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Purification and Characterization of Polyamine Aminotransferase of *Arthrobacter* sp. TMP-1[1]

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Polyamine aminotransferase of *Arthrobacter* sp. TMP-1 was induced by 1,3-diaminopropane (DAP), *N*-3-aminopropyl-1,3-diaminopropane (norspermidine), spermidine, and spermine, but not by putrescine. The enzyme was purified to homogeneity. Its molecular weight and subunit size were 129,000 and 64,000, respectively. Its absorption spectrum had maxima at 280 and 420 nm and a shoulder at about 350 nm, and changes were observed upon the addition of DAP, putrescine, and sodium borohydride. The spectrum and its changes indicated that the enzyme contained pyridoxal-5′-phosphate as the coenzyme. The coenzyme content was found to be 1 mol per mol of subunit. DAP, putrescine, norspermidine, spermidine, and spermine were active amino donors and gave relative rates of 100, 73, 24, 30, and 23%, respectively. Pyruvate was the most active amino acceptor, while 2-ketoglutarate and oxaloacetate were inert. The equilibrium constant of the DAP-pyruvate transamination was 0.34. DAP was suggested to be a minor product of the norspermidine-pyruvate reaction.

Key words: *Arthrobacter*, 1,3-diaminopropane, polyamine aminotransferase, pyruvate, spermidine.

Putrescine, spermidine, and spermine are biogenic polyamines occurring in virtually all living things (*1*). In addition, 1,3-diaminopropane (DAP) is present in certain enterobacteria and *Acinetobacter* strains (*2-4*) as the major polyamine component. *N*-3-Aminopropyl-1,3-diaminopropane (norspermidine) is a major polyamine in *Vibrio* strains (*5, 6*).

The bacterial metabolism of putrescine has been studied extensively and shown to correlate with the metabolism of L-arginine and polyamines (*7*). Spermidine is degraded first by the action of spermidine dehydrogenase [EC 1.5.99.6] in *Serratia* (*8*), *Pseudomonas* (*9*), and *Citrobacter* (*10*) strains. In addition, amine dehydrogenase of a *Pseudomonas* (*11*) and putrescine oxidase [EC 1.4.3.10] of *Micrococcus rubens* (*12*) were reported to act on spermidine, although the induction of these enzymes by polyamines has not been demonstrated. All the above enzymes oxidize spermidine at its imino group to yield 4-aminobutyraldehyde and DAP. This is in contrast to the reaction catalyzed by animal plasma amine oxidase [EC 1.4.3.6], which converts spermidine to *N*-4-aminobutyl-3-aminopropionaldehyde and ammonia (*1, 13, 14*).

No enzymes degrading DAP have been reported, to our knowledge, although the conversion of DAP to β-alanine by *P. aeruginosa* has been reported (*15*). We have isolated strain TMP-1, a Gram-positive bacterium, and reported preliminarily that the DAP-grown cells of this strain contain a novel enzyme, diaminopropane aminotransferase, which catalyzed the transamination from DAP to pyruvate (*16*). This strain was found later to belong to the genus *Arthrobacter*, and hence the strain is designated as *Arthrobacter* sp. TMP-1. An aminotransferase similar to the *Arthrobacter* enzyme was found to be involved in the degradation of agmatine and putrescine in *Nocardioides simplex* IFO 12069 (*Arthrobacter simplex* ATCC 6946, type strain). This enzyme was partially purified and shown to transfer the amino groups of DAP, putrescine, agmatine, cadaverine, spermidine, and spermine to pyruvate (*17*).

This paper describes the induction, purification, and properties of the novel *Arthrobacter* enzyme. This enzyme has been found to participate in the degradation of various diamines and polyamines, and hence we propose to designate it as polyamine aminotransferase.

MATERIALS AND METHODS

Chemicals and Enzymes—Norspermidine (3,3′-iminobispropylamine or *N*-3-aminopropyl-1,3-diaminopropane), spermidine, spermine, sodium pyruvate, and sodium borohydride were purchased from Sigma Chemical (St. Louis, USA); *N,N′*-bis(3-aminopropyl)-1,3-diaminopropane (thermine), *N*-2-aminoethyl-1,3-diaminopropane from Aldrich Chemical (Milwaukee, USA); *N,N′*-bis(3-aminopropyl)-piperazine, bis(3-aminopropyl)ether, and 3-aminopropionaldehyde diethylacetal from Tokyo Kasei Kogyo (Tokyo); *N*-3-aminopropyl-2-aminoethanol from Fluka (Buchs, Switzerland); DAP, sodium pyridoxal-5′-phosphate (PLP),

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Abbreviations: ABAPAL, *N*-4-aminobutyl-3-aminopropionaldehyde; APAPAL, *N*-3-aminopropyl-3-aminopropionaldehyde; DAP, 1,3-diaminopropane; PLP, pyridoxal-5′-phosphate; PMP, pyridoxamine-5′-phosphate.

and *N,N*-bis(2-hydroxyethyl)glycine (Bicine) from Nacalai Tesque (Kyoto). DEAE-Toyopearl 650M, Butyl-Toyopearl 650M, Toyopearl HW-55F, and a TSK-GEL 3000SW column were obtained from Tosoh (Tokyo). 3-Aminopropionaldehyde was prepared by the hydrolysis of the diethylacetal in 0.01 N HCl at 30°C for 30 min. Alanine dehydrogenase [EC 1.4.1.1] of *Bacillus megaterium* (9.2 U/mg) was prepared by the method of Ohashima and Soda (*18*). Partially purified aminopropionaldehyde dehydrogenase [EC 1.2.1.-] (100 U/mg) was prepared from DAP-grown cells of *Arthrobacter* sp. TMP-1; the purification of this enzyme will be reported elsewhere. The M_r standard proteins for gel filtration chromatography were obtained from Sigma, and those for SDS-PAGE (cytochrome *c* and its oligomers) were from Oriental Yeast (Tokyo).

