Bovine liver mitochondria have been found to contain an enzyme which will catalyze the formation of δ-aminolevulinic acid via a transamination reaction rather than via the condensation of glycine and succinyl coenzyme A. The enzyme, L-alanine:γ,δ-dioxovaleric acid aminotransferase (γ,δ-dioxovalerate transaminase) was isolated and purified to apparent homogeneity.

γ,δ-Dioxovalerate transaminase is quite stable, has optimal activity at pH 6.9, requires pyridoxal phosphate as a cofactor and has an apparent molecular weight of 240,000. The enzyme has high specificity for both substrates. The \( K_m \) for L-alanine is \( 3.7 \times 10^{-3} \text{ M} \) and the \( K_m \) for γ,δ-dioxovalerate is \( 2.4 \times 10^{-4} \text{ M} \). Plots of 1/\( K_m \) against varying alanine concentrations suggested a ping-pong reaction mechanism. Although the enzyme appeared to be a typical transaminase, exhaustive experiments failed to demonstrate reversibility of the reaction.

The capacity of γ,δ-dioxovalerate transaminase to synthesize δ-aminolevulinic acid appears to be far greater than the capacity of δ-aminolevulinic acid synthase from the same source. The possibility that γ,δ-dioxovalerate transaminase plays a role in the biosynthesis of δ-aminolevulinic acid in vivo must be considered.

Shemin and Russell, in 1953, demonstrated that the first committed precursor of protoporphyrin IX is the aminoketone δ-aminolevulinic acid (1). Isotopic labeling studies established glycine and succinyl coenzyme A as the precursors of δ-aminolevulinic acid (2, 3). The enzyme responsible for catalyzing the condensation of glycine and succinyl-CoA δ-aminolevulinic acid synthase (EC 2.3.1.37). δ-Aminolevulinic acid synthase has been described in a wide variety of tissues and organisms (4). Because of its wide distribution in nature and demonstrated role as a regulatory enzyme (5), δ-aminolevulinic acid synthase has generally been considered to represent the sole enzymic pathway for the synthesis of δ-aminolevulinic acid and for the regulation of porphyrin and heme synthesis.

Several points have led us to question these conclusions. First, in their initial publication describing the role of δ-aminolevulinic acid in porphyrin synthesis Shemin and Russell suggested other metabolic fates for δ-aminolevulinic acid (1). From experiments utilizing δ-aminole[14C]levulinic acid, a series of reactions termed the succinate-glycine cycle was described (1, 6, 7) and they found that δ-aminole[5,14C]levulinic acid gave rise to purines. They postulated that δ-aminolevulinic acid might be deaminated yielding γ,δ-dioxovalerate which, upon losing the terminal carbonyl group, would regenerate succinate and a one-carbon fragment capable of undergoing further reactions in the biosynthesis of purines. The existence of such a cycle has been confirmed by others (8, 9) and has been regarded primarily as a pathway for the catabolism of excess δ-aminolevulinic acid. However, Gibson et al. (10) and Neuberger and Turner (11) working with a partially purified system from Rhodopseudomonas spheroides suggested that the "reverse reaction," namely the transamination of γ,δ-dioxovalerate yielding δ-aminolevulinic acid, was favored.

Second, and of equal importance, is the fact that δ-aminolevulinic acid synthase has not yet been demonstrated in green plants (12, 13), even though it has been established that δ-aminolevulinic acid is an obligatory precursor in the biosynthesis of chlorophyll. Evidence continues to accumulate that plants form δ-aminolevulinic acid directly from a 5-carbon precursor by means of a transamination reaction (13-15).

In this report we describe the isolation and characterization of an enzyme from mammalian liver mitochondria which catalyzes the transamination reaction between alanine and γ,δ-dioxovalerate yielding δ-aminolevulinic acid and pyruvate. The capacity of this enzyme, L-alanine: γ,δ-dioxovalerate transaminase to form δ-aminolevulinic acid appears to be far greater than the capacity of δ-aminolevulinic acid synthase from the same mitochondria.
dicarbonyl compounds, \( \gamma,\delta \)-dioxovalerate and methylglyoxal, were the only compounds found to interfere. The degree of interference by each of these compounds was determined in separate experiments and correction factors were applied when necessary.

