δ-Aminolevulinic Acid Formation

PURIFICATION AND PROPERTIES OF ALANINE:4,5-DIOXOVALERATE AMINOTRANSFERASE AND ISOLATION OF 4,5-DIOXOVALERATE FROM CLOSTRIDIUM TETANOMORPHUM*

(Received for publication, August 17, 1981)

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1-Alanine:4,5-dioxovalerate aminotransferase, the enzyme that catalyzes the transamination between alanine and 4,5-dioxovalerate to yield δ-aminolevulinic acid and pyruvate, has been purified from extracts of Clostridium tetanomorphum by acetone precipitation and successive chromatography on Sephadex G-150, hydroxyapatite, Octyl-Sepharose, and SP-Sephadex C-50. The enzyme is pure by the criterion of disc gel electrophoresis with varying polyacrylamide concentrations. It is dimeric, and has an apparent molecular weight of 111,000. Each molecule contains 2 molecules of pyridoxal 5-phosphate. The apparent $K_m$ values for 4,5-dioxovalerate and l-alanine are 0.28 and 1.96 mM, respectively. In addition to alanine, glutamate also is an effective amino group donor. The enzyme is inhibited by various keto acids as well as by inhibitors of pyridoxal phosphate-containing enzymes. It was possible to show that 4,5-dioxovalerate is formed by cultures of C. tetanomorphum when grown in the presence of 0.2 M levulinate, an inhibitor of 5-aminolevulinic dehydratase.

Shortly after the discovery of the committed tetrapyrrole precursor, ALA* (1), extracts of avian erythrocytes (2) and of the photosynthetic bacterium Rhodopseudomonas spheroides (3) were shown to form this compound from succinyl-CoA and glycine. The enzyme that carries out this reaction, ALA synthase (EC 2.3.1.37), has since been found in a variety of tissues (for list, see Ref. 4). Although it is often difficult or impossible to detect this enzyme in organisms that make tetrapyrroles (5), it was assumed for over 20 years that, under physiological conditions, ALA is always made via this condensation reaction. About 8 years ago, however, it was found that organisms that contain the aminotransferase (8). Studies with Scenedesmus have indicated 4,5-dioxovalerate to be an intermediate in the biosynthesis of ALA (9). Very recently, the field has become more complex with the suggestion that in R. spheroides ALA may be formed by as many as three pathways (11). The possibility that an alternate pathway to ALA might not be confined to photosynthetic organisms is suggested by the occurrence of 4,5-dioxovalerate and of l-alanine:4,5-dioxovalerate aminotransferase in nonphotosynthetic organisms. Thus, the compound has been found in rat urine (12), and the enzyme has recently been obtained pure from bovine liver extracts (13).

In the present study, a specific irreversible 4,5-dioxovalerate aminotransferase has been purified from a nonphotosynthetic microorganism, Clostridium tetanomorphum. In addition, 4,5-dioxovalerate has been shown to occur in this organism.

EXPERIMENTAL PROCEDURES

Materials

Chemicals were obtained from the following sources: yeast extract, Difco; 5,5-dibromolevulinic acid, Porphyrin Products, Logan, UT; ethylacetococetic acid, Aldrich; hydroxypatite (Fast Flow), Calbiochem; and Blue Dextran, Pharmacia. Proteins used for molecular weight markers were obtained from Mann, Gallard-Schlesinger, Carle Place, NY, and Sigma. All other reagent grade chemicals were products of Sigma.

Methods

Growth of C. tetanomorphum— Cultures of C. tetanomorphum (ATCC 15920) were grown in a medium based on medium 163, American Type Culture Collection, containing per liter: 6 g of yeast extract, 17 g of sodium glutamate, 0.5 g of sodium thiglycolate in 25 mM potassium phosphate buffer, pH 7.4, and the following salts: MgSO$_4$, 1 mM; FeSO$_4$, 4 x 10$^{-5}$ M; MnCl$_2$, 1 x 10$^{-5}$ M; Na$_2$MoO$_4$, 1 x 10$^{-5}$ M; CaCl$_2$, 1 x 10$^{-5}$ M; and CoCl$_2$, 1 x 10$^{-5}$ M. After 18 h of growth at 37 °C, the bacteria were harvested at 0 °C and stored at -20 °C. The yield per liter of medium was approximately 4 g of packed cells.