Microorganisms and Culture Methods—*Arthrobacter* sp. TMP-1 was isolated from garden soil and partially characterized in our previous study (*16*). Further chemotaxonomic studies revealed that this bacterium belongs to the genus *Arthrobacter* (T. Yorifuji, unpublished), and thus this strain is described below as *Arthrobacter* sp. TMP-1. *B. megaterium* IFO 13498 was obtained from the Institute for Fermentation, Osaka (Juso, Osaka). Utilization of amines for cell growth was tested in a basal medium (*19*) supplemented with an amine (0.2%, w/v) as the sole carbon and nitrogen source; the tests for amines as the sole nitrogen source were done by the use of 0.05% (w/v) monosodium succinate and 0.45% (w/v) disodium succinate as the carbon sources. Culture was done in 8.0 ml of the medium at 30°C under aeration and the growth was monitored by turbidometry at 570 nm. When amines were tested for the ability to induce the aminotransferase, bacterial cells were grown in the basal medium (700 ml) containing 0.2% (w/v) NH_4Cl as the sole nitrogen source at 30°C for 31 h. A 100-ml portion of the culture was then mixed with 100 ml of 20 mM potassium phosphate buffer (pH 7.0) containing each amine at a concentration of 0.1% (w/v). The mixture was incubated at 30°C for 24 h under aeration and then the cells (about 0.3 g wet weight) were collected by centrifugation.

Cells used for the purification of the DAP-induced enzyme were grown in 15 liters of the peptone-glycerol medium (*20*) at 30°C for 12 h. To the culture, DAP·2HCl was added as an inducer to a concentration of 0.15% (w/v). After 24 h of additional cultivation, the cells were harvested, washed with 0.85% (w/v) NaCl, and stored at −20°C. When the norspermidine-, spermidine-, and spermine-induced enzymes were examined, cells were grown first in 1,400 ml of the peptone-glycerol medium, and the subsequent induction was done under conditions similar to those described above.

Assay of Enzymes—The standard assay mixture (1.0 ml) for the aminotransferase contained 100 mM Tris-HCl buffer (pH 9.0), 5.0 mM DAP, 10.0 mM pyruvate, and enzyme. The reaction was run at 30°C for 30 min and then stopped by the addition of 0.5 ml of 3.0 N HCl. The L-alanine formed was measured enzymatically with NAD^+ and alanine dehydrogenase (*16*). One unit (U) of the aminotransferase is defined as the amount that produces 1 μmol of L-alanine per min.

The aminopropionaldehyde dehydrogenase assay mixture (1.0 ml) contained 100 mM sodium Bicine buffer (pH 9.0), 0.5 mM 3-aminopropionaldehyde, 2.0 mM NAD^+, and enzyme. The reaction was run at 25°C and the A_{340} of the NADH formed in the mixture was monitored. One U of the dehydrogenase is defined as the amount that produces 1 μmol of NADH per min.

The specific activities of the above enzymes are defined as units per mg of enzyme protein (U/mg).

Purification of Polyamine Aminotransferase—Unless stated otherwise, all operations described below were done at 4°C; 20 mM potassium phosphate (pH 7.0) was used as the buffer; centrifugation was run at 15,000×*g* for 30 min. Polyamine aminotransferase was purified as follows. DAP-grown cells obtained from 60 liters of the culture (173 g wet weight) were suspended in 360 ml of 50 mM potassium phosphate buffer (pH 7.0) and disrupted with a sonic disintegrator (Kaijo Denki, Tokyo) at 2-10°C. The sonicate was centrifuged and, to the supernatant (445 ml), 100 ml of 25 mM Tris-HCl buffer (pH 8.0) containing 0.5 M $MnCl_2$ was gradually added. The mixture was stirred for 30 min and the precipitate formed was removed by centrifugation. The supernatant was dialyzed thoroughly against buffer and then put on a column (5×30 cm) of DEAE-Toyopearl 650M equilibrated with buffer. The column was washed thoroughly with buffer containing 0.1 M KCl, and then the enzyme was eluted at a KCl concentration of 0.15 M. The enzyme in the active eluate (440 ml) was dialyzed thoroughly against the buffer and then applied to a DEAE-Toyopearl 650M column (2.2×45 cm). The column was washed and the enzyme was eluted in a manner similar to that described above. The active eluate (150 ml) was mixed with 1.0 M potassium phosphate buffer (pH 7.0) to adjust the phosphate concentration to 50 mM. Then the enzyme was made 1.2 M with solid ammonium sulfate and applied to a Butyl-Toyopearl 650M column (1.8×47 cm) equilibrated with 50 mM potassium phosphate (pH 7.0) containing 1.2 M ammonium sulfate. The enzyme was eluted from the column with a 600-ml linear gradient of 1.2-0.2 M ammonium sulfate dissolved in 50 mM potassium phosphate (pH 7.0). The active eluate (147 ml) was made 1.2 M with ammonium sulfate and then applied to another Butyl-Toyopearl 650M column (1.8×27 cm) equilibrated as described above. The enzyme was eluted with a 300-ml linear gradient of 1.2-0.2 M ammonium sulfate in 50 mM potassium phosphate (pH 7.0). The active eluate (112 ml) was concentrated to about 1.0 ml by ultrafiltration and then applied to a Toyopearl HW-55F column (0.9×111 cm) equilibrated with buffer containing 0.1 M KCl. The enzyme was eluted with the equilibration buffer. The active eluate (10 ml) was diluted with buffer to adjust its KCl concentration to 0.05 M and then applied to a DEAE-Toyopearl 650M column (0.9×32 cm) equilibrated with buffer. The enzyme was eluted with buffer containing 0.15 M KCl. The active eluate (55 ml) was concentrated by ultrafiltration to 5.0 ml, then mixed with 5.0 ml of buffer containing 50% (v/v) glycerol, and stored at −20°C.