**Definition of Units and Specific Activity**—One unit of \( \gamma,\delta \)-dioxovalerate transaminase is defined as the amount of enzyme that will catalyze the formation of 1 \( \mu \)mol of \( \delta \)-amino-levulinic acid/\( h \) at 37°C. Specific activity is expressed as units/mg of protein. Protein was determined by the method of Lowry et al. (17) or, in the later stages of purification, spectrophotometrically by absorbance at 280 nm.

**Identification of Reaction Products**—That \( \delta \)-amino-levulinic acid is a product of the reaction when alanine and \( \gamma,\delta \)-dioxovalerate serve as substrates was established by four methods. First the \( \delta \)-amino-levulinic acid was converted to the pyrrole (16) which then was adsorbed on Dowex 50-wx8 (20). The solution was mixed with an equal volume of 0.01 M Tris-HCl, pH 7.2, containing 0.25 M sucrose and homogenized in a Waring Blender. Mitochondria were isolated by the method of Johnston and Lardy (24). The packed mitochondria were suspended in an equal volume of the same buffer, this time without sucrose, freeze-thawed, sonicated, and centrifuged at 10,000 \( \times \)g for 30 min. The pellet was washed twice with 0.5 volume of the same buffer and the three supernatant solutions combined (Fraction 1).

**Step 2: Heat Denaturation**—Aliquots (250 ml) of Fraction 1 were heated to 60°C in a stainless steel container and maintained at that temperature for 6 min with vigorous stirring. The material then was cooled rapidly and centrifuged at 10,000 \( \times \)g for 15 min. All subsequent steps were carried out at 4°C. The supernatant solutions (Fraction 2) were combined for the next step.

**Step 3: Calcium Phosphate Gel Fractionation**—Two columns (2.5 \( \times \) 55 cm) were prepared with calcium phosphate (25). The crude enzyme preparation (Fraction 2) was divided into two equal fractions which were placed on the columns. The material had run entirely into the gel, an equal volume of 0.01 M sodium phosphate buffer, pH 6.9, was used as an initial wash. The enzyme was eluted using a linear gradient of sodium phosphate going from 0.05 to 0.3 M. The flow rate was 36 ml/h and the total volume of applied buffer was 1 liter. The peak of enzyme activity was eluted from both columns at a sodium phosphate concentration of 0.19 M.

The fractions with highest specific activity were pooled and concentrated to one-eighth of the original volume in an Amicon model 6000 centrifugal cell using an XM 50 Diaflow filter. The enzyme was diluted with the original volume of 0.01 M Tris-HCl buffer, pH 7.6, containing 1 mM EDTA and reconcentrated to one-eighth of the original volume (Fraction 3). This dilution-reconcentration procedure was repeated once more.

**Step 4: DEAE-Cellulose Chromatography**—The enzyme solution (Fraction 3) was applied to a column of DEAE-cellulose (2.5 \( \times \) 26 cm) equilibrated with 10 mM Tris-HCl buffer, pH 7.6, containing 1 mM EDTA. After the enzyme had run into the DEAE, the column was washed with 50 ml of the same buffer. The enzyme then was eluted with a linear gradient of KCl going from 0 to 0.2 M in the same buffer. The flow rate was 30 ml/h. The total volume of the gradient was 1 liter and the enzyme eluted approximately in the middle of the gradient. The fractions with highest specific activity (3 units/mg of protein) were pooled and concentrated by pressure filtration (XM 50 Diaflow filter). The material was washed several times with the 10 mM Tris-HCl buffer, pH 7.6, during which time the specific activity increased by approximately 30%. The final product (Fraction 4) represented a 54-fold purification from the starting mitochondria and a 270-fold purification from the starting crude liver homogenate. This material was used for most of the characterization studies.

The purification procedure is summarized in Table I.
TABLE I
Purification of L-alanine:γ,δ-dioxovaleric acid aminotransferase from bovine liver

<table>
<thead>
<tr>
<th>Fraction number</th>
<th>Volume ml</th>
<th>Protein g</th>
<th>Units¹</th>
<th>Specific activity</th>
<th>Purification fold</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Mitochondria</td>
<td>250</td>
<td>11.5</td>
<td>20.6×10²</td>
<td>0.18</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>2. Heat denatura-</td>
<td>250</td>
<td>1.5</td>
<td>11.6×10²</td>
<td>0.77</td>
<td>4.3</td>
<td>56</td>
</tr>
<tr>
<td>tion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Concentrated</td>
<td>50</td>
<td>0.25</td>
<td>8.0×10²</td>
<td>3.2</td>
<td>18.5</td>
<td>39</td>
</tr>
<tr>
<td>G-200 column eluate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Concentrated</td>
<td>80</td>
<td>0.054</td>
<td>5.2×10²</td>
<td>9.6</td>
<td>54</td>
<td>25</td>
</tr>
<tr>
<td>DEAE column eluate</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

¹ One unit is the amount of enzyme that will catalyze the formation of 1 μmol of δ-aminolevulinic acid/h at 37°C.

