Purification and Properties of 7,8-Diaminopelargonic Acid
Aminotransferase

AN ENZYME IN THE BIOTIN BIOSYNTHETIC PATHWAY*

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SUMMARY

The enzyme 7,8-diaminopelargonic acid aminotransferase utilizes S-adenosyl-L-methionine to transaminate the biotin precursor 7-keto-8-aminopelargonic acid and form the next intermediate in the pathway, 7,8-diaminopelargonic acid. The enzyme has been purified nearly 1000-fold from an extract of a regulatory mutant of Escherichia coli which is derepressed for the enzymes of the biotin operon. The extract was treated with protamine sulfate, ammonium sulfate, and subjected to acid and heat treatments. Subsequently, the enzyme was chromatographed on columns of DEAE-cellulose, phosphocellulose, hydroxylapatite, and two Sephadex G-100. The resulting purified preparation was judged 86% homogeneous by the scanning of a stained disc gel. The enzymatic activity was associated with the major band in gels run at two different gel concentrations and two different pH values.

The cofactor, pyridoxal phosphate, can be resolved from the enzyme in the presence of phosphate buffer after incubation with the amino donor, S-adenosyl-L-methionine.

A molecular weight estimation of 94,000 ± 10,000 has been obtained by gel filtration and sucrose gradient sedimentation studies. Gel electrophoresis in the presence of sodium dodecyl sulfate, shows a single subunit with a molecular weight of 47,000 ± 3,000 indicating a dimeric enzyme.

A neutral compound was detected in the acidified reaction mixture which was derived from the methionine moiety of S-adenosyl-L-methionine and was present in amounts equivalent to the 7,8-diaminopelargonic acid produced in the reaction mixture. It is suggested that the keto product of the reaction, i.e. S-adenosyl-2-oxo-4-methylthiobutyric acid, may decompose nonenzymatically under the conditions of the reaction to form 5'-methylthioadenosine and the neutral compound, 2-oxo-3-butenolic acid.

An early intermediate in the synthesis of biotin in Escherichia coli is 7-keto-8-aminopelargonic acid, a condensation product of pimeloyl-CoA and L-alanine (1). We have previously shown that 7-keto-8-aminopelargonic acid is transaminated in the presence of S-adenosyl-L-methionine and a cell-free E. coli extract to 7,8-diaminopelargonic acid (2). Carbonylation of DAPA2 to form the ureido ring and the subsequent addition of a sulfur atom to form the tetrahydrothiophene ring complete the synthesis of the bicyclic molecule (3) as shown in the following reaction sequence;

\[
\begin{align*}
\text{pimeloyl-CoA} + \text{L-Ala} & \xrightarrow{\text{PLP}} \text{7-keto-8 amino pelargonic acid} \xrightarrow{\text{Ado-Met, PLP}} \\
\text{7,8-diamino pelargonic acid} & \xrightarrow{\text{HCO}_3^-, ATP}} \text{Dethiobiotin} \\
\end{align*}
\]

The letters under the arrows indicate the cistrons of the biotin operon of E. coli which specify the respective enzymes (4). The purification and characterization of 7-keto-8-aminopelargonic acid synthetase (the bioE gene product) (5) and dethiobiotin synthetase (the bioD gene product) have been reported (6, 7). We now report the purification and characterization of the bioA gene product, to which we have given the trivial name DAPA aminotransferase. While this work was in the process of completion, Itzumi et al. (8) reported on the purification of the DAPA aminotransferase enzyme from Brevibacterium divaricatum. Although the enzyme was purified 5000-fold, it had only 60% of the specific activity of our enzyme. Neither the properties of the enzyme nor the kineties of the reaction were discussed.

Since this is, to our knowledge, the only transaminase reaction which utilizes Ado-Met as an amino donor, extensive purification of DAPA aminotransferase was undertaken in order to establish the following: (a) that the observed activity is, in fact, due to a single enzyme and therefore Ado-Met interacts directly with

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‡ The abbreviations used are: DAPA, 7,8-diaminopelargonic acid; Ado-Met, S-adenosyl-L-methionine; PLP, pyridoxal phosphate; PMP, pyridoxamine phosphate.

§ The systematic name for this enzyme would be S-adenosyl-L-methionine:7-keto-8-aminopelargonic acid aminotransferase.
DAPA aminotransferase, and (b) that the previously observed ability of ATP and L-methionine to replace Ado-Met in the cell-free system (2) was due to the intermediate formation of Ado-Met and not to an intrinsic activity of DAPA aminotransferase with these substrates. In addition, experiments with the purified enzyme and radioactively labeled Ado-Met have permitted the detection and quantitation of a degradation product of Ado-Met deamination indicating the liability of the keto product formed from Ado-Met.