Assay of l-Alanine:4,5-Dioxovalerate Aminotransferase—The 4,5-dioxovalerate was prepared from 3,5-dibromolevulinic acid by the method of Gruenev et al. (14) as described by Varticovski et al. (13). The yield based on the assay described by Jerzykowski et al. (16) was 42%. Stock solutions were stored frozen.

l-Alanine:4,5-dioxovaleric acid aminotransferase activity was assayed by formation of the product, ALA, using the colorimetric method of Mauzerall and Granic (16). The standard assay mixture consisted of 20 mM neutralized 4,5-dioxovalerate and 10 mM l-alanine in 30 mM potassium phosphate buffer, pH 6.9, containing 0.06 mM pyridoxal phosphate, and enzyme (typically 100 μl) in a total volume of 0.5 ml. Enzyme was added last. After incubation at 37 °C for 30 min, 50 μl of ethyl acetocetate and 0.5 ml of 30 mM potassium phosphate buffer, pH 6.9, were added and the mixture was immediately placed in a boiling water bath for 10 min. Under these conditions, ALA was converted to the Ehrlich chromophore, which was measured at 553 nm, after addition of 1 ml of 2% p-dimethylaminobenzaldehyde in a mixture of acetic acid: perchloric acid (42:8) (16). In the early stages of the purification, enzyme samples had to be dialyzed against...
50 mm potassium phosphate buffer, pH 6.9, because of large amounts of endogenous ALA. All results were corrected for unreacted 4,5-dioxo- dioxovalerate and endogenous ALA. One unit of L-alanine:4,5-dioxo- dioxovalerate aminotransferase was taken as the amount of enzyme that catalyzed the formation of 1 μmol of ALA/h at 37 °C and pH 6.9 (13). Protein was based on the Coomassie Brilliant Blue G-250 protein assay. The Protein Aminotransferase was obtained from Bio-Rad. Ovalbumin was used as the standard.

Enzyme Purification—L-Alanine:4,5-dioxovalerate aminotransferase from French pressure cell extracts of the bacteria, after nucleic acid removal, was purified to homogeneity by acetone precipitation and column chromatography on Sephadex G-150, on hydroxypatite, on Octyl-Sepharose CL-4B, and on SP-Sephadex C-25. All purification steps were performed at 4 °C and all centrifugations were carried out at 48,000 X g for 15 min, unless otherwise stated.

Bacteria from the freezer (50 g) were dispersed uniformly without frothing, using a nonaerating stirrer (Kraft Apparatus, Inc., Mineola, NY), in 200 ml of a cold solution of 50 mm potassium phosphate buffer, pH 6.9, containing 0.1 mm pyridoxal 5-phosphate. The cells were disrupted in a French Pressure cell at 19,000 p.s.i. and the resulting homogenate was centrifuged. To each volume of the cooled supernatant solution, 0.1 volume of a 2% aqueous solution of protamine Sulfate was added with stirring. After the mixture had stood for 10 min, it was centrifuged. The pellet was discarded.

To the clear supernatant solution, an equal volume of acetone, prechilled to -10 °C, was added with stirring. The resulting precipitate, containing most of the aminotransferase activity originally present in the extract, was collected by centrifugation and dissolved in a minimum volume of 50 mm potassium phosphate buffer, pH 6.9, containing 0.1 mm pyridoxal 5-phosphate. The suspension was gently stirred overnight. Insoluble material was removed by centrifugation. The clear supernatant solution was concentrated to 10 ml by ultrafiltration through an Amicon YM-10 membrane.

The concentrate was applied to a Sephadex G-150 column (1.6 × 62 cm) that had been equilibrated with 50 mm potassium phosphate, pH 6.9. The flow rate was 24 ml/h. Fractions of 4 ml were collected. Fractions containing appreciable amounts of the aminotransferase were pooled and concentrated as before to 5 ml.

The concentrated sample from the Sephadex G-150 column was applied to a hydroxypatite column (1.5 × 24 cm) which had been equilibrated with 50 mm potassium phosphate buffer, pH 6.9. The column was then washed with 3 bed volumes of the phosphate buffer. A 400-ml linear gradient from 50 mm to 0.3 m potassium phosphate, pH 6.9, was applied to the column. The flow rate was 34 ml/h and fractions of 2.5 ml were collected. The aminotransferase was eluted from the column toward the end of the gradient. The fractions containing the enzyme were pooled, concentrated to 5 ml, and dialyzed overnight against 50 mm potassium phosphate buffer, pH 6.9, containing 4 m NaCl. Material that precipitated upon dialysis was removed by centrifugation.