The enzymes induced by norspermidine, spermidine, and spermine were partially purified separately as follows. Cells (3.8-4.3 g, wet weight) grown on each polyamine were disrupted and the crude enzyme was prepared in a manner similar to that used in the case of the DAP-grown cells. The enzyme was purified with a DEAE-Toyopearl 650M column (0.9×7.9 cm) and successively with a Toyopearl HW-55F column (0.9×111 cm); the conditions of the column chromatography were similar to those applied in the case of the

DAP-induced enzyme.

Analytical Methods—Amino compounds were measured with ninhydrin (*21*). Amines in growth media were measured with 2,4-dinitrofluorobenzene (*22*). Aldehydes were measured by the method of Friedemann (*23*). Aminoaldehydes were measured with aminopropionaldehyde dehydrogenase as described in our previous paper (*24*). L-Alanine was measured with alanine dehydrogenase (*16*). Protein in crude enzyme was measured by the method of Lowry *et al.* (*25*) with bovine serum albumin as the standard, and that in purified enzyme preparations was calculated from the A_{280} using an extinction coefficient $A^{1\%}_{280}$ of 10.0. The $A^{1\%}_{280}$ of the homogeneous polyamine aminotransferase protein was measured by refractometry (*26*) with bovine serum albumin as the standard. PAGE was done by the method of Davis (*27*). The M_r of polyamine aminotransferase was measured with a TSK-GEL G3000-SW gel filtration column (0.75 × 30 cm) and the subunit M_r of the enzyme was measured by PAGE in the presence of 0.2% SDS (*28*); the standard proteins used are described in the "*Chemicals and Enzymes*" section. TLC was done on cellulose-plastic sheets (Art 5577, E. Merck, Darmstadt, Germany) with a solvent system of methanol/pyridine/12 N HCl/water (80 : 17.5 : 2.5 : 10, v/v/v/v). Amino compounds were located with 1% (w/v) ninhydrin in ethanol/acetone/pyridine (50 : 50 : 1, v/v/v). Carbonyl compounds were located by successive use of 0.3% (w/v) 2,4-dinitrophenylhydrazine in ethanol and 5% (w/v) aqueous KOH. Absorption spectra were taken with a Hitachi U-3210 recording spectrophotometer.

RESULTS

Induction of Polyamine Aminotransferase—Growth of *Arthrobacter* sp. TMP-1 cells and the induction of polyamine aminotransferase were studied with several diamines and polyamines. As shown in Table I, the strain utilized DAP, putrescine, norspermidine, spermidine, and spermine both as sole carbon and nitrogen sources and as sole nitrogen sources. Table I also shows the activities of these amines to induce the enzyme. DAP induced the enzyme most effectively, while putrescine did not induce it. Putrescine and cadaverine induced putrescine oxidase [EC 1.4.3.10] (data not shown).

TABLE I. **Amines as growth substrates and polyamine aminotransferase inducers.** Cells were grown on the amine tested under the conditions given in "MATERIALS AND METHODS." The value of consumption was calculated from the concentration of the amine tested in the growth medium at the early stationary growth phase by taking the initial amine concentration as 100%. The specific activity was measured for the sonic extract of the cells under the standard assay conditions. Diaminoethane, 1,6-diaminohexane, and agmatine did not support growth, and they were not tested for enzyme induction.

Compound	Consumption as CN source (%)	Consumption as N source (%)	Specific activity (U/mg)
Ammonia	–	–	0.006
Norspermidine	52	31	0.059
Spermidine	38	29	0.030
Spermine	100	100	0.051
1,3-Diaminopropane	58	47	0.110
Putrescine	60	46	0.008
Cadaverine	10	10	0.031

Purification and Molecular Properties of Enzyme—The purification of polyamine aminotransferase is summarized in Table II. The final preparation, obtained with a yield of 21%, had a specific activity of 11.6 U/mg. It showed a single protein band on PAGE (data not shown). Its extinction coefficient $A^{1\%}_{280}$ was 7.7. The M_r and subunit M_r of the enzyme were 129,000 and 64,000, respectively, indicating that the enzyme is a homo-dimer.

The absorption spectrum of the enzyme in 125 mM potassium phosphate buffer (pH 8.0) had maxima at 280 and 420 nm and a shoulder at about 350 nm (Fig. 1, curve 1); the A_{280}/A_{420} was 12.0. The spectra in 125 mM potassium phosphate buffer (pH 7.0) and in 125 mM Tris-HCl buffer (pH 9.0) were almost the same as curve 1 (data not shown). When putrescine was added to the enzyme solution (pH 8.0) to a concentration of 0.56 mM, the A_{420} greatly decreased and a new peak with a maximum at about 340 nm appeared (curve 2). This spectral change was completed within 70 s at 25°C. The addition of pyruvate to the curve 2 sample to a concentration of 2.0 mM, followed by incubation for 70 s at 25°C gave the spectrum of curve 3, which was similar to that of the native enzyme. The addition of sodium borohydride to the native enzyme gave

TABLE II. **Purification profile of polyamine aminotransferase.** The experimental details are given in "MATERIALS AND METHODS." The protein of the 3rd DEAE-Toyopearl fraction was measured based on an $A^{1\%}_{280}$ of 7.7.

