymethyltyrosine were from Sigma Chemical (St. Louis, MO). Pyridoxal 5′-phosphate and formaldehyde solution were from Nacalai Tesque (Kyoto, Japan). L-2-Aminobutyric acid and D-2-aminobutyric acid were from Tokyo Chemical Industry (Tokyo, Japan). A formaldehyde test kit was from Wako Pure Chemical Industries (Osaka, Japan). A HiLoad 26/10 Q sepharose column, HiLoad 16/10 Phenyl sepharose column, HiLoad 16/60 Superdex 200 pg column, Gel Filtration LMW Calibration Kit, and Gel Filtration HMW Calibration Kit were from Amersham Bioscience (Piscataway, NJ). Nutrient broth was obtained from BD Biosciences (Franklin Lakes, NJ).

Microorganisms and culture conditions. Variovorax paradoxus strains AJ110406,[5)] NBRC15149, and NBRC15150 were grown on nutrient broth at 30 °C. In order to test the utilization of α-methyl-L-serine, nutrient broth containing 0.2% (w/v) α-methyl-DL-serine was used.

Enzyme assay. α-Methylserine aldolase activity was measured as follows: Formaldehyde release activity was

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Abbreviations: PLP, pyridoxal 5′-phosphate; EDTA, *N*,*N*,*N*′,*N*′-ethylenediaminetetraacetic acid; IPTG, isopropyl-β-D-thiogalactopyranoside

assayed in 50 mM potassium phosphate buffer (pH 7.4), 10 mM α-methyl-L-serine containing 0.1 mM PLP, and an appropriate amount of enzyme in a total volume of 0.1 ml. After incubation at 30 °C for 10 min, the reaction was arrested by the addition of the alkaline solution (5 N sodium hydroxide) from the formaldehyde test kit, and then the formaldehyde concentration was determined according to the instructions in the manual. One unit of enzyme activity was defined as the amount of enzyme required to catalyze the release of 1 micromole of the product per min under the abovementioned conditions.

To perform the enzyme activity assay for α-methyl-L-serine synthesis, a mixture containing 100 mM potassium phosphate buffer (pH 7.4), 0.1 mM PLP, 5–100 mM L-alanine, and 5–50 mM formaldehyde in a final volume of 0.2 ml was used. Incubation was performed at 30 °C for 5 min. The reaction was arrested by thoroughly mixing in 0.2 ml of 20 mM ice-cold copper sulfate. α-Methyl-L-serine was then determined by high-performance liquid chromatography (HPLC) analysis, as described below.

For the alanine racemase assay, a reaction mixture comprising 100 mM D- or L-alanine, 0.1 mM PLP, 50 mM potassium phosphate buffer (pH 7.6), and the enzyme in a total volume of 0.1 ml was used. The reaction was carried out at 30 °C for 10 min, and terminated by the addition of 0.02 ml of 160 mM perchloric acid. Further, 0.02 ml of 160 mM potassium hydroxide was added for neutralization. Then 0.11 ml of the detection solution (3.4 mM 4-aminoantipyrine, 5.7 mM *N*-ethyl-*N*-(3-sulfopropyl)aniline sodium salt, 10.2 U/ml peroxidase, 1.7 U/ml D-amino acid oxidase or L-amino acid oxidase, and 450 mM potassium phosphate buffer, pH 7.6) was added. The reaction was performed at 30 °C for 60 min, and the absorbance was measured at 561 nm to determine the resulting oxidized condensation product.

Cloning of the gene encoding α-methylserine aldolase. The 1.3-kb DNA fragment encoding α-methylserine aldolase from *Ralstonia* sp. AJ110405 was amplified with pSKA04098[5] as the template and synthesized oligonucleotide primers (5′-CGGAATTCGAGAGGAACTGAGCATGTTGAACGC-3′ and 5′-AACTGCAGTTAGCGCAGGAAATGCAGCTTGTTG-3′). Genomic DNA was extracted from *V. paradoxus* strains AJ110406, NBRC15149, and NBRC15150 using Qiagen Genomic-tip 500/G (Qiagen, Hilden, Germany) and digested with *Pst*I. Subsequently, 2–3 kb fragments were collected and ligated to the *Pst*I site of pUC118. *E. coli* JM109 was then transformed with the plasmids, and the library was screened by colony hybridization using the abovementioned probes. Positive clones that carried plasmids pUCB2-B2, pUC15149, and pUC15150 were obtained from strains AJ110406, NBRC15149, and NBRC15150 respectively in the *V. paradoxus* gene libraries.