The dialyzed supernatant solution was applied to an Octyl-Sepha- rose CL-4B column (1.6 × 6 cm) that had been equilibrated with the above phosphate buffer containing 4 m NaCl. The flow rate was 36 ml/h. Fractions of 1 ml were collected. The aminotransferase did not bind to the material although 90% of the protein that had been applied to the column was retained. Fractions containing the aminotransferase were pooled and dialyzed against 50 mm sodium acetate buffer, pH 5.1, for 6 h.

The dialysate was applied to a SP-Sepharose C-25 column (1.6 × 6 cm) that had been equilibrated in the acetate buffer. Fractions of 2 ml were collected. The flow rate was 12 ml/h. The purified 4,5-dioxovalerate aminotransferase was eluted from the column with 50 mm sodium acetate buffer, pH 5.1, containing 0.25 m NaCl. The elution pattern revealed a single protein peak with a symmetrical activity profile. The enzyme was dialyzed overnight against 50 mm potassium phosphate buffer, pH 6.9. It was kept at 4 °C for immediate use or was stored in small aliquots at -20 °C for later use.

Molecular Weight Determination—The molecular weight of the aminotransferase was determined by molecular exclusion chromatography using a Sephadex G-150 column (1.5 × 29 cm) that had been equilibrated with 50 mm potassium phosphate buffer, pH 6.9, and calibrated with known standards. These included Blue Dextran 2000 (Mr > 1,000,000), catalase (Mr = 230,000), bovine serum albumin (Mr = 68,000), ovalbumin (Mr = 43,000), and pancreatic trypsin inhibitor (Mr = 20,500). The column was run at a flow rate of 16 ml/h, and fractions were collected. The peak fraction of each protein was determined by absorbance measurement at 280 nm.

In addition, the molecular weight of the enzyme was determined by electrophoresis on dodecyl sodium sulfate-polycrylamide as outlined by Weber and Osborn (18) and by sedimentation equilibrium centrifugation as described by Bothwell et al. (19). Molecular weight markers for the electrophoresis (Mr = 41,300 to 71,500) were obtained from Gallard-Schlesinger. For sedimentation equilibrium, the protein sample was diluted (1.5 mg/ml) with 5% bovine serum albumin, ovalbumin, and pancreatic trypsin inhibitor. Solutions were centrifuged at 42,000 rpm for 18 h, and values of log F were determined by absorbance measurements at 210 and 280 nm. For the purified aminotransferase, log F was also determined by enzyme activity.

Polyacrylamide Gel Electrophoresis—Alkaline and acidic disc gel electrophoresis was performed according to the methods of Davis (20) and Reisfeld et al. (21), respectively. Gels were run at 2 mA/tube for 2 h and stained according to the method of Blakesley and Boezi (22). As a further check on purity, electrophoresis was also performed at several different gel concentrations (23).

Determination of Bound Pyridoxal 5-Phosphate—The purified aminotransferase was incubated with 4,5-dioxovalerate for 30 min at 37 °C to convert any bound pyridoxamine phosphate to the pyridoxal form. 4,5-Dioxovalerate was then removed from the enzyme by chromatography on Sephadex G-25. The enzyme was separated from the protein as described by Harwood et al. (24) and determined by the method of Ward and Schmidt (25).

Identification and Quantitation of 4,5-Dioxovalerate—4,5-Dioxo- valerate was identified and quantitated in extracts of Clostridial Alanine:4,5-dioxovalerate aminotransferase by a modification of the procedure of Porra et al. (10). The bacteria were harvested, suspended in 4 parts by weight of 50 mm sodium acetate buffer, pH 5.0, at 0 °C, and passed through a chilled French pressure cell (19,000 p.s.i.). The mixture was placed in a boiling water bath for 5 min. The suspension, after adjustment to pH 8.0 with 1 m Tris-HCl, pH 8.0, was centrifuged at 45,000 X g for 5 min. The supernatant solution was passed through a small column (volume = 3 ml) of Florisil (100 to 200 mesh) to remove flavins which would interfere in the determination of 4,5-dioxovalerate. To the eluant was added ethanol to a final concentration of 20%, followed by 5 μg/ml of solid 2,3-diaminonaphthalene. The condensation between 4,5-dioxovalerate and 2,3-diaminonaphthalene and the isolation of the benzoquinazoline 2-propionic acid were performed as described by Porra et al. (10), except that the citrate extraction was eliminated. The derivative was identified by thin layer chromatography on Whatman Linear High Performance (LHP-K) silica plates. The developing solvent was toluene-acetic acid (3:1) (v/v). The benzoquinazoline derivative was quantitated from the UV absorption spectrum using known extinction coefficients (10).