Step	Protein (mg)	Activity (U)	Sp. act. (U/mg)	Yield (%)
1. Crude extract	8,520	560	0.07	100
2. Mn-treatment	6,630	640	0.10	114
3. DEAE-Toyopearl (1st)	630	390	0.62	70
4. DEAE-Toyopearl (2nd)	160	360	2.25	64
5. Butyl-Toyopearl (1st)	26	280	10.8	50
6. Butyl-Toyopearl (2nd)	21	240	11.4	43
7. Toyopearl HW-55F	10.2	135	13.2	24
8. DEAE-Toyopearl (3rd)	10.4	120	11.6	21

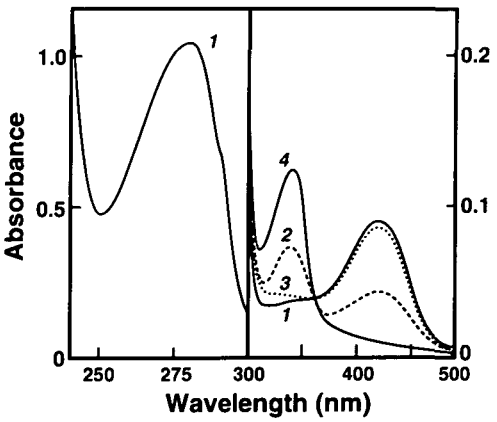

Fig. 1. **The absorption spectra of polyamine aminotransferase.** The solvent of the enzyme was 0.13 M potassium phosphate buffer (pH 8.0), and incubation was done at 25°C. Curve 1, the purified native enzyme, 1.1 mg (8.53 nmol) in 0.8 ml of buffer; curve 2, the enzyme after incubation with 1.0 mM putrescine for 70 s; curve 3, 70 s after the addition of pyruvate to the enzyme for curve 2 to a concentration of 2.0 mM (a correction was made for the absorption by pyruvate); curve 4, after the reaction of the native enzyme, 1.1 mg in 0.8 ml, with 86 nmol of sodium borohydride.

a spectrum with a maximum at 340 nm (curve 4), which had the A_{280}/A_{340} of 8.4. When DAP was added to the native enzyme to a concentration of 0.5 mM, it caused the same spectral change as that observed with putrescine just after the addition. However, further incubation of the enzyme resulted in a slow increase in the A in the 270–310 nm range (data not shown). The cause of this spectral change is unknown. No apo-form of the enzyme was obtained by any of our attempts to dissociate the chromophore, such as incubation of the enzyme with DAP or with hydroxylamine, followed by dialysis. However, the absorption spectrum of the enzyme and its changes under various conditions described above led us to the conclusion that the enzyme contained PLP as the prosthetic group and this coenzyme was converted to pyridoxamine-5′-phosphate (PMP) on incubation with putrescine, because the spectrum of the enzyme and its changes coincided well with those of many aminotransferases that contain PLP as the cofactor (*29*). The concentration of the borohydride-reduced enzyme in the solution for curve 4 in Fig. 1 was 10.7 μM, and its A_{340} was 0.126. It is most likely that the absorption at 340 nm can be attributed to the bound ε-pyridoxyllysine residue and that its molar absorption coefficient is close to the value, 5,800 mol^{-1} at 323 nm, of free ε-pyridoxyllysine (*30*). On the basis of these values, the concentration of the reduced coenzyme was calculated to be 22 μM. This indicates that the native dimeric enzyme protein contains 2 molecules of bound PLP.

Putrescine, when added to a solution of the native enzyme to give amine and enzyme subunit concentrations of 0.5 mM and 12.4 μM, respectively, caused a 43.8% decrease in the A_{420}. From this result, an equilibrium constant of 8.5×10^{-3} was obtained for the reaction,

Subunit-PLP + Putrescine =
Subunit-PMP + 4-Aminobutyraldehyde.

This scheme does not contain no dissociation steps of PLP and PMP from the subunits, because no apoenzyme was obtained by various treatments of the enzyme as described above.

Effect of pH on Activity and Stability of Enzyme—The effect of pH on the enzyme activity was examined under the standard assay conditions except for the use of various buffers. As shown in Fig. 2, the enzyme was most active at pH 9.0.

The enzyme was heated at 75°C for 10 min in each of the buffers (20 mM) used in the experiment shown in Fig. 2 and then the remaining activity was measured under the standard assay conditions. It was found that the enzyme was most stable at pH 7.0-8.0 (in the phosphate buffer), at which the heating resulted in a 50% loss of its activity. Heating of the enzyme at pH 4.0-6.0 resulted in a complete loss of the activity. The enzyme heated at 65°C for 10 min in 20 mM potassium phosphate buffer (pH 7.0) retained the original activity. PLP did not activate the enzyme or enhance its stability under various heating conditions tested (data not shown).

Substrate Specificity and Kinetic Parameters—Various amines and 2-keto acids were tested as amino donors and acceptors, respectively, in the enzyme reaction. As shown in Table III, DAP was the most active amino donor. Putrescine, norspermidine, spermidine, and spermine gave relatively high reaction rates. β-Alanine, 4-aminobutyrate, and other amino acids were inert. The test for the 2-keto acids was done with DAP as the amino donor. Pyruvate was the most active amino acceptor. 2-Ketoglutarate and oxaloacetate were inert.

The apparent K_ms for the amines, which acted as both inducers and substrates of the enzyme, and putrescine were obtained from double reciprocal plots of reaction rate against substrate concentration; the rate was measured under the standard assay conditions with various amino donors at various concentrations (data not shown). The apparent K_ms measured with 10.0 mM pyruvate for DAP,

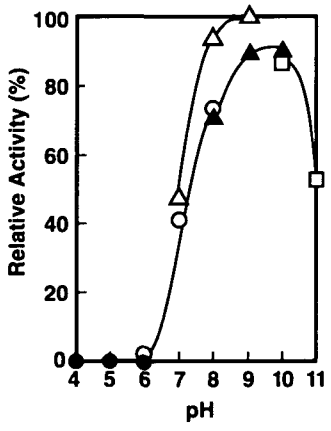

Fig. 2. **Effect of pH on enzyme activity.** The polyamine aminotransferase assays were done under the standard assay conditions except for the buffer used. The buffers were sodium acetate (●), potassium phosphate (○), Tris-HCl (△), sodium Bicine (▲), and sodium carbonate (□).

TABLE III. **Substrate specificity of the enzyme.** The activity with the amino substrates (5.0 mM) and pyruvate (10.0 mM) was obtained by measurement of the rate of L-alanine formation, and that with DAP (5.0 mM) and the carbonyl substrate (10.0 mM) was obtained by measurement of the rate of 3-aminopropionaldehyde formation. The following compounds were inert as amino donors: 1-amino-*n*-alkanes with 2-6 methylene groups, L-ornithine, L-lysine, β-alanine, and 4-aminobutyrate. 2-Ketoglutarate and oxaloacetate were inert as amino acceptors. AEDP, *N*-2-aminoethyl-1,3-diaminopropane; APAE, *N*-3-aminopropyl-2-aminoethanol; BAE, bis-(3-aminopropyl)ether; BAPP, *N,N′*-bis(3-aminopropyl)piperazine.

