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Action of Polyamine Aminotransferase on Norspermidine[1]

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The norspermidine-pyruvate reaction catalyzed by polyamine aminotransferase from *Arthrobacter* sp. TMP-1 formed *N*-3-aminopropyl-3-aminopropionaldehyde (APAPAL), L-alanine, 1,3-diaminopropane (DAP), allylamine, and acrolein, and the relative rates of formation of the latter four products were 24, 3.3, 2.3, and 1.2%, respectively, of the rate of the DAP-pyruvate transamination. The identification of APAPAL was done by ^{13}C-NMR after it had been enzymatically oxidized to *N*-3-aminopropyl-β-alanine followed by isolation of the oxidized product. The DAP was also isolated and identified by ^{13}C-NMR. The allylamine and acrolein were identified by HPLC and a specific color reaction with *m*-aminophenol, respectively. In the absence of pyruvate, the enzyme catalyzed the elimination of DAP from norspermidine to yield allylamine, and the addition of DAP to allylamine to yield norspermidine with relative rates of 0.007 and 0.095%, respectively. When allylamine was incubated with the enzyme as the sole substrate, it was converted to *N*-allyl-1,3-diaminopropane and an unidentified product.

Key words: acrolein, 1,3-diaminopropane, elimination and addition, norspermidine, polyamine aminotransferase.

The properties of polyamine aminotransferase of *Arthrobacter* sp. TMP-1 were reported in our previous paper (*1*). 1,3-Diaminopropane (DAP), norspermidine (*N*-3-aminopropyl-1,3-diaminopropane), spermidine, and spermine were effective amino donors for the enzyme reaction, and pyruvate was the most effective amino acceptor. In the presence of excess pyruvate, the amounts of L-alanine formed with norspermidine and spermine were 1.6 and 2.1 times (mol/mol), respectively, as much as the amounts of the amino donors consumed. Such an L-alanine overproduction was not observed with DAP or spermidine, and this indicated that one of the two amino groups of these amines was transferred. In addition, DAP was suggested to be a minor product of the norspermidine-pyruvate reaction (*1*).

Plasma amine oxidase forms *N*-4-aminobutyl-3-aminopropionaldehyde (ABAPAL) from spermidine. Because of its instability, this compound was identified after chemical reduction to *N*-4-aminobutyl-3-aminopropanol (*2*). At a high temperature, this amino aldehydic product undergoes a nonenzymatic elimination reaction to yield putrescine and acrolein, although the rate at 37°C is very low (*3, 4*).

N-3-Aminopropyl-3-aminopropionaldehyde (APAPAL) was expected to be a product of the norspermidine-pyruvate reaction. It is likely that the stability of APAPAL is comparable with that of ABAPAL. It seemed to be possible that DAP was formed from APAPAL by a nonenzymatic elimination reaction, and this compound caused the L-alanine overproduction. However, the L-alanine overproduction occurred at an early reaction stage (*1*), and it was difficult to explain this only in terms of the nonenzymatic elimination of DAP from APAPAL. A part of the DAP seemed to be formed by the catalytic action of the aminotransferase.

This paper reports the elimination of DAP from norspermidine in the presence and absence of pyruvate, its reverse reaction, and other related reactions catalyzed by the enzyme.

MATERIALS AND METHODS

Chemicals and Enzymes—Acrolein was prepared from its diethylacetal obtained from Aldrich Chemical (Milwaukee, USA). *m*-Aminophenol hydrochloride and allylamine were purchased from Nacalai Tesque (Kyoto). Norspermidine (3,3′-iminobispropylamine) was obtained from Sigma Chemical (St. Louis, USA); its trihydrochloride salt was purified by crystallization from ethanol. *N*-(3-Bromopropyl)phthalimide and 3-amino-1-propanol were products of Tokyo Kasei Kogyo (Tokyo). Cation exchange resin AG 50W-X8 was from Bio-Rad Laboratories (Richmond, USA). Other chemicals and enzyme preparations were obtained as described in our previous paper (*1*).