**EXPERIMENTAL PROCEDURE**

**Materials**

7-Keto-8-aminopelargonic acid and 7,8-diaminopelargonic acid were synthesized by the methods previously reported (4). L-Methionine, S-methyl-L-methionine, tria(hydroxymethyl)amino- methane (enzyme and buffer grade), ammonium sulfate (enzyme and buffer grade), sucrose (enzyme grade), ovalbumin, and y-globulin (human) were obtained from Schwarz/Mann. The following were obtained from Sigma: S-adenosyl-L-ethionine, S-adenosyl-L-homocysteine, ATP, PLP, PMSF, diethytoxil, adenosine, and deoxythymidine triphosphate, sphingolipid sulfate (grade 1). Maleate dehydrogenase, furamase, and L-amino acid oxidase (Crotalus terricess terricess) were obtained from Boehringer Mannheim. Alkaline phosphatase (Escherichia coli) was a product of Worthington. Bovine serum albumin was from Pentex. Thiamine hydrochloride and 2-mercaptoethanol were supplied by Eastman.

All of the chemicals required for the preparation of polyacrylamide gels, including sodium dodecyl sulfate, were obtained from Bio-Rad. DEAE-cellulose (DE52) and cellulose phosphate (P11) were products of Whatman. Sephadex G-100 and G-200 were supplied by Pharmacia. Bio-Rad was the source of hydroxylapatite and Dowex 50 (AO 50W-X8, 200 to 400 mesh).

DL-[2-14C]Methionine was supplied by New England Nuclear. All of the other chemicals were reagent grade.

**Methods**

**Growth of Cells**—Cells of *E. coli* strain 10 were grown overnight in 2 liters of tryptone broth (model F-130, New Brunswick Scientific Co.). The cells were grown with stirring (200 rpm) and aeration (5 liters/min) to late log phase (200 Klett units) and then harvested. The cellfree system of the purification steps described below all of the chemicals were reagent grade.

**Tryptone Broth**—Tryptone broth was prepared as follows: 10 g of Bactotryptone (Difco) and 2.5 g of NaCl were autoclaved in 1 liter of glass-distilled water and 0.1 ml of a 0.5% (w/v) solution of thiamine hydrochloride was added aseptically. The tryptone-maltose medium utilized for growth of 50-liter batches of bacteria in the fermenter contained in 48 liters of deionized water: 500 g of Bactotryptone, 250 g of NaCl, 125 g of MgSO4·7H2O, and 25 ml of 10% NaOH. After sterilization in the fermenter, 500 ml of filter-sterilized 20% maltose solution was added aseptically.

**Bacterial Strains**—A regulatory mutant, which is not repressible by biotin, referred to as strain 10, was utilized for the purification of DAPA aminotransferase. This strain was selected from parent strain *E. coli* K-12 strain Y10-1 by virtue of its resistance to the antibiotic action of the biotin antagonist a-dehydrobiotin (3).

**Preparation and Analysis of S-Adenosyl-L-methionine**—Ado-Met of higher purity than available commercially was prepared by the peridodic acid extraction of yeast according to the method of Schlenk, et al. (11). The Ado-Met was purified on a column of Dowex 50-H+ by a modification of the method of Schlenk and Ehninger (12). The purity of Ado-Met was established by thin layer chromatography with the following solvents: water 4:3:1, 7,8-diaminopelargonic acid-water (60:15:25) as developing solvent. On the basis of absorption at 260 nm (13) the preparation was 95 to 98% pure.

**Preparation of S-Adenosyl-L-(2-hydroxy-4-methylthio)butyric Acid**—The 2-hydroxy derivative of Ado-Met was prepared by nitrous acid deamination according to the method of Zappenda et al. (14). A small amount of the doubly-deaminated derivative of Ado-Met, S-inosyl-L-(2-hydroxy-4-methylthio)butyric acid was obtained as by a product of this procedure.

**Preparation of S-Adenosyl-L-[2-14C]Methionine**—Ado-Met labeled in the 2-position of the methionine moiety was prepared enzymatically from 200 μCi (7.6 mg) of [2-14C]methionine. Methionine adenyltransferase from *E. coli* B was prepared according to the procedure of Tabak and Tabak (15). The reaction mixture employed was that described by Lombardini et al. (16), except that 0.013 M NaCl was included to inhibit the decarboxylation of the newly synthesized Ado-Met. The S-adenosyl-L-[2-14C]methionine was purified as previously described. The product on analysis by thin layer chromatography showed only one ultraviolet-absorbing compound, which also contained 98.5% of the radioactive material. Unlabeled Ado-Met was added to give a specific activity of 2.32 x 106 cpm/μmol.

**Polyacrylamide Disc Gel Electrophoresis**—The purification of DAPA aminotransferase was monitored by polyacrylamide disc gel electrophoresis in 7% gels (pH 9.5) prepared according to the method of Davis (17). Nonstandard gels, a 15% gel running at pH 9.5 and a 7% gel running at pH 8.0, were prepared according to the method of Martin and Ames (18).