RESULTS

Purification—Table I summarizes the purification of L-alanine:4,5-dioxovalerate aminotransferase from 50 g (packed cell weight) of C. tetanomorphum. Although added pyridoxal phosphate did not stimulate the activity of the transaminase, it appeared to stabilize the enzyme, especially in the early stages of purification.

| Table I | Purification of L-alanine:4,5-dioxovalerate aminotransferase |
|---------|----------------------------------------------------------|
| Purification step | Volume | Total protein | Total activity | Specific activity | Purification Yield |
| | ml | mg | units | mg | -fold |
| Crude extract | 216 | 4082 | 1069 | 0.36 | 100 |
| 0-50% acetone frac | 41 | 1156 | 998 | 0.86 | 3.3 |
| 50-80% acetone frac | 43 | 424 | 998 | 2.33 | 8.9 |
| Sephadex G-150 | 41 | 298 | 987 | 34 | 130 |
| Hydroxypatite | 37 | 311 | 407 | 131 | 500 |
| Octyl-Sepharose | 6.5 | 0.46 | 212 | 461 | 1759 |

* One unit is defined as the amount of enzyme that will catalyze the formation of 1 pmol of 8-aminolevulinic acid/h at 37 °C and at pH 6.9.
purification steps. It was, therefore, routinely included in buffer at a final concentration of 0.1 mM in the initial stages of the purification.

Fast flow hydroxyapatite proved far superior to other hydroxyapatites tried, since it decreased the time required for chromatography and gave increased yields. Fig. 1 shows a typical elution profile given by the pooled fractions from the Sephadex G-150 column upon chromatography on a column of fast flow hydroxyapatite.

Purity—Elution from the SP-Sephadex C-25 column produced a single, symmetrical protein peak with constant specific activity. The purified 4,5-dioxovalerate transaminase was homogeneous as determined by disc gel electrophoresis on polyacrylamide at pH 4.5 (7.5% acrylamide), and at pH 8.5 with varying concentrations of acrylamide (Fig. 2). In addition, the purified enzyme gave a single, sharp protein band on polyacrylamide gel electrophoresis in the presence of dodecyl sodium sulfate.

Enzyme Assay—Under the standard assay conditions the rate of formation of ALA is constant for up to 1 h. It was shown by the methods used by Varticovski et al. (13) that ALA and pyruvate were produced in equimolar amounts.

$K_{m}$ values for 4,5-dioxovalerate and alanine were determined from double-reciprocal plots of rates versus substrate concentrations. Fig. 3 shows a family of such plots obtained for a range of constant concentrations of alanine in the presence of varying concentrations of 4,5-dioxovalerate. A series of essentially parallel lines was obtained suggesting a double displacement mechanism. The $K_{m}$ values for 4,5-dioxovalerate and alanine were calculated to be 0.26 mM and 1.96 mM, respectively.

Various attempts were made to demonstrate the reversibility of the reaction, i.e. the enzymatic transamination between ALA and pyruvate to yield 4,5-dioxovalerate and alanine. It was attempted to show the disappearance of ALA by carrying out the enzyme incubation in 50 mM potassium phosphate, pH 6.9, and in 50 mM sodium acetate, pH 4.1, both in the presence and in the absence of 60% MeOH (v/v) over a substrate concentration range of 10 μM to 5 mM. Under none of these conditions could any reversibility of the system be demonstrated.

Molecular Weight—The molecular weight of L-alanine:4,5-dioxovalerate aminotransferase was determined by a variety of methods. On dodecyl sodium sulfate-polyacrylamide gel electrophoresis, a single band appeared with an apparent molecular weight of 55,500 (Fig. 4). By gel filtration, however,
the molecular weight of the native enzyme was found to be 82,000. Sedimentation equilibrium centrifugation in 50 mM potassium phosphate buffer, pH 6.9, indicated a molecular weight of 78,000, while in the presence of this buffer containing 4 M NaCl a value of 72,000 was obtained. However, the molecular weight observed in the presence of 50 mM sodium acetate, pH 5.6, was 53,000. When the molecular weight of the enzyme in the above phosphate buffer containing 4 M NaCl was redetermined after storage of the enzyme at 4 °C for 3 days, a value of 52,000, similar to that observed for the enzyme at pH 5.6, was obtained. Both of the low molecular weight species were inactive.