Reaction Rate Measurements—The standard assay conditions for polyamine aminotransferase, and the definition of the enzyme unit (U) were described in our previous paper (*1*). The common assay conditions for the various reactions catalyzed by the enzyme described below were as follows unless stated otherwise. The reaction mixture, 0.5 ml, contained 100 mM potassium phosphate buffer (pH 8.0), an appropriate amount of the enzyme (specific activity, above 10 U/mg), and the specified substrate(s) for each reaction.

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

After incubation of the mixture at 30°C for an appropriate time, a 0.25-ml portion of the mixture was withdrawn and mixed with 0.25 ml of 3 N HCl, and the whole was neutralized with 0.25 ml of 3 N NaOH.

Definition of Relative Rate—The relative reaction rates for various enzyme reactions described below were calculated by taking the rate of the DAP-pyruvate transamination measured in 100 mM potassium phosphate (pH 8.0) as 100%; corrections were made for enzyme concentration. In the standard enzyme assay, 100 mM Tris-HCl (pH 9.0) was used as the buffer (*1*). The rate of the DAP-pyruvate reaction in the phosphate buffer was 67% of that obtained in the standard assay.

Isolation of N-3-Aminopropyl-β-Alanine—Enzymatically formed *N*-3-aminopropyl-β-alanine (APBA) was isolated as follows. The reaction mixture (50 ml) contained 100 mM potassium phosphate buffer (pH 8.0), 0.5 mmol of norspermidine, 3.0 mmol of pyruvate, 1.5 mmol of NAD^+, 7.8 U of polyamine aminotransferase, and 20.8 U of aminopropionaldehyde dehydrogenase. The mixture was incubated at 30°C for 9 h, and the reaction was stopped by the addition of HCl to a concentration of 1.0 N. The mixture was then applied to an AG 50W-X8(H^+) column (1.6×25 cm). The basic components were eluted with a 5,000-ml linear 0-4 N HCl gradient and monitored by TLC. Evaporation of the eluate containing APBA gave 70 mg of a crude preparation. Recrystallization from ethanol as the dihydrochloride salt gave 45 mg of a purified APBA preparation.

Isolation of DAP—The DAP formed in the norspermidine-pyruvate reaction was isolated as follows. The reaction mixture (500 ml) contained 100 mM potassium phosphate buffer (pH 8.0), 5.0 mmol of norspermidine, 10.0 mmol of pyruvate, and 91 U of polyamine aminotransferase. The mixture was incubated at 30°C for 14 h. The reaction was stopped by the addition of HCl to a concentration of 1.0 N. The mixture was then applied to a column (1.6 cm×12.5 cm) of AG 50W-X8(H^+). The basic components were eluted with a 2,500-ml linear 0-4 N HCl gradient. A fraction of this eluate gave a crude DAP preparation (300 mg). Washing with 99% ethanol gave 132 mg of the pure crystals of DAP dihydrochloride.

Isolation of Norspermidine—Norspermidine formed by the enzymatic addition of DAP to allylamine was isolated as follows. The reaction mixture (100 ml) contained 100 mM potassium phosphate buffer (pH 8.0), 1.0 mmol of DAP, 1.0 mmol of allylamine, and 104 U of polyamine aminotransferase. The reaction was run at 30°C for 36 h and then stopped by the addition of 50 ml of 3 N HCl. The mixture was applied to an AG 50W-X8(H^+) column (1.0×13 cm), which was then washed with 100 ml of 1.5 N HCl. The components were eluted with a 700-ml linear 1.5-4.0 N HCl gradient. A fraction of the eluate gave a crude norspermidine preparation (17 mg). Recrystallization from water with ethanol gave 10 mg of the pure trihydrochloride salt of the compound.

Measurement of Rate Constant for Addition of DAP to Acrolein—The reaction mixture (0.5 ml) contained 100 mM potassium phosphate buffer (pH 8.0), 2.0 mM DAP, 2.0 mM acrolein, 10.0 mM NAD^+, and 1.0 U of aminopropionaldehyde dehydrogenase. The rate of nonenzymatic addition reaction to yield APAPAL was determined from the rate of NADH formation, which was monitored at 340 nm with a spectrophotometer (Model 100-40, Hitachi).