Substrate	Relative activity (%)	Substrate	Relative activity (%)
Amino donor:			
DAP	100	2-Phenylethylamine	14
Putrescine	73	3-Phenyl-1-propylamine	10
Cadaverine	30	4-Phenyl-1-butylamine	33
1,6-Diaminohexane	10	*N*-Carbamoylputrescine	27
1,7-Diaminoheptane	27	Agmatine	62
1,8-Diaminooctane	20	Norspermidine	24
1,9-Diaminononane	17	Spermidine	30
1,10-Diaminodecane	23	Spermine	23
1-Pentylamine	22	Thermine	21
1-Octylamine	43	AEDP	84
1-Nonylamine	35	APAE	57
Allylamine	10	BAE	26
Benzylamine	19	BAPP	39
Amino acceptor:			
Pyruvate	100	Acrolein	0.4
2-Ketobutyrate	36		

putrescine, cadaverine, norspermidine, spermidine, and spermine were 0.54, 0.18, 0.67, 0.26, 0.17, and 0.20 mM, respectively. The K_m for pyruvate measured with 5.0 mM DAP was 1.9 mM. A calculation from the V_{max} for DAP obtained in the above experiment gave a value of 18 μmol/min as the maximum activity for 1 mg of enzyme. This value and the subunit M_r of the enzyme gave a turnover number of the DAP-pyruvate transamination of 13.6 s^{-1} for one subunit. The rates at various DAP concentrations with fixed pyruvate concentrations of 2.0, 5.0, and 10.0 mM were measured as described for aspartate aminotransferase [EC 2.6.1.1] (*31*) (data not shown). The double reciprocal plots of rate against DAP concentration gave a set of parallel lines. Parallel lines were also obtained by plotting the rates measured at various pyruvate concentrations with fixed DAP concentrations of 1.0, 2.0, and 5.0 mM. This result indicates that the transamination follows a ping-pong bi-bi reaction (*31, 32*).

Inhibitors—The effects of various compounds on the DAP-pyruvate reaction were examined. As shown in Table IV, carbonyl modifiers phenylhydrazine and hydroxylamine, and a thiol group modifier *p*-chloromercuribenzoate all strongly inhibited the enzyme. No marked inhibition was observed with any of the amines, amino acids, or keto acids tested.

Stoichiometry and Time Course Studies of Enzyme Reaction—A reaction mixture (4.0 ml) containing 5.0 mM DAP, 10.0 mM pyruvate, and 0.0025 U of the enzyme was incubated under the standard assay conditions, and samples, 0.5 ml each, were taken every 10 min. It was shown for all samples that the amount of L-alanine formed was almost equimolar to that of 3-aminopropionaldehyde formed, and both were almost equimolar to that of pyruvate consumed (data not shown). The reaction reached equilibrium after incubation of the mixture for 60 min, and the concentrations of L-alanine, 3-aminopropionaldehyde, and pyruvate were found to be 2.4, 2.6, and 7.6 mM, respectively. The equilibrium constant of the reaction was calculated to be 0.34.

Another time course study was done with 1.0 mM each of DAP, norspermidine, spermidine, and spermine in the presence of excess (10.0 mM) pyruvate. As shown in Fig. 3, the concentrations of L-alanine formed with DAP and spermidine were about 1.0 mM even after prolonged incubation. In contrast, with norspermidine and spermine, the L-alanine concentrations after 8 h of incubation were 1.6 and 2.1 mM, respectively. These values are considerably higher than the initial concentrations of the amino donors.

The results of the above experiments indicate that only one of the two terminal amino groups of DAP, as well as that of spermidine, is transferred to pyruvate. The structures of spermidine and norspermidine are similar to each other. While only norspermidine gave L-alanine overproduction, the structural similarity suggests that only one amino group of norspermidine also undergoes the transamination.

TLC Analysis of Products from Norspermidine—The above L-alanine overproduction led us to conduct a TLC study on the norspermidine-pyruvate reaction. A reaction mixture similar to that used for Fig. 3 was incubated and samples were taken at various times and developed on a TLC plate. As shown in lanes 2-4 in Fig. 4, the mixtures incubated for 30 min or more contained the spots of L-alanine formed (R_f, 0.48) and unreacted norspermidine (R_f, 0.19), while the sample taken at 120 min (lane 4) showed four further ninhydrin-positive spots with R_fs 0.23, 0.30, 0.56, and 0.62. When the spots for the lane 4 sample were located with 2,4-dinitrophenylhydrazine, there were the spots of pyruvate (R_f, 0.75) and another with R_f 0.30 (data not shown). The latter spot was developed at the same position as that of the small ninhydrin-positive spot in lane 4 (R_f, 0.30), and hence the responsible compound was suggested to be *N*-3-aminopropyl-3-aminopropionaldehyde (APAPAL). The R_f-0.23 spot was clear in lane 4 and present in lanes 2 and 3. This R_f is close to that of DAP; authentic DAP co-chromatographed with norspermidine (lane 6) gave a slightly higher R_f than that of the spot in lane 4. These observations suggest that DAP is a minor product of the norspermidine-pyruvate reaction.