The expression vector pTV118Nd was constructed as follows: The *Nde*I site was inserted into pTV118N with the Quik Change site-directed mutagenesis kit (Promega, Madison, WI) with primers (5′-CACACAGGAAACAGCATATGGCCATGATTACG-3′ and 5′-CGTAATCATGGCCATATGCTGTTTCCTGTGTG-3′) according to the manual provided by the manufacturer. To construct expression plasmid pTVVHMT01, the gene was amplified with pUCB2-B2 by primers (5′-GGAATTCCATATGCCTGCCGCCGCCCTGCA-3′ and 5′-AACTGCAGTCAGCGCACGATGTAGCGCAGTTCG-3′), digested with *Nde*I/*Pst*I, and inserted into the *Nde*I/*Pst*I site of pTV118Nd. Similarly, pTVVHMT02 (template DNA, pUC15149; primers, 5′-GGAATTCCATATGCCCGCCGCCCTCCAACG-3′ and 5′-AACTGCAGTCAGCGCACGATGTAGCGCAGGCCG-3′), and pTVVHMT03 (template DNA, pUC15150; primers, 5′-GGAATTCCATATGCCCGCAGCCCTTCACCG-3′ and 5′-AACTGCAGTCAGCGCACGATGTAGCGCAGGCCG-3′) were constructed. The DNA sequence of the PCR products was determined to confirm that no error occurred during amplification by the DNA sequencer (ABI-3100, Applied Biosystems, Foster City, CA).

Purification of α-methylserine aldolase. *E. coli* JM109/pTVVHMT03 was precultured in 100 ml of Luria Bertani (LB) medium containing 100 μg/ml of ampicillin sodium salt (amp) at 30 °C for 24 h, and then inoculated into 1 liter of LB medium containing 100 μg/ml of amp and 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). The cells were grown at 37 °C for 16 h, harvested by centrifugation (8,000 × *g*, 10 min), and washed twice with 25 mM Tris–HCl buffer containing 20 μM PLP and 1 mM EDTA (pH 7.4) (buffer A). The cell suspension in buffer A was sonicated with Insonator 201 (Kubota, Tokyo), and centrifuged at 12,000 × *g* for 20 min. To remove the insoluble fraction, the supernatant was ultracentrifuged at 200,000 × *g* for 30 min, and the resulting supernatant was used as the cell-free extract. All procedures were carried out at 4 °C or on ice.

The cell-free extract was applied to a HiLoad 26/10 Q sepharose column equilibrated with buffer A. The enzyme was eluted with a linear gradient of 0–1 M sodium chloride.

The active fractions were collected and dialyzed against 25 mM potassium phosphate buffer containing 20 μM PLP and 1 mM EDTA (pH 7.0) (buffer B). The dialyzed solution was mixed with an equal volume of buffer B containing 2 M ammonium sulfate and applied to a HiLoad 16/10 Phenyl sepharose column that was equilibrated with buffer B containing 1 M ammonium sulfate. The enzyme was then eluted with a linear gradient of 1–0 M ammonium sulfate.

The active fractions were pooled and dialyzed against 2.5 mM potassium phosphate buffer containing 20 μM PLP (pH 7.0). The dialyzed enzyme was loaded onto a Cellulofine HAp column (1.6 × 10 cm) (Seikagaku corporation, Tokyo), equilibrated with the dialysis buffer, and then eluted with a linear gradient of 2.5–250 mM potassium phosphate buffer (pH 7.0).

The purified enzyme was dialyzed against 25 mM potassium phosphate buffer containing 1 mM EDTA and 20 μM PLP (pH 7.4) (storage buffer) and stored at 4 °C for about 1 month. In order to store the enzyme for a longer period (3 months), an equal volume of glycerol was added, and the solution was preserved at −20 °C.

Protein analysis. Protein concentrations were determined by the Bradford method[9] with bovine serum albumin as the standard. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed on a 10–20% polyacrylamide gel (Daiichi Pure Chemicals, Tokyo) with the Precision Protein Marker (Bio-Rad Laboratories, Hercules, CA) as the marker proteins. The native relative molecular mass was determined with a HiLoad 16/60 Superdex 200 pg column with Gel Filtration LMW and Gel Filtration HMW calibration kits.

PLP content. After the purified enzyme was thoroughly dialyzed against 25 mM potassium phosphate buffer containing 1 mM EDTA and 20 μM PLP (pH 7.4), the concentrations of PLP inside and outside the dialysis bag were measured using phenylhydrazine,[10] and the difference between inside and outside was determined as the PLP content of the enzyme.[11]

Absorption spectra. The absorption spectra of the purified enzyme were measured in cuvettes of 10-mm path length at 25 °C with a spectrophotometer (DU800, Beckman Coulter, Fullerton, CA). Each spectrum was taken using the corresponding buffer as the blank.