HPLC Conditions—Amines in enzyme reaction mixtures were measured by HPLC as follows. The reaction mixture to be analyzed (1.0 ml) was filtered through a membrane (diameter, 0.4 cm; pore size, 0.45 μm), and then a 20-μl portion of the filtrate was injected into an HPLC system equipped with a Guanidino-Pak cation exchange column (JASCO, Tokyo). Elution was done with 0.04 M potassium phosphate buffer, pH 12.4, in the measurement of DAP and allylamine or with the buffer, pH 13.0, in that of norspermidine. The column was used repeatedly without washing with alkaline solution. The amino compounds in the eluate were monitored with a spectrophotometric detector (UVIDEC 100-VI, JASCO) at 570 nm after color development with a ninhydrin reagent (Jas-Nin-Kit, JASCO). The output from the detector was recorded and integrated with a processor (DS-L300, JASCO).

Analytical Methods—The conditions for the analyses of amino compounds with ninhydrin, aldehydic compounds with 2,4-dinitrophenylhydrazine, amino aldehydes with aminopropionaldehyde dehydrogenase, and L-alanine with alanine dehydrogenase, and the conditions for TLC of amines on cellulose-coated plastic sheets were described in our previous paper (*1*). Acrolein was measured by a colorimetric method based on the specific reaction of this compound with *m*-aminophenol in the presence of hydroxylamine (*5*). ^{13}C-NMR analyses were done in D_2O with a Bruker AC250 (62.89 MHz) or a Bruker DRX-500 (129.18 MHz) instrument using sodium 2,2-dimethyl-2-silapentane-5-sulfonate as the external standard.

RESULTS

Identification of Amino Aldehyde from Norspermidine—The norspermidine-pyruvate reaction catalyzed by polyamine aminotransferase produced complex products and the amino aldehydic product from the amino donor could not be isolated because of its instability, as reported for the plasma amine oxidase reaction (*2, 4*). However, NAD^+-dependent aminopropionaldehyde dehydrogenase obtained from *Arthrobacter* sp. TMP-1 (*1*) was found to be active toward the amino aldehyde. Thus, this enzyme was used together with NAD^+ to convert the aldehyde to the corresponding carboxylate.

The reaction mixture contained norspermidine, pyruvate, NAD^+, the aminotransferase, and an excess amount of the dehydrogenase. The amount of the L-alanine formed in the mixture was essentially equal to that of the NADH formed throughout the reaction (data not shown), showing that the aldehydic product was nearly completely dehydrogenated under the conditions applied. The relative rate of the aldehyde formation calculated from the rate of the NADH formation was 21.6%. In our previous study, the relative rate of 24.0% was obtained for the L-alanine formation in the absence of the dehydrogenase and NAD^+ (*1*). These results indicate that the amino aldehyde, as well as L-alanine, is a major product of the reaction. The reaction mixture yielded 45 mg of a crystalline preparation of the carboxylate. Its ^{13}C-NMR spectrum in D_2O at 62.89 MHz showed six carbon signals and was compatible with that it was APBA dihydrochloride (Fig. 1). Its ^1H-NMR spectrum supported this identification (data not shown). Thus the amino aldehyde formed as the major reaction product was identified as APAPAL.

Identification of DAP as a Norspermidine-Pyruvate Reaction Product—The reaction mixture contained 5.0 mmol of norspermidine, 10.0 mmol of pyruvate, and the aminotransferase. When the reaction was stopped by adding HCl after incubation for 14 h, the mixture contained 3.8 mmol of L-alanine. The mixture yielded 132 mg of the hydrochloride salt of an amine preparation, which gave a single ninhydrin-positive spot on TLC. Its ^{13}C-NMR spectrum at 62.89 MHz, which showed two signals at chemical shifts of 27.6 and 39.4 ppm, was essentially the same as that of authentic DAP dihydrochloride.