Enzymes Induced by Polyamines—The *Arthrobacter* enzymes, which are capable of catalyzing the transamination of DAP and are induced by polyamines (Table I), were

TABLE IV. **Effect of various compounds on the enzyme reaction.** The enzyme was preincubated with a test compound in 200 mM Tris-HCl buffer (pH 9.0) at 30°C for 10 min and then its activity was measured under the standard conditions. No marked inhibition was observed with the following compounds (concentration in mM): methylamine (5.0), ethylamine (5.0), 1-propylamine (5.0), 1-butylamine (5.0), 2-ketoglutarate (5.0), 4-aminobutyrate (5.0), β-alanine (5.0), L-lysine (5.0), $CuSO_4$ (2.0), $MgCl_2$ (2.0), $NiSO_4$ (2.0), $FeCl_2$ (2.0), $ZnSO_4$ (2.0), $MnCl_2$ (2.0), EDTA (10.0), and *N*-ethylmaleimide (1.0). DTNB, 5,5'-dithiobis(2-nitrobenzoate); PCMB, *p*-chloromercuribenzoate.

Compound	Conc. (mM)	Relative activity (%)
None	—	100
1-Hexylamine	5.0	77
L-Ornithine	5.0	88
$CoCl_2$	2.0	67
Phenylhydrazine	0.1	74
Phenylhydrazine	1.0	39
Hydroxylamine	0.1	43
Hydroxylamine	1.0	0
DTNB	1.0	58
PCMB	1.0	1

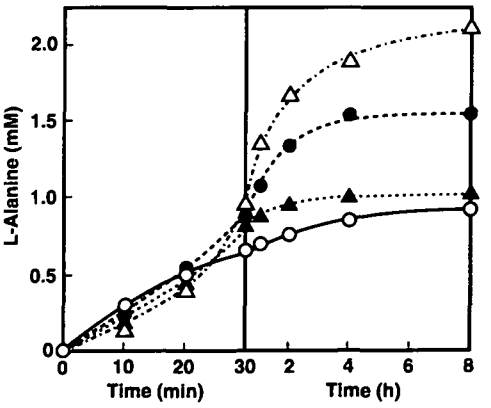

Fig. 3. **Time course study of L-alanine formation from amines and pyruvate.** The reaction mixture (2.0 ml) containing 100 mM potassium phosphate buffer (pH 8.0), 1.0 mM amine, 10.0 mM pyruvate, and 1.1 U of polyamine aminotransferase was incubated at 30°C, and a 0.1-ml portion was withdrawn at the time indicated to measure L-alanine. The amines added were: ○, DAP; ●, norspermidine; ▲, spermidine; △, spermine.

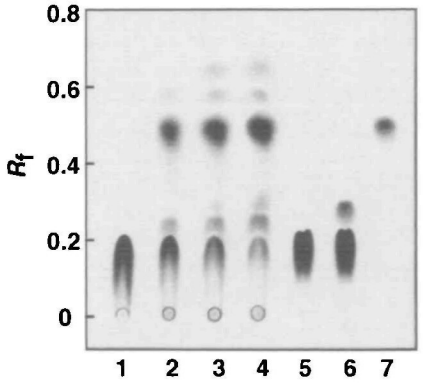

Fig. 4. **TLC of norspermidine-pyruvate reaction mixture.** The mixture (1.0 ml) contained 100 mM potassium phosphate buffer (pH 8.0), 10.0 mM norspermidine, 30.0 mM pyruvate, and 2.4 U of polyamine aminotransferase. The reaction was run at 30°C. Lanes 1, 2, 3, and 4: a 10-μl portion of the mixture after incubation for 0, 30, 60, and 120 min, respectively, was spotted. Lanes 5, 6, and 7: authentic norspermidine, norspermidine plus DAP, and L-alanine were spotted, respectively. The spots were located with ninhydrin. R_f, retardation factor.

partially purified separately by a procedure including two column chromatographic steps (data not shown). The purified enzymes induced by norspermidine, spermidine, and spermine had specific activities of 0.86, 1.47, and 1.43 U/mg, respectively. Their activities were examined under the standard assay conditions by the use of DAP, putrescine, cadaverine, 1,6-diaminohexane, norspermidine, spermidine, and spermine as the amino donors (data not shown). Their amino donor specificities were all found to be very similar to that of the DAP-induced enzyme. This indicates that the enzymes induced by these amines are identical to the DAP-induced enzyme.

DISCUSSION

DAP, norspermidine, spermidine, and spermine, which serve as the growth substrates of *Arthrobacter* sp. TMP-1, induce a single aminotransferase and are active as amino donors for the enzyme reaction. This shows that the enzyme catalyzes the initial steps of the degradation pathways for these compounds. We therefore propose to designate the enzyme as polyamine aminotransferase. Putrescine is active as a substrate, but not as an inducer. Polyamine aminotransferase is the first enzyme found in Gram-positive bacteria to catalyze the initial polyamine degradation steps, to our knowledge. The *N. simplex* strain utilizes agmatine and putrescine, and produces an aminotransferase for their degradation (*17*). The amino donor specificity of the partially purified enzyme (*17*) is similar to that of the *Arthrobacter* enzyme. We have found that norspermidine and spermidine are also inducers of the *Nocardioides* enzyme (T. Yorifuji, unpublished). On the other hand, the *Arthrobacter* strain does not utilize agmatine and seems to degrade putrescine by utilizing putrescine oxidase (Table I). The agmatine degradation *via* putrescine in *A. globiformis* IFO 12137 (ATCC 8010) (*19*) and *Rhodococcus* sp. strain C-x (*33*) involves agmatine deiminase [EC 3.5.3.12], carbamoylputrescine amidase [EC 3.5.1.53], and putrescine oxidase, and no polyamine aminotransferase. All the above bacteria are members of the phylogenetic Gram-positive high G+C bacterial group (*34*). The above observations show the diversity in the metabolism of diamines and polyamines in this bacterial group and in the distribution of polyamine aminotransferase among a certain class (not all) of the strains in this group. The relationship between the diversity of amine metabolism in this group of bacteria including *Arthrobacter* sp. TMP-1 and the ribosomal RNA sequences are under investigation in our laboratory.