Stability of the Enzyme

Aliquots of the purified enzyme were freeze-thawed or kept on ice for 72 h. No loss of enzyme activity was detected in either case. We routinely maintained the enzyme at −20°C for periods up to 6 months without any significant loss of activity.

Molecular Weight

The molecular weight of the enzyme was estimated by chromatography using a column (2.5 × 50 cm) of Sephadex G-200 (superfine) equilibrated with 0.05 M Tris-HCl, pH 7.4, containing 1 mM EDTA and 10 mM NaCl. The operating pressure was 12 cm of water and the flow rate was 13 ml/h. Fractions of 6 ml were collected. The calculations were made following the method of Andrews (26). Void volume was established with a 0.2% solution of blue dextran. Approximately 20 units of enzyme were placed on the column. In each of several runs the peak enzyme activity emerged at approximately the same elution volume. The standards used were: horse heart cytochrome c, human hemoglobin, rabbit muscle lactic dehydrogenase, bovine liver catalase, and horse spleen ferritin. The molecular weight of γ,δ-dioxovalerate transaminase was thus estimated to be 240,000 (range, 230,000 to 250,000).

Definition of Purity of γ,δ-Dioxovalerate Transaminase

Polyacrylamide gel electrophoresis was performed on a sample of Fraction 4 and a major band and several smaller bands were observed (Fig. 1). However, the material that passed through the Sephadex G-200 column showed only two bands: the same major band and one smaller one (Fig. 1). This material had approximately twice the activity of Fraction 4. Aliquots of the Sephadex eluates were passed through Shaltiel hydrophobic columns (Kit 1, agarose-C₄, series) (27). The longer the alkyl side chain, the greater was the observed increase in specific activity. Gel electrophoresis of the Shaltiel column eluates (10 μg of protein) indicated that the major band was γ,δ-dioxovalerate transaminase since the increase in specific activity coincided with the removal of virtually all but the main protein band (Fig. 1).

Fraction 4 was also subjected to the crystallization technique of Jacoby (28). Crystals were observable in the 60% saturated ammonium sulfate wash. The specific activity of the crystalline enzyme was equivalent to that attained by chromatography on the hydrophobic gel (agarose-C₄ - 10) described above.

Enzyme Characterization

Localization—Upon clearly identifying γ,δ-dioxovalerate transaminase activity in mammalian liver homogenates, experiments were undertaken to localize the enzyme within the cell. Liver tissue was homogenized in a Teflon Potter-Elvehjem homogenizer and the mitochondria were separated by the method indicated in Step 1 of the enzyme purification procedure. The cytosol contained minimal enzymic activity. The mitochondria were sonicated and freeze-thawed and centrifuged in a Spinco model L ultracentrifuge at 144,700 × g for 30 min. The enzymic activity appeared only in the supernatant fraction, indicating that the enzyme is present in mitochondria, but is not tightly membrane bound.

Buffer, Ionic Strength, and pH—The enzymic activity was relatively insensitive to changes in ionic strength. The activity of the bovine enzyme was studied over the pH range 6.0 to 8.0. Sodium phosphate and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffers (0.1 M) were employed so that an overlap at the same pH was possible. The enzyme has optimal activity at pH 6.9 with a broad peak exhibiting 90% activity at pH 6.6 and at pH 7.4. The enzyme was virtually inactive when 0.1 M Tris HCl was used as the assay buffer. The low concentrations of Tris used in preparative steps had no effect on enzyme activity as measured in the standard assay mixture containing 50 mm sodium phosphate. Studies with rat liver enzyme yielded similar results.

Pyridoxal Phosphate Studies—Spectral evidence consistent with the presence of pyridoxal phosphate was obtained in the most highly purified enzyme preparations (29). Incubations with KCN, isoniazide, and hydroxylamine, followed by dialysis, resulted in complete loss of enzymic activity. Enzymic activity could not be restored by the addition of pyridoxal.
phosphate suggesting that the cofactor is tightly bound. This suggestion was supported by the observation that it was not possible to enhance enzymic activity by the addition of pyridoxal phosphate at any stage of the purification procedure. This is also consistent with the lack of enzymic activity in Tris buffer.