Time Course Study of Reaction of Norspermidine with Pyruvate—Norspermidine, pyruvate, and polyamine aminotransferase were incubated in a mixture under common assay conditions, and samples were withdrawn from the reaction mixture at various times. As shown in Fig. 2, the concentrations of L-alanine, DAP, allylamine, and acrolein increased linearly up to 5 min after the start of the reaction, and the relative rates of their formation were found to be 24, 3.3, 2.3, and 0.9%, respectively. The rate of formation of DAP was therefore about 13% of that of L-alanine. In this experiment, DAP and allylamine were identified by TLC and HPLC. Acrolein was identified and measured by the *m*-aminophenol method (5).

Elimination of DAP from Norspermidine without Pyruvate—Norspermidine was stable under physiological conditions, that is, we found no ninhydrin-positive product derived from it after prolonged incubation at 30°C in 100 mM potassium phosphate buffer (pH 8.0) without any enzyme. This compound, 5.0 mM, was incubated with 10.1 U of the aminotransferase for 30 min under the common assay conditions and then the products were analyzed by HPLC. The chromatogram contained peaks due to DAP and allylamine (data not shown). The relative rate of DAP formation was found to be 0.007%. This value is considerably lower than that, 3.3%, observed in the presence of pyruvate (Fig. 2).

Addition of DAP to Allylamine—A reaction mixture (100 ml) containing DAP, allylamine, and the aminotransferase was incubated under the common assay conditions, and a reaction product (10 mg) was isolated under the conditions described in the "MATERIALS AND METHODS" section. Its ^{13}C-NMR spectrum at 62.89 MHz showed three carbon signals at the chemical shifts of 26.5, 39.3, and 47.4 ppm. This spectrum was identical with that of authentic

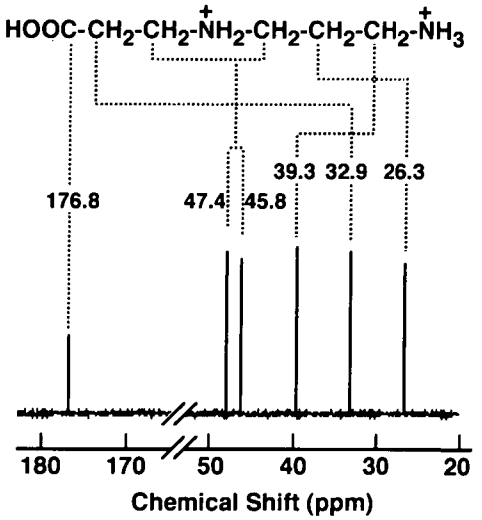

Fig. 1. **^{13}C-NMR spectrum of isolated *N*-3-aminopropyl-β-alanine.**

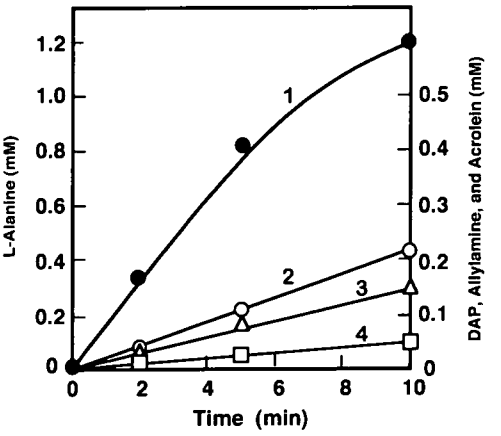

Fig. 2. **Time course study of reaction of norspermidine in the presence of pyruvate.** The reaction mixture (5.0 ml) contained 100 mM potassium phosphate buffer (pH 8.0), 5.0 mM norspermidine, 10.0 mM pyruvate, and 5.0 U of polyamine aminotransferase. The reaction was run at 30°C. At the time indicated, a 1.0-ml portion of the mixture was withdrawn and analyzed for L-alanine (●), DAP (○), allylamine (△), and acrolein (□).