To prepare apo-form of the enzyme, the enzyme in 25 mM potassium phosphate buffer containing 1 mM EDTA (pH 7.4) (buffer C) was treated with 1 mM hydroxylamine at 25 °C for 30 min, and then dialyzed against buffer C.[11–13]

Sodium borohydride treatment was carried out according to the method of Matsuo and Greenberg.[14] The purified enzyme (0.5 mg/ml) was dialyzed at 4 °C for 30 min against buffer C plus 5 mM sodium borohydride. Then the enzyme was dialyzed twice against buffer C.

When the spectrum of the enzyme with L-alanine was taken, the enzyme (1.0 mg/ml) was extensively dialyzed at 4 °C against buffer C. Then an equal volume of 1–500 mM of L-alanine in the same buffer was added. The spectra data with other compounds were collected in the same manner.

HPLC analysis. α-Methyl-L-serine, α-methyl-D-serine, L-alanine, D-alanine, L-2-aminobutyric acid, and D-2-aminobutyric acid were detected using a high-performance liquid chromatograph equipped with Sumichiral OA-6100 (4.6 × 150 mm) (Sumika Chemical Analysis Service, Osaka, Japan). Detection was performed at 215 nm at 30 °C using 0.5 mM copper sulfate as the mobile phase.

Results

Cloning and expression of the gene encoding α-methylserine aldolase and purification of the recombinant enzyme

When *V. paradoxus* strains AJ110406, NBRC15149, and NBRC15150 were grown in nutrient broth containing 0.2% (w/v) α-methyl-DL-serine, only α-methyl-L-serine was utilized in the medium, although the D-isomer remained almost unutilized after 24 h of culturing. About 0.01 U/mg of formaldehyde release activities from α-methyl-L-serine was detected in the cell-free extracts from these strains.

By screening with a DNA fragment corresponding to α-methylserine aldolase of *Ralstonia* sp. AJ110405 as the probe, pUCB2-B2, pUC15149, and pUC15150 were obtained from the gene libraries of AJ110406, NBRC15149, and NBRC15150 respectively. Sequence analysis of the 1,993-, 1,985-, and 1,990-bp inserts in pUCB2-B2, pUC15149, and pUC15150 respectively, revealed open reading frames (1,323-, 1,320-, and 1,320-bp respectively); their deduced amino acid sequence had 55.5%, 55.3%, and 56.2% similarity respectively to α-methylserine aldolase of *Ralstonia* sp. AJ110405[5] (Fig. 1).

E. coli JM109 harboring pTVVHMT01, pTVVHMT02, and pTVVHMT03 were grown at 37 °C in LB medium containing 100 μg/ml of amp with 0.1 mM IPTG induction. The formaldehyde release activities of the enzyme in the cell-free extracts were at almost same level as or somewhat higher than that from the corresponding wild-type strain (0.009, 0.013, and 0.050 U/mg respectively). In further experiments, the recombinant enzyme from *V. paradoxus* NBRC15150 was purified from the cell-free extract of *E. coli* JM109/pTVVHMT03. A typical summary of the enzyme purification procedure from cell-free extract is shown in Table 1. Heterologously expressed α-methylserine aldolase from *V. paradoxus* NBRC15150 was purified through three steps of column chromatography with 29.4-fold purification. The purified enzyme was homogeneous, as demonstrated by SDS–PAGE analysis (Fig. 2).

Protein analysis

The apparent native molecular mass of the recombinant α-methylserine aldolase was approximately 73 kDa, as determined by gel filtration.

The PLP content of the enzyme was calculated to be approximately 0.8–1.0 mol of the subunit. The enzyme exhibited absorption maxima at 280 nm and 429 nm at pH 7.4, with an A_{280}/A_{429} ratio of approximately 5.4 (Fig. 3). The hydroxylamine-treated enzyme showed no activity, with disappearance of the absorption maximum at 429 nm. By subsequent re-dialysis against a buffer with PLP (25 mM potassium phosphate buffer containing 1 mM EDTA and 20 μM PLP, pH 7.4), 66.7% of the enzyme activity was recovered. Reduction of the enzyme with sodium borohydride also resulted in a loss