**Molecular Weight Estimation**—The molecular weight of the aminotransferase was determined by three procedures: (a) polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate according to the method of Weber and Osborn (19), (b) gel filtration on Sephadex G-200 by a modification of the method of Andrews (20), and (c) sucrose gradient centrifugation by a modification of the method of Martin and Ames (21).

**Isoelectric Focusing in Sucrose Density Gradient**—An LKB isoelectric focusing apparatus (110 ml capacity) was first utilized with 1% wide range (pH 3 to 10) amphotelytes. In a second experiment, the amphotelyte concentration was increased to 5%, while the pH range was narrowed to pH 3 to 6. In addition, 0.1% 2-mercaptoethanol was added to the gradient to stabilize the enzyme.

**Purification**

In the sequence of the purification steps described below all of the buffers were prepared with glass-distilled water. Determina-
tions of buffer pH were made at ambient temperature. All procedures were carried out at 0-4℃, except where noted.

**Step 1: Preparation of Cell-free Extract**—The enzyme was prepared from 70-g cells of *E. coli* strain 30 which were grown as described under "Methods" and stored at -20℃ until used. The enzyme activity was found to be stable at -20℃ in the cells or at any stage of the purification for periods of up to 1 year or more. However, in the latter stages of the purification, the enzyme must be protected by freezing in the presence of 20% glycerol. The extract was prepared by sonication disruption (Branson, model W140D) of a suspension of the unwashed cells in Buffer A (0.05 M potassium phosphate buffer (pH 7.0), containing 10 mM 2-mercaptoethanol, 1 mM EDTA, and 0.05 mM PLP (4 ml of buffer per g cells)). The sonic extract was centrifuged at 30,000 rpm for 1 hour (30 rotor, Beckman, model L).

**Step 2: Protamine Sulfate Precipitation of Nucleic Acids**—The protamine sulfate (0.25 ml of a 2% solution prepared in Buffer A per ml of extract) was added dropwise while the extract was continuously stirred. The stirring was continued for 20 min after the addition was complete. The mixture was centrifuged at 12,000 rpm for 30 min (GSA rotor, Sorvall RC2-B).

**Step 3: Ammonium Sulfate Fractionation**—The protein fraction precipitating between 35 and 55% ammonium sulfate saturation was removed by centrifugation. To the supernatant (300 ml) was slowly added powdered ammonium sulfate (62.7 g) while the mixture was continuously stirred. Stirring was continued for 20 min, and after centrifugation as in Step 2, the precipitate was discarded. To the supernatant (325 ml) was added 42.6 g of ammonium sulfate as before. The precipitate was collected by centrifugation in 70 ml of Buffer B (0.05 M potassium phosphate buffer pH 7.0, containing 10 mM 2-mercaptoethanol and 0.01 mM PLP) and dialyzed overnight against 3 liters of the same buffer. The volume of the dialyzed ammonium sulfate fraction was 79 ml.

**Step 4: Acid Treatment**—Acetic acid (0.05 M) was added continuously to the dialyzed enzyme solution until the pH value fell to 5.1 as measured with the combination glass electrode (Corning). The solution was then allowed to stand undisturbed for 1 hour. After centrifugation at 18,000 rpm for 40 min (SS-34 rotor, Sorvall RC2-B), the phosphate concentration of the supernatant was raised to 0.05 M by the addition of 1 M potassium phosphate (pH 7.0) and the pH was adjusted to pH 7.0 by the addition of 0.1 N NaOH.

**Step 5: Heat Treatment**—The enzyme solution, contained in two 125-ml flasks, was heated with gentle swirling in a 65℃ water bath to a temperature of 59-60℃, and then transferred to a 60-61℃ bath for an additional 10 min. After rapid cooling in ice water, the precipitated protein was removed by centrifugation at 18,000 rpm for 60 min (SS-34 rotor, Sorvall RC2-B) and the supernatant was frozen at -20℃.

**Step 6: DEAE-cellulose Column Chromatography**—The heated enzyme was thawed and centrifuged at 12,000 rpm for 20 min (GSA rotor, Sorvall RC2-B). The enzyme solution was then placed in a 50℃ water bath for 20 min. This treatment has been found to activate the enzyme and thus eliminate a shoulder on the main activity peak eluted from the DEAE-cellulose column. After chilling to 0℃, NaCl was added to a final concentration of 0.05 M and the solution was applied to a DEAE-cellulose column (2.6 X 60 cm) which had been equilibrated with Buffer C (0.05 M potassium phosphate buffer, pH 7.0, containing 10 mM 2-mercaptoethanol, 0.01 mM PLP, and 0.05 M NaCl). The enzyme solution (82 ml) was applied to the column at a flow rate of 2 ml/min. Fractions of about 3 ml were collected at a flow rate of 1.5 ml/min. The absorbance of each fraction was determined at 280 nm, and even-numbered fractions containing 74% of the total activity were combined and concentrated to 1.4 ml by ultrafiltration. 2-Mercaptoethanol was added to a final concentration of 10% and the mixture incubated for 30 min at 37℃. The denatured protein was removed by centrifugation at 35,000 rpm for 20 min (40 rotor, Beckman model L).