Substrate Specificity Studies—The specificity of $\gamma,\delta$-dioxovalerate transaminase with regard to the amino donor was examined by replacing alanine with other amino donors. As is evident from Table II, the enzyme is quite specific for alanine, and is stereospecific for the $\beta$ isomer. Studies on the specificity of amino acceptors other than $\gamma,\delta$-dioxovalerate were technically difficult because the product of the transamination was not $\delta$-aminolevulinic acid. This problem was dealt with by examining possible amino acceptors as potential inhibitors of the $\gamma,\delta$-dioxovalerate transaminase reaction.

Kinetic Studies—The enzyme demonstrated Michaelis-Menten kinetics for both substrates. $K_m$ values were determined under the standard assay conditions. Lineweaver-Burk plots were constructed using a computer program. The apparent $K_m$ for L-alanine was $3.7 \times 10^{-4}$ m and the apparent $K_m$ for $\gamma,\delta$-dioxovalerate was $2.4 \times 10^{-4}$ m. Plots of $1/v$ at varying alanine concentrations generated a series of parallel lines indicative of a ping-pong reaction mechanism characteristic of transaminases (Fig. 2).

Two types of compounds were studied as inhibitors of the $\gamma,\delta$-dioxovalerate transaminase reaction. These compounds were either reaction products (pyruvate and $\delta$-aminolevulinic acid) or substrate analogs ($\alpha$-ketoglutarate, succinic semialdehyde, and methylglyoxal). The kinetic data generated in this series of experiments are complex and have been treated in detail in another publication (30). The concentration of each compound producing 50% inhibition of the enzymic reaction is shown in Table III.

Of the substrate analogs only methylglyoxal was demonstrated to serve as an alternate amino acceptor. Transamination of methylglyoxal yielded aminoacetone which was detected as the Ehrlich chromophore and as a ninhydrin-positive spot on paper chromatography. Methylglyoxal was, however, less than 1% as efficient an amino acceptor as $\gamma,\delta$-dioxovalerate.

Studies on the Conversion of $\delta$-Aminolevulinic Acid and Pyruvate to $\gamma,\delta$-Dioxovalerate and Alamine

The "reverse" reaction (the formation of $\gamma,\delta$-dioxovalerate and alanine from $\delta$-aminolevulinic acid and pyruvate) was studied in three ways.

$\delta$-Aminolevulinic Acid Disappearance—These experiments were done over a concentration range of 1 $\mu$M to 10 $\mu$M for $\delta$-aminolevulinic acid and included a similar range of concentrations of pyruvate. The amount of enzyme (Fraction 4) varied from 0.2 to 10 units of activity. Parallel experiments in the "forward" direction (the formation of $\delta$-aminolevulinic acid and pyruvate from $\gamma,\delta$-dioxovalerate and alanine) were always run to confirm the activity of the enzyme preparation. $\delta$-Aminolevulinic acid concentration was followed at intervals up to 4 h by conversion to the pyrrole and measurement of the Ehrlich chromophore. No significant decrease in $\delta$-aminolevulinic acid concentration was observed.

The reverse reaction as determined by the measurement of $\delta$-aminolevulinic acid disappearance has been reported by others using crude enzyme preparations. We therefore repeated our $\delta$-aminolevulinic acid disappearance studies using an unfractiated liver homogenate as the enzyme source. We were able to detect a decrease in $\delta$-aminolevulinic acid concentration as a function of time. However, when porphobilinogen formation was simultaneously measured, it was possible to account for the decrease in $\delta$-aminolevulinic acid by the synthesis of porphobilinogen via $\delta$-aminolevulinic acid dehydrase, a reaction apparently not considered in previous reports (31, 32).

Formation of $\gamma,\delta$-Dioxovalerate—Under the conditions described above (using highly purified enzyme preparations) we attempted to measure the formation of $\gamma,\delta$-dioxovalerate by the $\alpha$-phenylenediamine reaction (22). Because we observed that the pyruvate-$\alpha$-phenylenediamine complex interfered with the detection of the $\gamma,\delta$-dioxovalerate-$\alpha$-phenylenediamine complex at 336 nm, we removed pyruvate at the end of the incubation with lactic acid dehydrogenase and NADH (20). $\gamma,\delta$-Dioxovalerate did not serve as a substrate for lactic dehydrogenase. Even with the removal of pyruvate we were unable to detect $\gamma,\delta$-dioxovalerate formation.