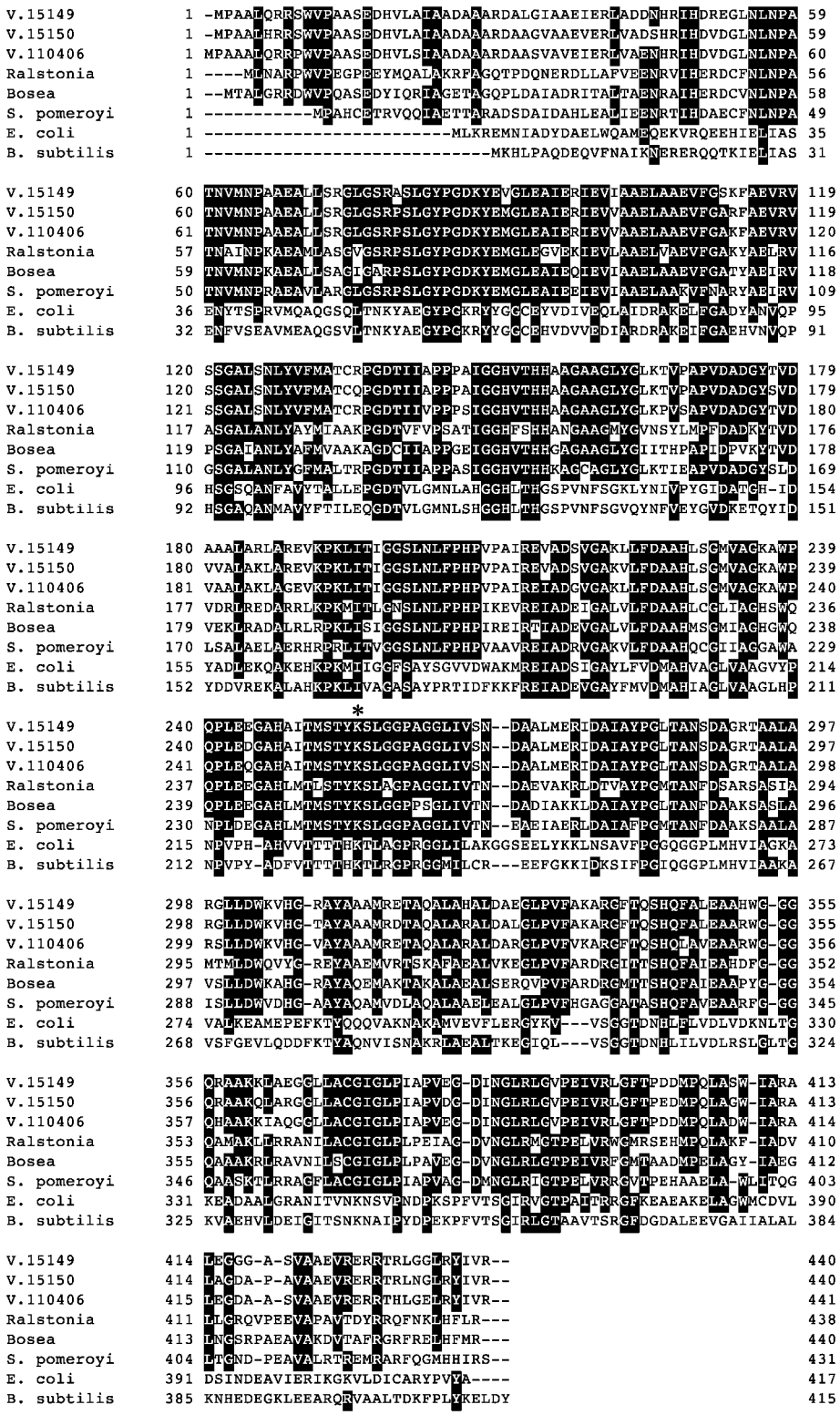

Fig. 1. Primary Structure Alignment.

Putative PLP-binding lysine residue is indicated with an asterisk. V.15149, α-methylserine aldolase from *V. paradoxus* NBRC15149 (GenBank Accession no. AB426474); V.15150, α-methylserine aldolase from *V. paradoxus* NBRC15150 (no. AB426475); V.110406, α-methylserine aldolase from *V. paradoxus* AJ110406 (no. AB426473); Bosea, α-methylserine aldolase from *Bosea* sp. AJ110407 (no. AB426472); Ralstonia, α-methylserine aldolase from *Ralstonia* sp. AJ110405 (no. AB426471); S. pomeroyi, the putative serine hydroxymethyltransferase (glyA-3) from *Silicibacter pomeroyi* (no. AAV96754); E. coli, serine hydroxymethyltransferase from *E. coli* (no. AAA23912); B. subtilis, serine hydroxymethyltransferase from *B. subtilis* (no. I40483).

Table 1. Purification of Recombinant α-Methylserine Aldolase from *Variovorax paradoxus* NBRC15150

Fraction	Total protein (mg)	Specific activity (U/mg)	Total activity (U)	Yield (%)
Cell-free	940	0.050	42.3	100
Q sepharose	58.3	0.89	51.8	122
Phe sepharose	37.4	1.40	52.4	123
Cellulofine HAp	35.2	1.47	51.7	122

Formaldehyde release activity was measured as described in "Materials and Methods."