Glycerol was added to the supernatant to a final concentration of 10% and the 2-mercaptoethanol, which interferes with the bioassay for DAPA, was removed by chromatography on the smaller Sephadex column. The enzyme was eluted from the column with Buffer C and fractions of 2.2 ml were collected at a flow rate of 0.2 ml/min. A single protein peak which coincided with the enzyme activity was observed. Each fraction was made 20% in glycerol and stored frozen at -20℃.

**RESULTS**

**Purification**

A summary of the purification procedure is shown in Table 1 which indicates an over-all purification of nearly 1000-fold with a 5% yield. After chromatography on the first Sephadex G-100 column, only two major bands are observed on polyacrylamide disc gel electrophoresis as shown in Fig. 1A. After the second Sephadex G-100 column only a single major protein band with several minor components is seen. A gel electrophoretic pattern of the peak fraction is shown in Fig. 1B. In order to estimate the percentage purity of this fraction, the gel was scanned at a wavelength of 550 nm using a linear transport attachment to the Gilford Spectrophotometer (model 2400). The enzyme band constituted 56% of the total protein as estimated from the areas under the protein peaks on the scan.

That the enzyme activity coincided with the major protein band observed on the gel (Fig. 1B) was demonstrated by slicing the gel and assaying one half for activity, and staining the other half for protein. In standard gels (7% run at pH 9.5, as well as in two nonstandard gels, one of 15% run at pH 9.5 and one of 7% run at pH 8.0), the peak activity was found in the slice containing...
stained with Coomassie blue (0.09%) and destained in 70% acetic acid. The Sephadex G-200 column in the presence of four marker proteins; electrophoresis at 8 mA/gel for 4 hours.

G-100 column (50 ml containing 1.2 mg protein) was subjected to gel filtration of DAPA aminotransferase which had been preincubated with 7-keto-8-aminopelargonic acid, and to phosphatase marker and from a plot of the elution volume of each marker protein versus the logarithm of its molecular weight, the molecular weight of DAPA aminotransferase enzyme was estimated to be 84,000.

In a second gel filtration experiment, DAPA aminotransferase (purified through Step 9) was incubated 20 min at 37°C with 4 mM Ado-Met in the presence of 0.12 mM Tris-HCl (pH 8.5), in order to convert the enzyme to the readily resolved PMP form (see below). It was then chromatographed as before in the presence of potassium phosphate buffer (0.10 M, pH 8.0) on the same Sephadex G-200 column, except that the markers run concurrently with the enzyme were γ-globulin, alcohol dehydrogenase, and ovalbumin. In this experiment the resolved DAPA aminotransferase eluted from the column just before ovalbumin. The elution volume corresponded to a molecular weight of 47,000. The DAPA aminotransferase recovered from the column represented apoenzyme since it was inactive unless the fractions were assayed in the presence of PLP.

Molecular Weight Estimation by Sucrose Gradient Sedimentation—DAPA aminotransferase, pretreated in two different ways, was sedimented in separate sucrose density gradients (5 to 20%). DAPA aminotransferase sedimented in an identical manner relative to alcohol dehydrogenase regardless of whether the enzyme was preincubated with 7-keto-8-aminopelargonic acid and PLP or preincubated with Ado-Met. The latter treatment converted the enzyme to the PMP form and resulted in the dissociation of the cofactor from the enzyme as shown by the fact that the fractions from only this gradient were inactive when assayed in the absence of PLP. The sedimentation behavior of the enzyme which was not preincubated with either substrate was identical with that observed in the previous two gradients. Like the enzyme preincubated with 7-keto-8-aminopelargonic acid, it was active both in the presence and absence of PLP. From the sedimentation rate of DAPA aminotransferase relative to that of alcohol dehydrogenase, an estimation of 104,000 daltons was obtained from these gradients.

Subunit Molecular Weight Estimation by Sodium Dodecyl Sulfate Gel Electrophoresis—Sodium dodecyl sulfate gel electrophoresis of DAPA aminotransferase after the final step in the purification revealed a single protein band, as shown in Fig. 2. This result indicates that if the subunits of the enzyme are nonidentical, they are nonetheless of similar molecular weight and are not resolved by this technique. In order to obtain an accurate estimate of the molecular weight of the enzyme subunit, electrophoresis of the enzyme in triplicate sodium dodecyl sulfate gels was carried out concurrently with that of six standard proteins. From a plot of the average mobility of the standards against their molecular weight, it was determined that the average mobility of the enzyme subunit corresponds to a molecular weight of 47,000 ± 3,000 indicating the presence of two subunits in the native enzyme.