### Table II

Comparison of aminodonors for L-alanine:$\gamma,\delta$-dioxovaleric acid aminotransferase

<table>
<thead>
<tr>
<th>Amino Donor</th>
<th>% Enzyme Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Alanine</td>
<td>100</td>
</tr>
<tr>
<td>DL-Alanine</td>
<td>89</td>
</tr>
<tr>
<td>$\beta$-Alanine</td>
<td>9</td>
</tr>
<tr>
<td>$\epsilon$-Aminocaproic acid</td>
<td>22</td>
</tr>
<tr>
<td>4-Aminobutyric acid</td>
<td>10</td>
</tr>
<tr>
<td>Lysine</td>
<td>3</td>
</tr>
<tr>
<td>Glycine</td>
<td>9</td>
</tr>
<tr>
<td>L-Ornithine</td>
<td>6</td>
</tr>
<tr>
<td>L-Valine</td>
<td>2</td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table III

Inhibitors of L-alanine:$\gamma,\delta$-dioxovaleric acid aminotransferase

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration producing 50% inhibition (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction products</td>
<td></td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.10</td>
</tr>
<tr>
<td>$\delta$-Aminolevulinic acid</td>
<td>0.30</td>
</tr>
<tr>
<td>Substrate analogs</td>
<td></td>
</tr>
<tr>
<td>$\alpha$-Ketoglutarate</td>
<td>30.0</td>
</tr>
<tr>
<td>Saccinic semialdehyde</td>
<td>0.15</td>
</tr>
<tr>
<td>Methylglyoxal</td>
<td>0.60</td>
</tr>
</tbody>
</table>
unable to demonstrate the formation of γ,δ-dioxovalerate.

We next attempted to demonstrate the "reverse" reaction using δ-aminolevulinic acid as a substrate (3pmol over a range of 1 μM to 10 mM). At the end of the incubation (30 min to 2 h) between 2 and 4 μmol of unlabeled γ,δ-dioxovalerate were added and three insoluble derivatives were prepared: the 2,4-dinitrophenylhydrazone, the thiosemicarbazone, and the semicarbazone.

The 2,4-dinitrophenylhydrazone was prepared and recrystallized twice from acetone. The derivative was chromatographed on Silica Gel thin layer plates. Direct counting of the plates was done with a Packard model 7200 strip counter. No significant radioactivity could be detected on the plates was done with a Packard model 7200 strip counter. The carbazones of γ,δ-dioxovalerate-phenylhydrazone derivative band. Liquid scintillation counting of the phenylhydrazone derivative revealed a major problem with quenching but even with appropriate external standard ratio curves we could detect no formation of γ,δ-dioxovalerate.

The thiosemicarbazone and semicarbazone derivatives of γ,δ-dioxovalerate were also studied. At the end of the incubation 2 and 4 μmol of γ,δ-dioxovalerate and 5 to 10 μmol of an aqueous saturated solution of thiosemicarbazide hydrochloride were added. The carbazones were crystallized and recrystallized from water and counted in a liquid scintillation counter. No significant quenching was observed and recovery studies done with authentic [4-14C]γ,δ-dioxovalerate solutions indicated a 70 to 75% recovery. Using δ-aminolevulinic acid as a substrate and highly purified enzyme we were unable to detect radioactivity in either of the γ,δ-dioxovalerate-carbazones.

Finally, in our studies of the reverse reaction, using δ-aminolevulinic acid as a substrate, we attempted to isolate nonderivatized [4-14C]γ,δ-dioxovalerate by an ion exchange chromatographic method that clearly separates γ,δ-dioxovalerate from δ-aminolevulinic acid. Mixtures of authentic δ-aminolevulinic acid and [4-14C]δ,γ-dioxovalerate were applied to Dowex 50 where the δ-aminolevulinic acid was adsorbed (33). Recovery of δ-aminolevulinic acid from the Dowex 50 was found to range from 80 to 95%. Washes from the Dowex column (which contained the [4-14C]δ,γ-dioxovalerate) were then applied to Dowex 2 (PH 3.9).