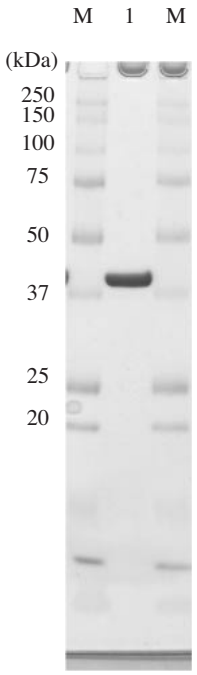

Fig. 2. SDS–PAGE of Purified α-Methylserine Aldolase.
Lane M, standard proteins; lane 1, purified recombinant α-methylserine aldolase from *V. paradoxus* NBRC15150.

of activity, with disappearance of the absorption maximum at 429 nm and an increase in the absorbance at 340 nm, but the addition of PLP (final 20 μM) did not restore the enzyme activity.

Effects of pH and temperature

The optimal pH for the enzyme activity toward α-methyl-L-serine was 7.4–8.0. When the enzyme activity was measured at 30–70 °C by chasing formaldehyde release within 5 min, maximum activity was found at 50 °C (212% of activity relative to that at 30 °C). The enzyme was stable for 30 min when heated to 40 °C in the storage buffer, although 14.8% of the enzyme activity remained after treatment at 50 °C for 30 min.

Substrate specificities

The V_{max} and K_m values were 1.89 U/mg and 1.2 mM respectively toward α-methyl-L-serine, and L-alanine was detected as a product by HPLC analysis, but no D-isomer was detected. The enzyme did not catalyze formaldehyde release from α-methyl-D-serine, L-serine, D-serine, (*S*)-2-amino-1-propanol, (*R*)-2-amino-1-propanol, (*S*)-α-hydroxymethyltyrosine, (*R*)-α-hydroxymethyltyrosine, α-*iso*-butyl-DL-serine, α-*iso*-propyl-DL-serine, or α-benzyl-DL-serine.

Enzyme activity for α-methyl-L-serine synthesis was detected with L-alanine and formaldehyde as the substrates and α-methyl-D-serine was not detected as the product. No activity for α-methyl-L-serine synthesis was detected with D-alanine. The specific activity of the enzyme was 14.8 μmol min^{-1} mg^{-1} with 50 mM L-alanine and 10 mM formaldehyde as the substrates, but the activity was inhibited with formaldehyde of more than 4 mM (Fig. 4). The K_{eq} ([formaldehyde]·[L-alanine]/[α-methyl-L-serine]) was 0.04 when the reaction was chased with 10 mM of α-methyl-L-serine as the substrate at 30 °C in 50 mM of potassium phosphate buffer (pH 7.4).

Moreover, the specific activity of the enzyme was 2.2 μmol min^{-1} mg^{-1} for α-ethyl-L-serine synthesis when 50 mM L-2-aminobutyric acid and 10 mM formaldehyde were used as the substrates. Alanine racemase activity was detected as 0.28 and 0.04 μmol min^{-1} mg^{-1} respectively with D-alanine and L-alanine as the substrates.

Absorption spectra with substrates

The purified enzyme with L-alanine exhibited an absorption maximum at 498 nm at pH 7.4, and the absorbance peak at 498 nm depended on the concentration of L-alanine (Fig. 5A). The absorption peak was at 500 nm when L-2-aminobutyric acid was used (Fig. 5B). The reciprocal plot of the concentration to the absorbance showed linearity, and the affinity constants for L-alanine (Fig. 5A) and L-2-aminobutyric acid (Fig. 5B) were 1.6 mM and 4.2 mM respectively.

An absorption peak was also observed with 50 mM of L-serine, α-methyl-L-serine, but no peak was exhibited around 500 nm with 50 mM of glycine. The relative absorbance was 66.7% with L-serine, 49.7% with L-2-aminobutyric acid, and 13.4% with α-methyl-L-serine when the value with L-alanine was 100% (Fig. 6).

Effects of reagents and metals

At a concentration of 1 mM, the sulfhydryl reagent *N*-ethylmaleimide reduced formaldehyde release activity to 90%. The enzyme was also affected by 1 mM of iodoacetate amide (94%) and 1 mM of iodoacetic acid (92%). The addition of dithiothreitol and 2-mercaptoethanol had no effect on the formaldehyde release activity.