Isoelectric Focusing of DAPA Aminotransferase—The isoelectric point of DAPA aminotransferase as determined by isoelectric focusing was 4.7, but the recovery of activity was low due to the precipitation of the enzyme. Even with modification of the procedure, the maximum recovery attained was only 37% which was considered insufficient for the preparative use of this method.

Activity of Purified Enzyme with Mg⁺⁺-ATP and L-Methionine—The ability of Mg⁺⁺-ATP and L-methionine to replace the Ado-Met in the crude cell-free extract was previously ascribed to the presence of the Ado-Met synthetase enzyme. No activity could be detected with Mg⁺⁺-ATP and L-methionine in the puri-
Homocysteine. S-adenosyl-L-(2-hydroxy-4-methylthio)butyric acid due to the low level of PLP carried over in the dialysis buffer. Beyond 10 min of preincubation with Ado-Met; this was probably reversed. Resolution in the presence of Ado-Met and phosphate ions was nearly complete in 10 min; the half-time was about 1.5 min. A low level of activity persisted in the absence of PLP beyond 10 min of preincubation with Ado-Met; this was probably due to the low level of PLP carried over in the dialysis buffer.

Cofactor Requirement—The stimulation of DAPA aminotransferase activity in dialyzed cell-free extracts by the addition of PLP (2, 22), suggested a requirement for this cofactor by the enzyme. It was found that the enzyme could be resolved by preincubation in the presence of Ado-Met and 0.125 mM potassium phosphate buffer, pH 8.0, as indicated by the loss of activity when PLP was omitted from the incubation mixture (Fig. 3). In contrast to the effect of preincubation with Ado-Met, preincubation with 7-keto-8-aminopelargonic acid or H₂O had no effect upon the activity. Regardless of which substrate was present in the preincubation mixture when the remaining substrate was added in the presence of PLP, the same high level of activity was achieved, indicating that the resolution was rapidly and fully reversible. Resolution in the presence of Ado-Met and phosphate ions was nearly complete in 10 min; the half-time was about 1.5 min. A low level of activity persisted in the absence of PLP beyond 10 min of preincubation with Ado-Met; this was probably due to the low level of PLP carried over in the dialysis buffer.

Enzyme Specificity—Two keto analogs of 7-keto-8-aminopelargonic acid, S-keto-7-aminopelargonic acid and 7,8-diketopelargonic acid, can be transaminated by DAPA aminotransferase to form DAPA (2, 23). However, the enzyme appears to be highly specific for Ado-Met as the amino donor. Of the Ado-Met analogs tested, neither S-adenosyl-l-thionine, S-adenosyl-L-homocysteine, S-adenosyl-L-(2-hydroxy-4-methylthio)butyric acid, S-methyl-L-methionine, adenosine, nor methionine could support synthesis of detectable amounts of DAPA from 7-keto-8-aminopelargonic acid.

A much more sensitive test of the ability of an Ado-Met analog to donate an amino group to the enzyme is to test its ability to effect resolution of the enzyme. Since in this case the potential amino donor reacts with the enzyme in the absence of the other substrate, 7-keto-8-aminopelargonic acid, the latter substrate can exert no competitively inhibitory effect (see accompanying paper (23)). But more important, the potential amino donor need react with the enzyme molecule only once in order to inactivate it for the subsequent assay carried out in the absence of PLP.

Several analogs of Ado-Met, various amino acids, and S-keto-7-aminopelargonic acid were all tested for their ability to effect resolution of the aminotransferase under the same conditions used with Ado-Met. A greater than 10% decrease in activity compared to the control level was considered to represent significant resolution of the cofactor from the enzyme. By this criterion, the following compounds were unable to effect significant resolution of the enzyme (the concentrations employed are given in parentheses): S-adenosyl-l-homocysteine (4 mM), S-adenosyl-L-(2-hydroxy-4-methylthio)butyric acid (5 mM), S-methyl-L-methionine (4 mM), adenosine (4 mM), adenylic acid (2 mM), L-methionine (4 mM), L-leucine (8 mM), and S-keto-7-aminopelargonic acid (2 mM). The only compound, other than Ado-Met itself, which was found to effect the resolution of the enzyme was S-adenosyl-L-ethionine (1 mM). However, this resolution was very slow (only 50% in 30 min, compared to 90% in 10 min for Ado-Met) and may have been due to traces of Ado-Met in the commercial preparation of S-adenosyl-L-ethionine. In fact, a contamination of only 0.1% Ado-Met would explain the ability of this analog to resolve the enzyme very slowly since it was found that Ado-Met at a concentration of 4 μM also showed about 50% resolution in 30 min.