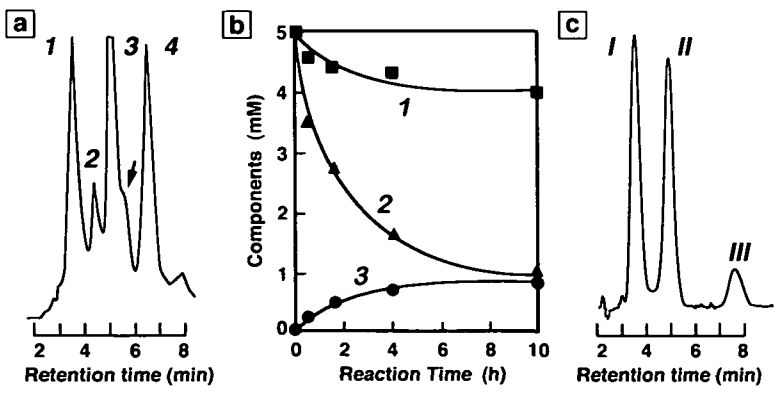

Fig. 3. **HPLC (a) and time course (b) studies of DAP-allylamine reaction, and HPLC of allylamine-alone reaction products (c).** a: The reaction mixture (1.0 ml) containing 5.0 mM DAP, 5.0 mM allylamine, and 10.6 U of the enzyme was incubated at 30°C for 24 h, and then analyzed by HPLC with 0.04 M potassium phosphate buffer, pH 13.0, as the eluate. Attribution of peaks: 1, unknown compound; 2, allylamine; 3, DAP; 4, norspermidine. b: The components of the above reaction mixture were measured by HPLC at the time indicated: curves 1, DAP; 2, allylamine; 3, norspermidine. c: The reaction mixture containing 5.0 mM allylamine as the sole substrate was incubated for 4 h; other conditions were the same as those in experiment a. HPLC was run with 0.04 M potassium phosphate buffer, pH 12.4. Peaks I, unknown compound; II, allylamine; III, unknown compound.

norspermidine trihydrochloride.

In another experiment, DAP, allylamine, and the enzyme were incubated under the common assay conditions and the components of the reaction mixture were monitored by HPLC. As shown in Fig. 3a, the HPLC of the mixture run with the pH 13.0 buffer showed the formation of norspermidine (Fig. 3a, peak 4), the peak of which was separated from those of the allylamine (peak 2) and DAP (peak 3). Figure 3b shows the relationships between the reaction time and the concentrations of norspermidine, DAP, and allylamine in the reaction mixture. The norspermidine concentration increased for 4 h in the initial part of the reaction with a relative rate of 0.095%, and then its formation decelerated (curve 3). Curves 1 and 3 showed that the consumption of DAP and the formation of norspermidine proceeded with a 1:1 stoichiometry. In contrast, the decrease in the concentration of the allylamine in 10 h was about 4 times that of DAP (curve 2), and the mixture in the final reaction stage contained considerable amounts of unknown products (Fig. 3a, peak 1 and a shoulder of peak 3, indicated by an arrow at the retention time of 5.5 min). This observation suggested that a large part of the consumed allylamine was converted to the unidentified products.

Figure 4 shows the result of TLC analysis of the ninhydrin-positive components of the above reaction mixture after incubation for 24 h. The formation of norspermidine was demonstrated by the spot that appeared at R_f 0.22 (lanes 2-4). The spots of the DAP and allylamine were located at R_fs 0.32 and 0.78, respectively.

Reaction of Allylamine—The above result (Fig. 3b) suggested that the unknown products were formed from allylamine alone. Thus, a reaction mixture containing allylamine as the sole substrate was incubated and the products were examined by HPLC with the pH 12.4 buffer; the other experimental conditions were similar to those in the experiment shown in Fig. 3a. The time course of the allylamine concentration was very similar to curve 2 of Fig. 3b (data not shown). Figure 3c illustrates the elution of the components of the mixture after incubation for 4 h. There were three peaks at the retention times of 3.6, 4.9, and 7.6 min, and their peak areas were 44, 46, and 10% of the total peak area, respectively. Peaks I, II, and III in the figure corresponded to peaks 1, 2, and the shoulder of peak 3 in Fig. 3a, respectively, and peak II was attributed to unreacted allylamine (data not shown). This result indicated that the same products as the unknown compounds found in the former experiment were formed from allylamine alone and gave peaks I and III.