Among the metal ions tested, activity was enhanced by 1 mM of manganese chloride (141%). Neither cobalt chloride nor nickel chloride at a concentration of 1 mM had an effect on the activity. The enzyme was inhibited by 1 mM of copper chloride (5%) and 1 mM of zinc chloride (72%), and slightly inhibited by 1 mM of calcium chloride (89%), 1 mM of magnesium chloride (92%), and 1 mM of ferrous sulfate (91%).

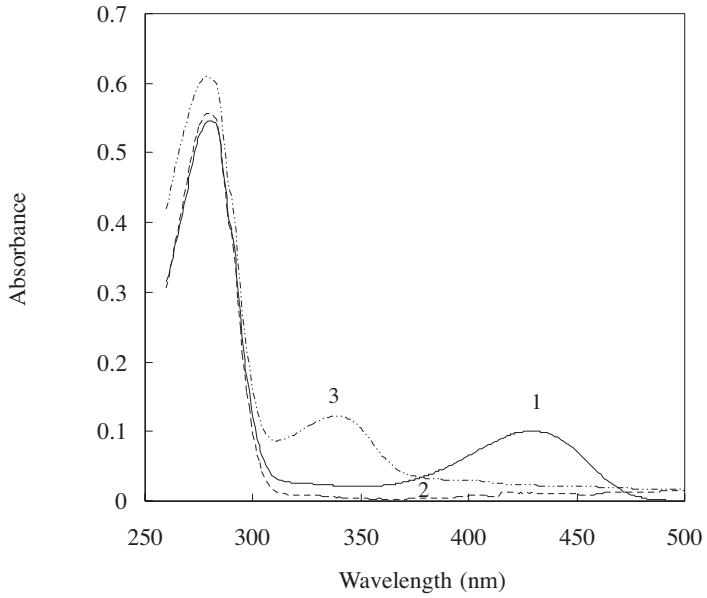

Fig. 3. Absorption Spectrum of Purified α-Methylserine Aldolase.
Absorption spectra were measured as described in "Materials and Methods." Curve 1, the purified enzyme (0.5 mg/ml) in 25 mM potassium phosphate buffer containing 1 mM EDTA (pH 7.4); curve 2, hydroxylamine-treated enzyme; curve 3, sodium borohydride-reduced enzyme.

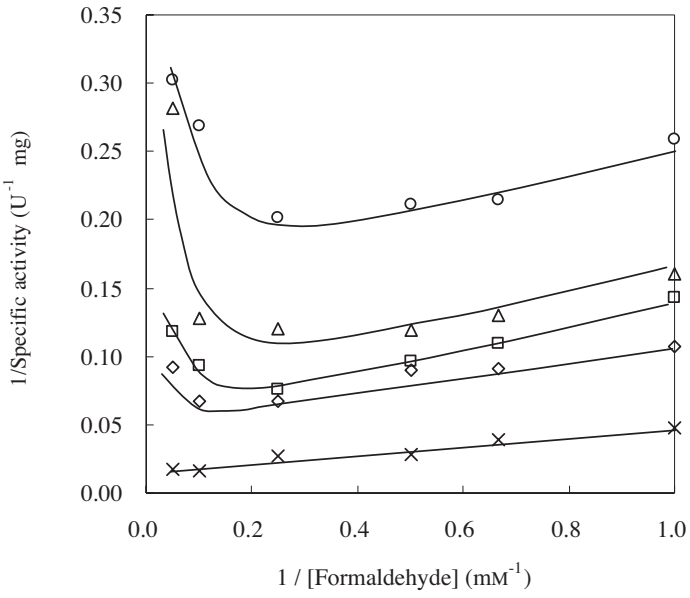

Fig. 4. Inhibition of α-Methyl-L-serine Synthesis Activity by Formaldehyde.
The activities were assayed with 1–20 mM of formaldehyde and 5 mM (○), 10 mM (△), 20 mM (□), 50 mM (◇) and 100 mM (×) of L-alanine as described in "Materials and Methods."

Discussion

Genes encoding α-methylserine aldolase from three strains of *V. paradoxus* were cloned in this study, and these enzymes showed amino acid sequence similarity to those from *Ralstonia* sp. AJ110405 (55.3–56.2%), *Bosea* sp. AJ110407 (62.8–63.0%)[5] and the putative serine hydroxymethyltransferase (*glyA-3*) from *Silicibacter pomeroyi* (65.1–66.9%).[15] Three *glyA* genes encoding serine hydroxymethyltransferase from *S. pomeroyi* were identified by genome sequence analysis, but two of *glyA* genes (*glyA-1* and *glyA-2*) encoded identical amino acid sequences, and showed 30.3–31.7% amino acid sequence similarity to α-methylserine aldolases from *V. paradoxus*. The polypeptide encoded by *glyA-3* from *S. pomeroyi* might have α-methylserine aldolase-like activity.