Keto Product of Reaction—Although the amino product of the reaction, DAPA, had been extensively identified with the aid of very sensitive biological assay procedures (4), the expected keto product of Ado-Met, S-adenosyl-2-oxo-4-methylthiobutyric acid, could not be detected. At the concentration levels attained in the enzymatic reactions, the use of commercial reagents for detection were not sufficiently sensitive. We also had to consider the possibility that the keto product was unstable at pH 8.5, as similar sulfinium compounds have been reported to undergo decomposition with the formation of an unsaturated keto acid (24). A similar decomposition of the expected keto product would yield 5'-methylthioadenosine and 2-oxo-3-butenic acid and the latter fragment should be readily separated from the former by passage of an acidified solution through a Dowex 50-H⁺ column. With the aid of S-adenosyl-L-[2-³H]methionine, the stability of the keto product could be ascertained and any fragments containing the carbon 2 quantitated. Reaction mixtures containing 1.25 mM of S-adenosyl-L-[2-³H]methionine (2.9 X 10⁶ cpm) were incubated for various periods of time at 37°. In addition, a zero time control as well as controls with boiled enzyme and without 7-keto-8-aminopelargonic acid were included. After the incubation, all of the tubes were heated at 100° for 1 min in order to promote the decomposition of the a-keto product. After chilling to 0°, 20-μl samples were removed for bioassay of DAPA. The 1 ml reaction mixture was then acidified by the addition of 2 ml of 0.1 N HCl and applied to a 2-ml Dowex 50-H⁺ column. The column was washed with a 2-ml portion of 0.01 N HCl and the combined effluent and eluate (5 ml) was added to 18 ml of Aquasol scintillation fluid and counted. The column was immediately washed with a second portion of 0.01 N HCl (5 ml) and collected and counted in the same manner. The results, presented in Table II, show that a nonenzymatic reaction was occurring as evidenced by the increased counts in both 40-min controls compared to the zero time control. On the assumption that the nonenzymatic reaction occurring during the 37° incubation was linear with time, the necessary correction was made at each time interval. The net counts thus obtained indicated the presence of 1.8 nmol, 3.0 nmol, and 3.7 nmol of the uncharged fragment after 10, 20, and 40 min, respectively. Bio-

![Fig. 3. Resolution of DAPA aminotransferase by preincubation with Ado-Met in phosphate buffer. The enzyme was preincubated for the indicated times with (a) 4.2 mM Ado-Met (squares) (b) 0.017 mM 7-keto-8-aminopelargonic acid (triangles); and (c) H₂O (circles). The incubation was then continued for 30 min after the addition of the remaining substrate(s) with PLP (closed figures) and without PLP (open figures).](http://www.jbc.org/)
TABLE II

Correlation of a deaminated derivative of Ado-Met with DAPA production

The reaction mixture contained in 1 ml of the following: 0.15 M Tris Cl buffer (pH 8.5), 0.02 mM 7-Keto-8-aminopelargonic acid, 1.25 mM [2-14C]Ado-Met, and 4.7 X 10⁴ units of DAPA aminotransferase (Step 10). To obtain the enzymatic counts, the non-enzymatic control reaction was assumed to be linear with time and the average of the net control counts was subtracted on that basis. The calculation of the nanomol of keto product is based on a specific activity for the labeled Ado-Met of 2.32 X 10⁶ cpm/μmol. Other details of the method are given in the text.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Gross counts</th>
<th>Net counts</th>
<th>Enzymatic counts</th>
<th>Keto product*</th>
<th>DAPA</th>
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<tbody>
<tr>
<td>Zero time</td>
<td>7,410</td>
<td>12,600</td>
<td>5,200</td>
<td>4,200</td>
<td>1.8</td>
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<tr>
<td>10 min</td>
<td>16,400</td>
<td>8,900</td>
<td>6,000</td>
<td>3.0</td>
<td>2.9</td>
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<tr>
<td>40 min</td>
<td>11,500</td>
<td>12,700</td>
<td>8,000</td>
<td>3.7</td>
<td>3.9</td>
</tr>
<tr>
<td>Control: 40 min (—7-keto-8-aminopelargonic acid)</td>
<td>11,400</td>
<td>4,100</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>Control: 40 min (boiled enzyme)</td>
<td>11,400</td>
<td>4,000</td>
<td>0</td>
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* Postulated product, 2-oxo-3-butenoic acid (see Fig. 4).


TABLE III

Effect of heating at 100° on stability of deaminated derivative of Ado-Met

The reaction mixture contained in 1 ml of the following: 0.15 M Tris Cl buffer (pH 8.5), 0.02 mM 7-keto-8-aminopelargonic acid, 1.25 mM [2-14C]Ado-Met, and 4.7 X 10⁴ units of DAPA aminotransferase (Step 10). To obtain the enzymatic counts, the non-enzymatic control reaction was assumed to be linear with time and the average of the net control counts was subtracted on that basis. The calculation of the nanomoles of keto product is based on a specific activity for the labeled Ado-Met of 2.32 X 10⁶ cpm/μmol. Other details of the method are given in the text.