The possibility that *N*-allyl-1,3-diaminopropane (ALDP) was one of the products formed by the addition of allylamine to allylamine was examined as follows. ALDP was synthesized from allylamine (2.0 mmol) and *N*-(3-bromopropyl)phthalimide (2.0 mmol) by the method of Oshima (*6*). The ^{13}C-NMR spectrum (129.18 MHz) of the prepared amine proved that it was ALDP dihydrochloride; its 6 carbons showed chemical shifts of 26.4, 39.3, 46.4, 52.3, 126.7, and 129.8 ppm. HPLC of ALDP gave a peak at the retention time of 7.6 min, which was the same as that of peak III in Fig. 3c. This showed that ALDP was one of the products. The other, which gave peak 1 in Fig. 3a, as well as peak I in Fig. 3c, could not be identified. The possibility that 3-amino-1-propanol was formed by the addition of water to allylamine was ruled out since this compound eluted at a retention time of 4.4 min on HPLC at pH 13.0.

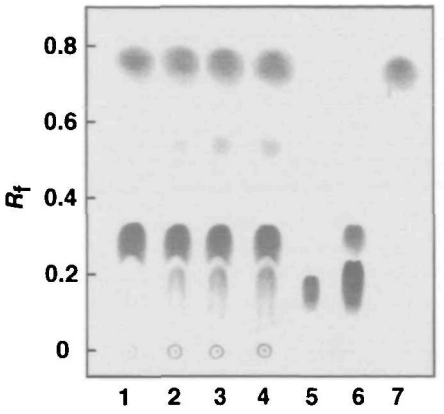

Fig. 4. **TLC of the DAP-allylamine reaction mixture.** The composition of the reaction mixture and the reaction conditions were the same as those for Fig. 3b. Lanes 1, the mixture at the start of the reaction; 2, after 6 h; 3, after 12 h; 4, after 24 h; 5, norspermidine; 6, a mixture of norspermidine and DAP; 7, allylamine. R_f, retardation factor.

Reaction of DAP, Acrolein, and L-Alanine—Our preliminary experiments showed that norspermidine, as well as allylamine, was formed in a reaction mixture containing DAP, acrolein, L-alanine and the enzyme, and that the nonenzymatic addition of DAP to acrolein to form APAPAL proceeded at a considerable rate. These observations suggested two possible routes for the norspermidine formation; Route A, in which the transamination-addition reaction catalyzed by the enzyme yielded norspermidine without the formation of free APAPAL as the intermediate, and Route B, in which the transamination from L-alanine to APAPAL, formed nonenzymatically from acrolein and DAP, yielded the product.

The rate constant k at 30°C for the nonenzymatic APAPAL formation was determined to be 4.3 $min^{-1} \cdot M^{-1}$ by measuring the rate of NADH formation in the reaction mixture containing DAP, acrolein, NAD^+, and aminopropionaldehyde dehydrogenase; the experimental details are described in "MATERIALS AND METHODS." Then mixtures (0.5 ml each) containing 2.0 mM DAP, 2.0 mM acrolein, 10 mM L-alanine, and various amounts of the aminotransferase were incubated under the common assay conditions. When the mixture contained 0.1 U or more of the enzyme and was incubated for 30 min, it contained 0.36 mM norspermidine and 0.30 mM allylamine. From the k value and the concentrations of the substrates, the concentration of APAPAL nonenzymatically formed in 30 min was anticipated to be 0.5 mM. Therefore, if the contribution of Route A were considerable and if excess enzyme were present, the concentration of norspermidine formed after 30 min should exceed 0.5 mM. However, the result of the experiment showed that this is not the case. Thus, the contribution of Route A to norspermidine formation remains obscure.