In addition, α-methylserine aldolases from *V. paradoxus* showed similarity in amino acid sequence to serine hydroxymethyltransferase from *E. coli* (28.7–

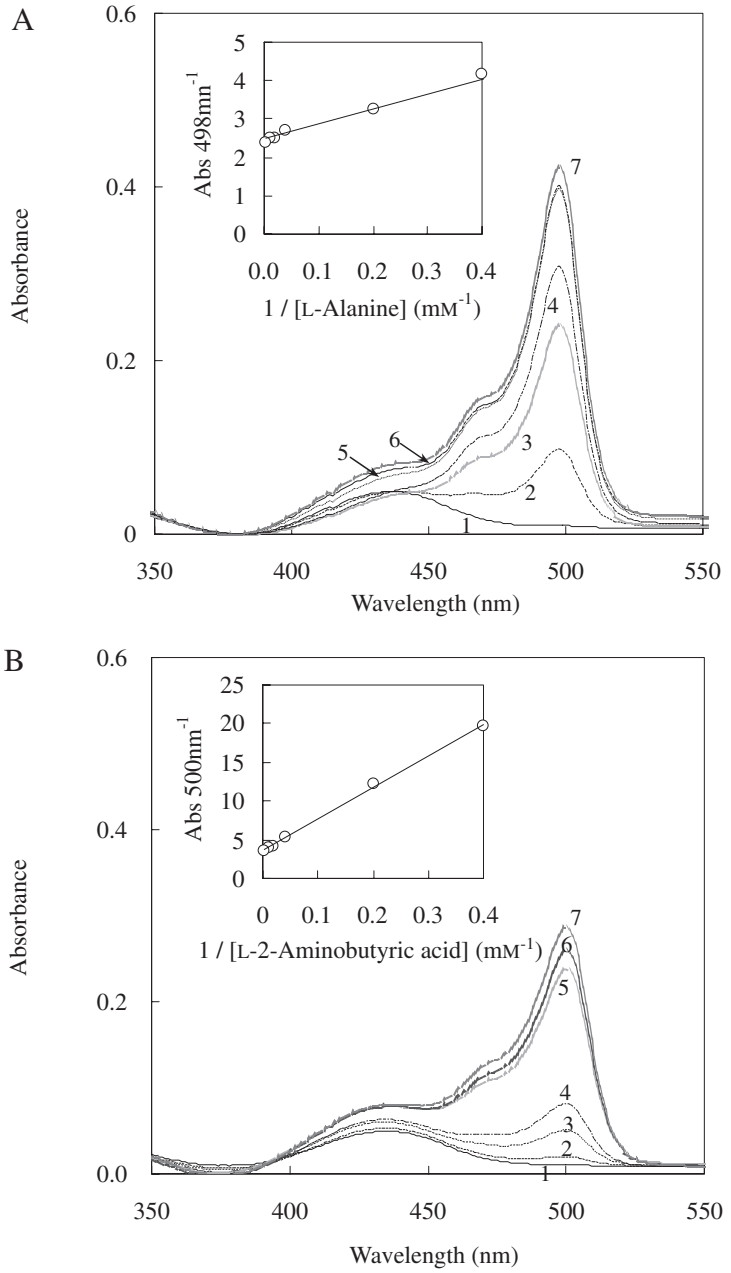

Fig. 5. Absorption Spectrum of Purified α-Methylserine Aldolase with L-Alanine and L-2-Aminobutyric Acid as Substrates.

The purified enzyme (0.5 mg/ml) in 25 mM potassium phosphate buffer containing 1 mM EDTA (pH 7.4) with 0.5–250 mM of L-alanine (A) or L-2-aminobutyric acid (B) was scanned as described in "Materials and Methods." Curve 1, no addition; curve 2, 0.5 mM; curve 3, 2.5 mM; curve 4, 5 mM; curve 5, 50 mM; curve 6, 100 mM; curve 7, 250 mM.

29.1%) and *B. subtilis* (27.4–28.9%) (Fig. 1). These catalyze interconversion between glycine and L-serine *via* tetrahydrofolate. Hydroxymethyl transfer from α-methyl-L-serine with tetrahydrofolate was shown by serine hydroxymethyltransferase, but its product was D-alanine.[16,17] L-Threonine aldolase, capable of catalyzing formaldehyde formation from L-serine,[18,19] appeared to be similar in having aldolase activity toward an amino acid, although its similarity to α-methylserine aldolase could be hardly recognized in amino acid sequence. Remarkably, α-methylserine aldolase from *V. paradoxus* NBRC15150 did not act on L-serine, but catalyzed interconversion between L-alanine and α-methyl-L-serine without tetrahydrofolate, indicating that this enzyme is clearly different from these enzymes, as explained above.