The optical properties of the *Arthrobacter* aminotransferase are similar to those of many aminotransferases containing PLP as the cofactor (*29*). Bacterial enzymes with activities toward diamines such as putrescine found so far are also active toward ω-amino acids such as 4-aminobutyrate, but inactive toward DAP (*35-38*). All strains producing enzymes of this class belong to the phylogenetic group of *Proteobacteria*, which is rather remote from the group of Gram-positive bacteria (*34*). ω-Amino acid:pyruvate aminotransferase of a *Pseudomonas* strain, one such enzyme, has an activity toward β-alanine comparable with those toward diamines (*38*). This enzyme and polyamine aminotransferase are similar in that pyruvate is the most active amino acceptor, and both enzymes transfer the amino group bound to the terminal methylene group of a substrate. However, polyamine aminotransferase is unique in that it does not act on ω-amino acids. In addition, the molecular properties of the *Arthrobacter* enzyme are considerably different from those of the *Pseudomonas* enzyme. The latter shows absorption maxima at 280, 345, and 390 nm and has a homo-tetrameric structure (*39*). Its subunit M_r (43,000) (*40*) is much lower than that (64,000) of the former.

The norspermidine-pyruvate reaction resulted in L-alanine overproduction (Fig. 3) and yielded three ninhydrin-positive products besides L-alanine and APAPAL (Fig. 4). One of them seemed to be DAP. Plasma amine oxidase yields *N*-4-aminobutyl-3-aminopropionaldehyde (ABAPAL) from spermidine (*13, 14*). This product undergoes a nonenzymatic β-elimination reaction at higher temperatures, 100°C for example, to yield putrescine and acrolein (*41-44*). This suggests that APAPAL formed from norspermidine by polyamine aminotransferase also undergoes the same reaction to form DAP and acrolein. However, as shown in Fig. 3, the L-alanine concentration of the reaction mixture, which initially contained 1.0 mM norspermidine, reached 1.0 mM after reaction for only 30 min. This observation suggests that the formation of DAP occurs at an early stage of the reaction and that the transamination from DAP to pyruvate causes the L-alanine overproduction. The half-life of APAPAL may be of the same order as that of ABAPAL, which was reported to be 40 min at 37°C (*43*). Thus, nonenzymatic elimination of DAP from APAPAL may not be the major reaction, especially at the early stage, in L-alanine overproduction, and therefore DAP may be formed, at least in part, by the enzyme action. Further results on DAP formation and related reactions will be presented in our accompanying paper (*45*).

REFERENCES

1. Tabor, C.W. and Tabor, H. (1984) Polyamines. *Annu. Rev. Biochem.* **53**, 749-790
2. Hamana, K. and Matsuzaki, S. (1992) Polyamines as a chemotax-