These results suggest that the transaminase is composed of two subunits with a monomer molecular weight of about 55,000 and that the native enzyme dissociates gradually at high ionic strength, and more rapidly at relatively low pH. The molecular weight of 78,000 to 82,000 for the native enzyme as determined by gel filtration or sedimentation equilibrium thus reflects a monomer-dimer equilibrium (28-31).

It is interesting to note that the major losses in the purification of the enzyme occurred at the steps where the protein was subjected to high salt concentration (Octyl-Sepharose) or to a low pH (SP-Sephadex).

Amino Acid Composition—The amino acid composition of the purified transaminase is presented in Table II. It was calculated from two analyses assuming a monomer molecular weight of 55,000. The purified enzyme contained two pyridoxal 5-phosphate groups per dimer.

Substrate Specificity—All of the common amino acids were tested as amino group donors in the enzyme-catalyzed transamination. Four amino acids in addition to L-alanine were found to be quite active. Table III gives a comparison of their activities to that of L-alanine. L-Glutamate was found to be as efficient an amino donor as L-alanine.

The question whether the purified 4,5-dioxovalerate aminotransferase exhibited any other kind of aminotransferase activity was explored. Thus, the enzyme was incubated with aspartate and α-ketoglutarate, substrates of glutamic-oxaloacetic aminotransferase, and with alanine and α-ketoglutarate, substrates of glutamic-pyruvate aminotransferase (32). The 4,5-dioxovalerate aminotransferase did not catalyze the transamination between these substances and it did not catalyze the reverse reaction when oxaloacetate and glutamate or pyruvate and glutamate were present. In addition, commercial preparations of glutamic-oxaloacetic aminotransferase and of glutamic-pyruvate aminotransferase did not catalyze the transamination between 4,5-dioxovalerate and L-alanine.

The keto acids that could be expected to be produced in the transamination between 4,5-dioxovalerate and four of the five amino acid donors were tested as potential inhibitors. The results are shown in Table IV. Oxaloacetate was found to be the most effective keto acid inhibitor of the transamination between 4,5-dioxovalerate and L-alanine.

Table IV in addition shows various substrate analogs and their inhibitory effects. Aminoxyacetic acid and diacetyl are known to inhibit other pyridoxal-containing transaminases (33, 34). Aminoxyacetic acid inhibited the transaminase most extensively.

Identification of 4,5-Dioxovalerate in Extract of C. tetanomorphum—In order to show that 4,5-dioxovalerate is formed by C. tetanomorphum, the bacteria were grown in the presence of 0.2 M levulinic acid for 18 h and then harvested. 4,5-Dioxovalerate was not present in extracts of the bacteria that had been grown without levulinate or with lower concentrations of levulinate. The benzoquinoline derivative of 4,5-dioxovalerate had an R_f value of 0.54 when chromatographed on silica thin layer plates as described under "Methods." One gram of bacteria (packed cell weight) was found to contain 230 nmol of 4,5-dioxovalerate.

### DISCUSSION

The present work was initiated to shed some light on the possible mode of formation of the pyrrole ring system in C. tetanomorphum. This organism was chosen since glutamate, its preferred carbon source, is also the putative carbon source for ALA formed by the 5-carbon pathway in plants (35). In this organism, as in other clostridia, the classical ALA synthase has never been detected. Indeed, this organism does not make porphyrins from glycine and succinate (36). Hence, C.

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**Table II**

Amino acid composition of L-alanine:4,5-dioxovalerate aminotransferase

| Amino acid         | Residues/monomer |
|--------------------|------------------|
| Asparagine/aspartic acid | 52.2 (52) |
| Threonine          | 22.2 (22)       |
| Serine             | 27.2 (37)       |
| Glutamine/glutamic acid | 54.7 (55)    |
| Proline            | 18.7 (22)       |
| Glycine            | 36.8 (37)       |
| Alanine            | 34.8 (35)       |
| Valine             | 32.9 (33)       |
| Methionine         | 9.3 (9)         |
| Isoleucine         | 38.8 (39)       |
| Leucine            | 38.9 (39)       |
| Tyrosine           | 19.9 (20)       |
| Phenylalanine      | 18.7 (39)       |
| Histidine          | 6.0 (6)         |
| Lysine             | 46.5 (47)       |
| Arginine           | 19.0 (19)       |
| Tryptophan         | 7.0 (7)         |