The affinity of the purified enzyme from *V. paradoxus* NBRC15150 for α-methyl-L-serine in the formaldehyde release reaction was higher than that of the enzymes from *Ralstonia* sp. AJ110405 and *Bosea* sp. AJ110407, but the specific activity of the enzyme was almost identical to those of these microorganisms.[5]

This enzyme can catalyze the stereospecific transfer of a hydroxymethyl group from formaldehyde to

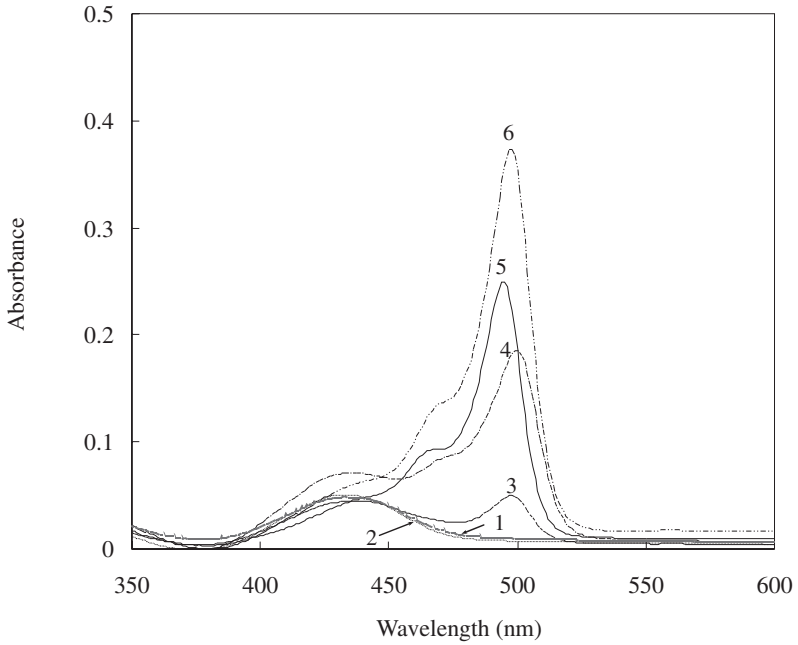

Fig. 6. Absorption Spectrum of Purified α-Methylserine Aldolase with Several Chemical Compounds.
The purified enzyme (0.5 mg/ml) in 25 mM potassium phosphate buffer containing 1 mM EDTA (pH 7.4) with 50 mM of the compounds described below was scanned as described in "Materials and Methods." Curve 1, no addition; curve 2, glycine; curve 3, α-methyl-L-serine; curve 4, L-2-aminobutyric acid; curve 5, L-serine; curve 6, L-alanine.

L-alanine and L-2-aminobutyric acid, leading to the formation of α-methyl-L-serine and α-ethyl-L-serine respectively, but no formaldehyde was released from the other tested compounds containing the α-hydroxymethyl group. This enzyme can also catalyze the racemization of alanine as reported for the PLP-bound enzymes,[20,21] although no alanine racemase assay was performed with the other α-methylserine aldolases in previous studies.[5]

α-Methylserine aldolase showed an absorption peak at 425 nm. The spectral change due to sodium borohydride and to hydroxylamine (Fig. 3) suggests that PLP is bound to the ε-amino group of a lysine, as reported for other PLP-bound enzymes.[21,22] By alignment of the amino acid sequence with serine hydroxymethyltransferases, Lys 255 in α-methylserine aldolase from *V. paradoxus* NBRC15150 of was found to be conserved among the three types of α-methylserine aldolase (Fig. 1), and it is expected to be a PLP-binding residue that forms a Schiff base.

The specific peak was exhibited at around 500 nm with L-alanine, L-2-aminobutyric acid, α-methyl-L-serine, and L-serine, suggesting that the quinonoid intermediate was formed (Figs. 5, 6).[20,23] No formaldehyde release was detected with L-serine as the substrate, but the enzyme with L-serine showed a specific spectrum change at 495 nm. This enzyme appeared to form the quinonoid intermediate with L-serine. Hence, it appeared interesting to investigate the hydroxymethyl transfer to L-serine and its racemization by the enzyme.

Further, enantioselectivity by the enzyme was strict in the aldol reaction, as reported previously.[5] This reaction can be applied in the synthesis of α-methyl-L-serine and its derivatives. Thus far, the role of the enzyme in microorganisms is unknown, and hence further metabolic and structural studies may help to gain insight into the role of α-methylserine aldolase.

Acknowledgments

We thank Ms. Mayuko Yoda for providing technical assistance.

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