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<th>Enzymatic counts</th>
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</tr>
</thead>
<tbody>
<tr>
<td>A. Heated at 100° for 1 min</td>
<td>5,790</td>
<td>15,400</td>
<td>9,610</td>
<td>7,940</td>
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<tr>
<td>Zero time</td>
<td>9,560</td>
<td>6,900</td>
<td>1,110</td>
<td>3.4</td>
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<tr>
<td>Control: 20 min (boiled enzyme)</td>
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<td>4,200</td>
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</tr>
<tr>
<td>Control: 40 min (boiled enzyme)</td>
<td>560</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. Not heated</td>
<td>9,560</td>
<td>1,110</td>
<td>460</td>
<td></td>
</tr>
<tr>
<td>Zero time</td>
<td>8,160</td>
<td>9,000</td>
<td>8,160</td>
<td></td>
</tr>
<tr>
<td>Control: 20 min (boiled enzyme)</td>
<td>1,400</td>
<td>840</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Postulated product, 2-oxo-3-butenoic acid (see Fig. 4).

ASSAY OF THE AMINO PRODUCT

An assay of the amino product produced at these times indicated an equivalent amount of DAPA formed. This finding would support the view that it is the 2-amino group of the methionine moiety of Ado-Met which is transferred to 7-keto-8-aminopelargonic acid in the DAPA aminotransferase reaction and that the product is unstable under the experimental conditions used.

It was of interest to determine whether the 2-keto derivative of Ado-Met would be sufficiently labile to decompose completely during the course of the reaction at 37° without the additional heating step. The reaction was run for 40 min with and without the 1-min heating period with the appropriate controls. In addition, a 20-min control (boiled enzyme) reaction was included to determine if the nonenzymatic reaction was indeed linear with time as had been assumed. The results are presented in Table III. It is clear that the omission of the heating reduces the zero time control value drastically (from 5790 cpm to 560 cpm) without reducing the net counts which represent the enzymatically-produced unchanged decomposition product. It can also be seen that the nonenzymatic reaction was approximately linear with time as assumed in the previous calculation. Since the decomposition of the sulfonium ion of the product is therefore virtually complete during the course of the reaction at 37° and does not require heating at 100° to bring it about, this experiment indicates that the deaminated Ado-Met derivative produced in the reaction is extremely labile in Tris-Cl buffer, pH 8.5.

DISCUSSION

On the basis of the experimental evidence presented above, the transamination of 7-keto-8-aminopelargonic acid by DAPA aminotransferase in the presence of Ado-Met can be formulated as shown in Fig. 4. The substrates for this enzyme are both unusual for a transamination reaction. The biotin vitamer which is aminated in the reaction, 7-keto-8-aminopelargonic acid, is an α-amino ketone, rather than the usual α-keto acid and, therefore, has the potentiality for acting as an amino donor as well as an amino acceptor. Ado-Met, which serves as the amino donor in the reaction, has not, to our knowledge, previously been reported to serve as a substrate of any transaminase. Ado Met is well known as the methyl donor in a wide variety of methylation reactions (25), and after decarboxylation, as a propylamine donor in spermidine and spermine biosynthesis (26). It has also been shown to be an activator of lysine-2,3-aminomutase, a PLP enzyme (27).
The amino product of 7-keto-8-aminopelargonic acid transamination was previously identified as DAPA by a comparison of its chromatographic and electrophoretic properties with those of an authentic standard (2). Its biological activity in feeding bioA mutants of Escherichia coli provides the basis for a sensitive, simple, and direct bioassay procedure.

The unexpected finding that Ado-Met is required as the amino-donating substrate in the reaction has now been well established with the successful purification of the enzyme. The contaminants in the Ado-Met preparation (2) and the nonenzymatic decomposition products of Ado-Met in alkaline solution have been ruled out as amino donors.

Several lines of evidence suggest that Ado-Met serves as the source of the amino group. In the first place, the ability of Ado-Met to promote the rapid resolution of the enzyme indicates that it has the ability to donate an amino group to the enzyme-bound PLP. Such a transfer would break the covalent aldimine linkage to the enzyme, and permit the cofactor to dissociate from the enzyme in the presence of phosphate ions which compete for the phosphate binding site of the PLP molecule. A similar technique, but with much higher phosphate concentrations, has been used by Scardil et al. (38) and by Taylor and Jenkins (29) to resolve aspartate aminotransferase and leucine aminotransferase, respectively. Particularly significant is the inability of S-adenosyl-L-(2-hydroxy-4-methylthio)butyric acid to promote the resolution of the enzyme, even though inhibition studies show it to bind more strongly and with greater specificity to the PLP form of the enzyme than any other analog tested (23). This suggests that the 2-amino group of the methionine moiety of Ado-Met is the one transferred to the enzyme-bound cofactor.