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**Table III**

Amino group donors for L-alanine:4,5-dioxovalerate aminotransferase

| Amino group donor | Concentration | Activity |
|-------------------|---------------|----------|
| L-Alanine         | 4.69          | 100      |
| L-Glutamate       | 4.22          | 96       |
| L-Histidine       | 4.57          | 74       |
| L-Phenylalanine   | 4.95          | 71       |
| L-Aspartate       | 4.81          | 67       |

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**Table IV**

Inhibition of L-alanine:4,5-dioxovalerate aminotransferase

| Inhibitor        | Concentration | Activity |
|------------------|---------------|----------|
| Ketoacids        |               |          |
| Pyruvate         | 2.69          | 33       |
| α-Ketoglutarate  | 1.81          | 73       |
| Phenylpyruvate   | 1.44          | 100      |
| Oxaloacetate     | 2.35          | 17       |
| Substrate analogs|               |          |
| Glyoxal          | 1.75          | 23       |
| Methylglyoxal    | 2.44          | 30       |
| Glyoxylic acid   | 2.02          | 52       |
| Diacetyl         | 3.16          | 64       |
| Succinate semialdehyde | 2.66 | 60     |
| Aminoxyacetic acid | 1.61 | 12      |
tetanomorphum seemed to be an excellent candidate for the utilization of a 5-carbon pathway for ALA formation. Furthermore, very little is known about the control of corrinoid formation, and a knowledge of the early steps in this pathway in an organism that does not make hemes may simplify a study of this process. Two properties of the aminotransferase studied in this paper make it a candidate on the path to ALA formation, namely its high specificity for 4,5-dioxovalerate as amino group acceptor, and the fact, noted before by other workers for this type of enzyme obtained from photosynthetic (37, 38) as well as nonphotosynthetic (13) sources, of its surprising irreversibility. This irreversibility also holds for the nonenzymatic transamination (39). The fact that 4,5-dioxovalerate is found in C. tetanomorphum under conditions where the further metabolism of ALA is blocked, when considered in conjunction with the irreversibility of the ALA-forming 4,5-dioxovalerate aminotransferase, would make it rather unlikely that this compound is formed from, rather than being a precursor to, ALA. Various experiments to investigate this point are being pursued.

The purification of L-alanine:4,5-dioxovalerate aminotransferase is straightforward and reproducible. It yields a protein that is pure by the criterion of electrophoresis on polyacrylamide gels at various polyacrylamide concentrations. The enzyme exists as a dimer with a monomer molecular weight of about 55,000. The data suggest that the dimer, molecular weight about 110,000, is the active species. Similar results have been observed for other aminotransferases (40). The enzyme, like other aminotransferases (41), is stabilized by pyridoxal 5-phosphate. It is destabilized at low pH and by salts at high ionic strength, suggesting that the dimer is held together by electrostatic forces.

The double reciprocal plots of initial velocities versus substrate concentrations suggest a double displacement mechanism, commonly exhibited by aminotransferases. The low $K_m$ values for 4,5-dioxovalerate are consistent with a high specificity of this enzyme for this substrate. The enzymes from R. sphaeroides (37) and from Chlorella vulgaris (42) also show lower $K_m$ values for 4,5-dioxovalerate than for alanine. The observation that amino acids besides alanine can act as amino donors is not surprising, since other aminotransferases also show multiple specificities (43). The purified liver 4,5-dioxovalerate aminotransferase, however, appears to exhibit a much higher specificity for the amino donor than the purified clostridial enzyme (13). The high specificity of the present enzyme for 4,5-dioxovalerate is in noteworthy contrast to the fact that 4,5-dioxovalerate is not a substrate for the conventional aminotransferases from pig heart.

The assay for 4,5-dioxovalerate was considerably simplified by the use of Florisil to remove interfering flavins. This step eliminated a tedious extraction with citrate buffer (10) that removed not only unwanted flavins but also appreciable amounts of 4,5-dioxovalerate.

When grown on different media, C. tetanomorphum shows large variations in the amounts of corrinoids produced, but no changes in the levels of ALA dehydratase and of porphobilinogen deaminase (44). There was also no significant difference in the levels of 4,5-dioxovalerate aminotransferase in these bacteria. It can be concluded that these three enzymes do not limit the rate of tetrapyrrole formation in this organism. Further work is in progress on steps leading to the formation of 4,5-dioxovalerate and its possible participation in ALA formation.

Acknowledgment—We are much indebted to Pamela Keim for the amino acid analysis of the purified enzyme.

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J. Biol. Chem. 1982, 257:2207-2211.

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