DISCUSSION

Polyamine aminotransferase of *Arthrobacter* sp. TMP-1 yielded 1.6 and 2.1 mol of L-alanine per 1 mol each of

norspermidine and spermine, respectively, in the presence of excess pyruvate (*1*). DAP was suggested to be formed as a minor product in the norspermidine-pyruvate reaction (*1*). Overproduction of L-alanine was scarcely observed with DAP or spermidine. The structures of norspermidine and spermine are similar to each other in that both have 1-aminopropane moieties on both sides of their linear molecules, and norspermidine is simpler than spermine. Therefore, in this study, we examined the reactions of norspermidine catalyzed by the enzyme. This paper presents evidence for the formation of APAPAL, DAP, allylamine, and acrolein, besides L-alanine, in the norspermidine-pyruvate reaction. It is clear that the molecule of norspermidine is cleaved in the reaction, although the rate is low.

Plasma amine oxidase forms ABAPAL from spermidine, and the product undergoes nonenzymatic elimination to form putrescine and acrolein (*2-4*). There is a possibility that APAPAL also undergoes nonenzymatic elimination to form DAP and acrolein, and this causes the L-alanine overproduction in the norspermidine-pyruvate reaction. However, we conclude that the minor products of the reaction, at least in part, were formed by the catalytic action of the aminotransferase, on the basis of the following observations: (i) even in the absence of pyruvate, the enzyme catalyzes the elimination of DAP from norspermidine to yield allylamine, (ii) the enzyme catalyzes the addition of DAP to allylamine to yield norspermidine, and (iii) in the course of the reaction, DAP, allylamine, and acrolein are formed without any lag phase (Fig. 3). The half-life of ABAPAL formed in the amine oxidase reaction was reported to be 137 min at pH 7 and 27°C (*4*). The half-life of APAPAL, which we estimated from the rate of DAP formation at pH 8 and 30°C, was 480 min; the preparation tested contained some impurities, but no detectable DAP (data not shown). This result suggests that APAPAL is rather stable in the absence of the enzyme, and thus the third observation above supports our conclusion.

Pyridoxal-5′-phosphate (PLP) was shown to be bound to polyamine aminotransferase (*1*). This coenzyme is present in most aminotransferases [EC class 2.6.1] found so far. Several PLP-containing enzymes other than aminotransferases catalyze transamination at lower rates; aspartate 1-decarboxylase [EC 4.1.1.11] (*7*) and arginine racemase [EC 5.1.1.9] (*8*) are examples. Many PLP-containing enzymes have been found to catalyze elimination reactions (*9*). However, polyamine aminotransferase is unique, to our knowledge, in that its major transamination activity is accompanied with the minor elimination-addition activity.

The generally accepted reaction mechanisms for PLP-containing enzymes (*9-11*) can explain most of the reactions catalyzed by polyamine aminotransferase in the absence of pyruvate, as shown in Scheme I in Fig. 5, although this report does not provide any direct evidence in this regard. The scheme works as follows. Norspermidine reacts with the enzyme-bound PLP (PLP-E) to form a Schiff base intermediate (Intermediate I). A part of this is then cleaved to yield APAPAL and the enzyme-bound pyridoxamine-5′-phosphate (PMP-E), while the other part undergoes elimination to yield DAP and an allylamine-PLP Schiff base (Intermediate II). A part of Intermediate II is hydrolyzed to allylamine and PLP-E, and the other part yields acrolein and PMP-E. The addition of DAP to allylamine to form norspermidine is the reverse of this reaction sequence. The addition of allylamine to Intermediate II yields an ALDP-PLP intermediate, which then yields ALDP. The transamination from L-alanine (10.0 mM) to acrolein (5.0 mM) was found to proceed at a relative rate of 2.7% (data not shown), and this seems to reflect the formation of Intermediate II.