onomic marker in bacterial systematics. *Crit. Rev. Microbiol.* **18**, 261-283
3. Busse, J. and Auling, G. (1988) Polyamine pattern as a chemotaxonomic marker within the *Proteobacter. System. Appl. Microbiol.* **11**, 1-8
4. Yamamoto, S., Tsuzaki, Y., Kishi, R., and Nakao, H. (1991) Occurrence of L-2,4-diaminobutyrate decarboxylase activity in *Acinetobacter. Chem. Pharm. Bull.* **39**, 2451-2453
5. Yamamoto, S., Shinoda, S., Kawaguchi, M., Wakamatsu, K., and Makita, M. (1983) Polyamine distribution in *Vibrionaceae*: norspermidine as a general constituent of *Vibrio* species. *Can. J. Microbiol.* **29**, 724-728
6. Yamamoto, S., Chowdhury, M.A.R., Kuroda, M., Nakano, T., Koumoto, Y., and Shinoda, S. (1990) Further study on polyamine compositions in *Vibrionaceae. Can. J. Microbiol.* **37**, 148-153
7. Cunin, R., Glansdorff, N., Piérard, A., and Stalon, V. (1986) Biosynthesis and metabolism of arginine in bacteria. *Bacteriol. Rev.* **50**, 314-352
8. Tabor, C.W. and Kellogg, P.D. (1970) Identification of flavin adenine dinucleotide and heme in a homogeneous spermidine dehydrogenase from *Serratia marcescens. J. Biol. Chem.* **245**, 5424-5433
9. Hisano, T., Abe, S., Wakashiro, M., Kimura, A., and Murata, K. (1990) Microbial spermidine dehydrogenase: purification and properties of the enzyme in *Pseudomonas aeruginosa* and *Citrobacter freundii. J. Ferment. Bioeng.* **69**, 335-340
10. Hisano, T., Murata, K., Kimura, A., Matsushita, K., Toyama, H., and Adachi, O. (1992) Characterization of membrane bound spermidine dehydrogenase of *Citrobacter freundii. Biosci. Biotech. Biochem.* **56**, 1916-1920
11. Shinagawa, E., Matsushita, K., Nakashima, K., Adachi, O., and Ameyama, M. (1988) Crystallization and properties of amine dehydrogenase from *Pseudomonas* sp. *Agric. Biol. Chem.* **52**, 2255-2263
12. Adachi, O., Yamada, H., and Ogata, K. (1966) Purification and properties of putrescine oxidase of *Micrococcus rubens. Agric. Biol. Chem.* **30**, 1202-1210
13. Tabor, C.W., Tabor, H., and Bachrach, U. (1964) Identification of the amino aldehydes produced by the oxidation of spermine and spermidine in purified plasma amine oxidase. *J. Biol. Chem.* **239**, 2194-2203
14. Tabor, H. and Tabor, C.W. (1972) Biosynthesis and metabolism of 1,4-diaminobutane, spermidine, spermine, and related amines. *Adv. Enzymol.* **36**, 203-268
15. Razin, S., Gery, I., and Bachrach, U. (1959) The degradation of natural polyamines and diamines by bacteria. *Biochem. J.* **71**, 551-558
16. Yorifuji, T., Yonaha, K., Shimizu, E., Ichikawa, H., Matsumoto, F., and Tabata, Y. (1991) Diaminopropane aminotransferase, a novel enzyme of a coryneform bacterium. *Agric. Biol. Chem.* **55**, 2187-2189
17. Kaneoke, M., Shimizu, E., and Yorifuji, T. (1994) Metabolism of L-arginine, agmatine, and related compounds in *Nocardioides simplex. Biosci. Biotech. Biochem.* **58**, 244-249
18. Ohashima, T. and Soda, K. (1979) Purification and properties of alanine dehydrogenase from *Bacillus sphaericus. Eur. J. Biochem.* **100**, 29-39
19. Kaneoke, M., Shiota, K., Kusunose, M., Shimizu, E., and Yorifuji, T. (1993) Function of the arginine oxygenase pathway in utilization of L-arginine-related compounds in *Arthrobacter globiformis* and *Brevibacterium helvolum. Biosci. Biotech. Biochem.* **57**, 814-820
20. Yorifuji, T., Hirabayasi, K., Nagashima, T., Inagaki, N., Shimizu, E., Imada, K., Katsumi, T., and Sawamura, S. (1989) Distribution of the arginine oxygenase pathway among coryneform bacteria. *Agric. Biol. Chem.* **53**, 1103-1110
21. Herman, A.C. and Vanaman, T.C. (1977) Automated micro procedures for peptide separations. *Methods Enzymol.* **47**, 220-236
22. Tabor, H., Tabor, C.W., and de Meis, L. (1971) Chemical synthesis of N-acetyl-1,4-diaminobutane, N^1-acetylspermidine, and N^8-acetylspermidine. *Methods Enzymol.* **17(B)**, 829-833
23. Friedemann, T.E. (1957) Determination of α-keto acids. *Methods Enzymol.* **3**, 414-418
24. Shimizu, E., Tabata, Y., Hayakawa, R., and Yorifuji, T. (1988) Specific measurement of putrescine with putrescine oxidase and aminobutyraldehyde dehydrogenase. *Agric. Biol. Chem.* **52**, 2865-2871
25. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265-275
26. Shimizu, E., Kiguchi, S., and Yorifuji, T. (1989) A simple approximation of weight-based protein measurement with HPLC apparatus. *Agric. Biol. Chem.* **53**, 2801-2803
27. Davis, B.J. (1964) Disc electrophoresis. II. Methods and application to human serum protein. *Ann. N.Y. Acad. Sci.* **121**, 404-427
28. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685
29. Braunstein, A.E. (1973) Amino group transfer in *The Enzymes* (Boyer, P.D., ed.) 3rd ed., Vol. 9, pp. 379-481, Academic Press, New York
30. Dempsy, W.B. and Christensen, H.N. (1962) The specific binding of pyridoxal 5′-phosphate to bovine plasma albumin. *J. Biol. Chem.* **237**, 1113-1120
31. Velick, S.F. and Vavra, J. (1962) A kinetic and equilibrium analysis of the glutamic oxaloacetate transaminase mechanism. *J. Biol. Chem.* **237**, 2109-2122
32. Cleland, W.W. (1970) Steady state kinetics in *The Enzymes* (Boyer, P.D., ed.) 3rd ed., Vol. 1, pp. 1-65, Academic Press, New York
33. Shoeb, S.M., Hirota, M., Shimizu, E., and Yorifuji, T. (1994) Diguanidinobutane (arcaine) degradation in *Rhodococcus* sp. C-x. *Biosci. Biotech. Biochem.* **58**, 859-863
34. Olsen, G.J., Woese, C.R., and Overbeek, R. (1994) The winds of (evolutionary) change: breathing new life into microbiology. *J. Bacteriol.* **176**, 1-6
35. Voellmy, R. and Leisinger, T. (1976) Role of 4-aminobutyrate aminotransferase in the arginine metabolism of *Pseudomonas aeruginosa. J. Bacteriol.* **128**, 722-729
36. Brohn, F. and Chen, T.T. (1971) A single transaminase for 1,4-diaminobutane and 4-aminobutyrate in a *Pseudomonas* species. *Biochem. Biophys. Res. Commun.* **45**, 578-582
37. Kim, K. (1964) Purification and properties of a diamine α-ketoglutarate transaminase from *Escherichia coli. J. Biol. Chem.* **239**, 783-786
38. Yonaha, K. and Toyama, S. (1979) Diamine transamination catalyzed by ω-amino acid:pyruvate aminotransferase of *Pseudomonas* sp. F-126. *Agric. Biol. Chem.* **43**, 1043-1048
39. Morita, Y., Aibara, S., Yonaha, K., Toyama, S., and Soda, K. (1979) Preliminary crystallographic study of ω-amino acid-pyruvate aminotransferase from *Pseudomonas* sp. F-126. *J. Mol. Biol.* **130**, 211-213
40. Yonaha, K., Toyama, S., Yasuda, M., and Soda, K. (1977) Properties of crystalline ω-amino acid:pyruvate aminotransferase of *Pseudomonas* sp. F-126. *Agric. Biol. Chem.* **41**, 1701-1706
41. Tabor, C.W., Tabor, H., McEwen Jr., C.M., and Kellogg, P.D. (1964) Further studies of the aminoaldehydes formed by the enzymatic oxidation of spermine and spermidine. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **23**, 385
42. Alarcon, R.A. (1970) Acrolein. IV. Evidence for the formation of the cytotoxic aldehyde acrolein from enzymatically oxidized spermine or spermidine. *Arch. Biochem. Biophys.* **137**, 365-372
43. Hölttä, E., Pulkkinen, P., Elfving, K., and Jänne, J. (1975) Oxidation of polyamines by diamine oxidase from human seminal plasma. *Biochem. J.* **145**, 373-378
44. Kimes, B.W. and Morris, D.R. (1971) Preparation and stability of oxidized polyamines. *Biochim. Biophys. Acta* **228**, 223-234
45. Yorifuji, T., Kondo, S., Naka, T., Ishihara, T., and Shimizu, E. (1997) Action of polyamine aminotransferase on norspermidine. *J. Biochem.* **122**, 544-549