The possibility existed that a molecule of 7-keto-8-aminopelargonic acid could transaminate a second molecule to form one molecule of 7,8-diaminopelargonic acid and one of 7,8-diketopelargonic acid with Ado-Met serving only to activate the enzyme. This alternative was discounted when it was found that 7,8-diketopelargonic acid, a biotin vitamer which has no potential for self-transamination, could be converted to DAPA in the presence of Ado-Met and in the absence of 7-keto-8-aminopelargonic acid by the purified enzyme (23). In this reaction the source of the two amino groups transferred to 7,8-diketopelargonic acid must be Ado-Met.

Third, kinetic studies have established the involvement of Ado-Met as a substrate in a ping-pong mechanism in which 7-keto-8-aminopelargonic acid is a substrate inhibitor (23). This evidence that Ado-Met binds at the active center of one of two stable enzyme forms in a manner which is competitive with the amino group of Ado-Met by Scardil et al. (38) and by Taylor and Jenkins (29) to resolve evidence that Ado-Met binds at the active center of one of two enzyme forms in a manner which is competitive with the amino group of Ado-Met, 7-keto-8-aminopelargonic acid could transaminate a second molecule to form one molecule of 7,8-diaminopelargonic acid and one of 7,8-diketopelargonic acid with Ado-Met serving only to activate the enzyme. This alternative was discounted when it was found that 7,8-diketopelargonic acid, a biotin vitamer which has no potential for self-transamination, could be converted to DAPA in the presence of Ado-Met and in the absence of 7-keto-8-aminopelargonic acid by the purified enzyme (23). In this reaction the source of the two amino groups transferred to 7,8-diketopelargonic acid must be Ado-Met.

Fourth, if Ado-Met does indeed donate its 2-amino group to 7-keto-8-aminopelargonic acid, then the product of the reaction, S-adenosyl-2-oxo-4-methylthiobutyric acid, must appear in amounts equivalent to the DAPA synthesized. With the aid of S-adenosyl-L-[2-14C]methionine as substrate we have found that the keto product readily decomposes under the experimental conditions used and a keto fragment containing C-2 of the methionine moiety accumulates in amounts equivalent to the DAPA produced. Certain α-sulfonium compounds, of which the 2-keto derivative of Ado-Met is an example, have been shown by Böhme and Heller to decompose readily in alkali by the following mechanism (24):

\[
\begin{align*}
\text{X-C-CH}_2\text{CH}_2\text{S}^\circ\text{R} & \rightarrow \text{X-C-CH}_2\text{CH}_2\text{O} \\
R' & + \text{R'SR}
\end{align*}
\]

Dimethylpropiothetin decomposes in alkaline solution to acrylic acid and dimethylsulfide (30), and an analogous decomposition of the 2-keto product of Ado-Met would yield 5'-methylthioadenosine and 2-oxo-3-butenolic acid. The firm identification of the products of Ado-Met transamination would be aided by the availability of the authentic compounds. Neither S-adenosyl-2-oxo-4-methylthiobutyric acid nor its decomposition product, 2-oxo-3-butenolic acid, have, to our knowledge, been synthesized.

The molecular weight estimates for DAPA aminotransferase obtained by two methods were 84,000 obtained by gel filtration, and 104,000 by sucrose gradient centrifugation. The average mass of 94,000 ± 10,000 is taken as an estimate of the molecular weight of the holoenzyme. The highly purified apoenzyme eluted from the Sephadex G-200 column much later than did the holoenzyme, at a position corresponding to a molecular weight of 47,000. This result suggested that resolution of the enzyme favors dissociation of the enzyme into subunits, as has been observed for aspartate aminotransferase (31, 32). The failure of the apoenzyme to dissociate on the sucrose gradient may have been due to the use of a partially purified enzyme preparation or to an effect of the sucrose itself. Sodium dodecyl sulfate disc gel electrophoresis established the molecular weight of the enzyme subunit as 47,000 ± 3,000. The fact that the subunits show a single band on a sodium dodecyl sulfate gel indicates that the two subunits are of very similar molecular weights. Genetic evidence on this question has been provided by the studies of Cleary and Campbell who found a pattern of intragenic complementation among 15 bioA mutants which suggested that bioA comprises a single strand (33). Cleary and Campbell also point out that intragenic complementation can result from interaction between heterologous monomers in an enzyme normally composed of identical subunits. Thus the genetic evidence supports the finding that DAPA aminotransferase is multimeric and indicates that the subunits, shown by sodium dodecyl sulfate disc gel electrophoresis to be of similar molecular weight are, in fact, identical.

REFERENCES


\[^{2}\text{G. L. Stoner and M. A. Eisenberg, unpublished data.}\]
Purification and properties of 7, 8-diaminopelargonic acid aminotransferase.
G L Stoner and M A Eisenberg


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