The DAP formation from norspermidine without pyruvate proceeded at the relative rate of 0.007%, which was considerably lower than the value, 3.3%, measured in the presence of pyruvate. To account for this discrepancy, it was initially suspected that PMP-enzyme incapable of catalyzing the elimination reaction was formed by the reaction of PLP-enzyme with norspermidine, and pyruvate affected the equilibrium to give a higher concentration of the active PLP-enzyme. However, the rate of the addition of DAP to allylamine, as well as that of the consumption of allylamine in the allylamine-alone reaction, was not enhanced by the addition of pyruvate or acrolein (data not

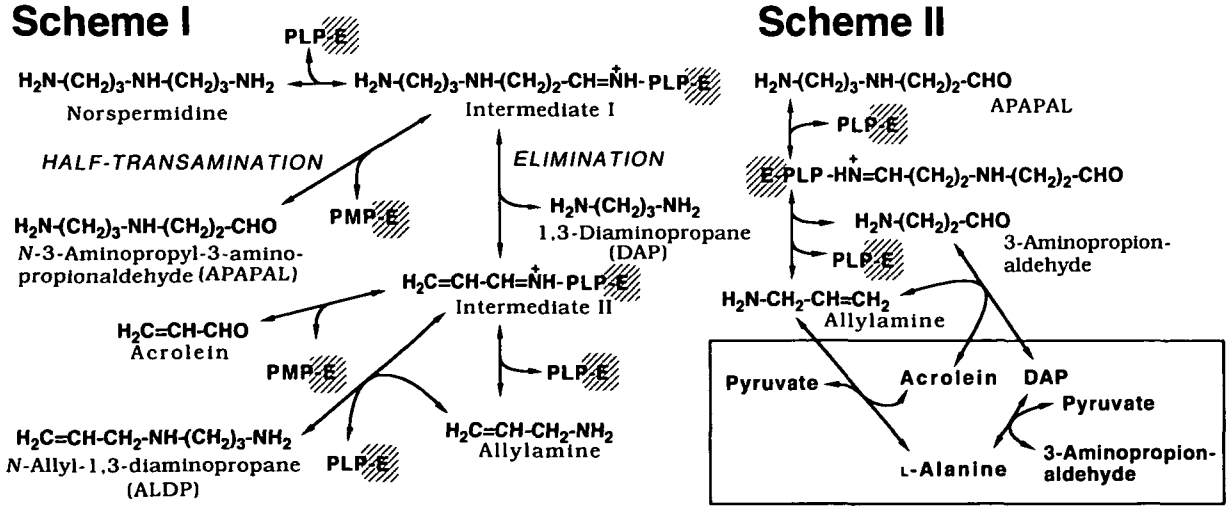

Fig. 5. **The proposed reaction schemes.** Scheme I, for the reactions of norspermidine in the absence of pyruvate; Scheme II, for the formation of DAP and allylamine in the norspermidine-pyruvate reaction.

shown). Allylamine was shown not to act as a suicide substrate of the enzyme (data not shown). Therefore, another route for the formation of DAP and allylamine was suggested to operate only in the presence of pyruvate. Scheme II in Fig. 5 shows a possible set of reactions. This scheme operates as follows. APAPAL, which is formed at a higher rate when pyruvate is present, reacts with PLP-E to form a Schiff base intermediate. This intermediate undergoes elimination to yield allylamine and 3-aminopropionaldehyde at a higher rate than that of the elimination reaction of Intermediate I in Scheme I. The transamination between these products may form DAP and acrolein. The L-alanine overproduction in the norspermidine-pyruvate reaction in the presence of excess pyruvate (*1*) may occur by transamination from DAP and allylamine to pyruvate (the routes in the box). Thus, the scheme can explain well the formation of the minor products, although this study provides no direct evidence for it. APAPAL seems to be rather stable under physiological conditions, and thus the nonenzymatic cleavage of this compound may not play an important role in the L-alanine overproduction.

The actions of the enzyme on spermidine and spermine, and the characterization of aminopropionaldehyde dehydrogenase are now under investigation in our laboratory.

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