The [4-14C]δ,γ-dioxovalerate was adsorbed under these conditions (9). The column was washed extensively with H2O and the [4-14C]δ,γ-dioxovalerate was eluted with 1 N HCl. Recovery of [4-14C]δ,γ-dioxovalerate was found to range from 80 to 90%. This technique yielded clear separation of [4-14C]δ,γ-dioxovalerate and δ-aminolevulinic acid as demonstrated by simultaneous liquid scintillation counting and colormetric measurements. In the experimental situation, in which the enzyme was incubated with δ-aminolevulinic acid and pyruvate over the concentration ranges mentioned above, we could not demonstrate the formation of [4-14C]δ,γ-dioxovalerate.

Formation of Alanine—In these studies pyruvate labeled with 14C in the carboxyl position was used as the amino acceptor over a range of concentrations (1 μM to 1 mM). After incubation the reaction was stopped with acid and the remaining pyruvate was chemically decarboxylated with H2O2 (34). No significant difference in residual counts, indicating alanine formation, could be detected between incubations with and without either purified or crude enzyme preparations. Further, no evidence of alanine formation could be detected using paper chromatography.

Discussion

The data presented describe the mammalian enzyme L-alanine:γ,δ-dioxovaleric acid aminotransferase which catalyzes the formation of δ-aminolevulinic acid via a transamination reaction. Indirect evidence for the biosynthesis of δ-aminolevulinic acid by a transamination reaction in mammalian tissues has been reported by others (32, 35, 36) but the present report is the first to characterize the enzyme responsible, γ,δ-Dioxovalerate transaminase from bovine liver mitochondria has been purified to near homogeneity.

Bovine γ,δ-dioxovalerate transaminase is a remarkably stable protein with an apparent molecular weight of 240,000. The molecular weight suggests that the enzyme was isolated as an oligomer. Most known transaminases have molecular weights of approximately 100,000. γ,δ-Dioxovalerate transaminase has distinct substrate specificity for both the amino donor (L-alanine) and the amino acceptor (γ,δ-dioxovalerate). Other amino donors and acceptors had only a fraction of the reactivity of these two compounds. None of the alternate amino donors studied appeared to inhibit the enzyme, whereas the alternate amino acceptors were inhibitory. Only one alternate amino acceptor, methylyglyoxal, yielded a detectable reaction product yet methylyglyoxal was a distinctly inferior substrate when compared to γ,δ-dioxovalerate. The interpretation of the significance of apparent K, values is always difficult. Nevertheless, low values are commonly expected for the natural substrates of biosynthetic enzymes. The apparent K, of 0.24 mM for γ,δ-dioxovalerate is compatible with this expectation but the apparent K, for L-alanine of almost 4 mM is not. However, it is important to point out that the apparent K, for glycine in the δ-aminolevulinic acid synthase catalyzed formation of δ-aminolevulinic acid is 10 mM (37).

The in vivo availability of substrates for γ,δ-dioxovalerate transaminase must be considered if a significant role in the biosynthesis of δ-aminolevulinic acid is assigned to the enzyme. L-Alanine is of course ubiquitous. The enzymic reduction of α-ketoglutarate to γ,δ-dioxovalerate has been reported in corn leaf extracts (15) and analogous reactions involving the reduction of a carboxyl group to an aldehyde are well known (35, 39). Thus it seems reasonable to assume that γ,δ-dioxovalerate might be a naturally occurring metabolite within mitochondria.

Classically transaminases catalyze readily reversible reactions with equilibrium constants approximating unity. Mechanistically these reactions are ping-pong in type (40) and our studies in which γ,δ-dioxovalerate and L-alanine concentrations were varied suggested a ping-pong reaction mechanism for γ,δ-dioxovalerate transaminase (Fig. 2). However, exhaustive experiments failed to demonstrate reversibility of the γ,δ-dioxovalerate transaminase reaction. Other workers, using bacterial systems have noted this lack of reversibility (9, 11). Shigisada suggested that the formation of γ,δ-dioxovalerate from δ-aminolevulinic acid might involve a different enzyme (9). However, this explanation avoids consideration of the molecular events involved in the γ,δ-dioxovalerate transaminase reaction. It is possible that γ,δ-dioxovalerate transaminase is nonclassical in its reaction mechanism and that it forms as a yet unidentified intermediate. One possibility for such an intermediate is glutamic-1-semialdehyde (41). Such an intermediate might even react at a second active site on the enzyme yielding δ-aminolevulinic acid and pyruvate. This point has been studied in our laboratory, so far without definitive results. Thus, although purified γ,δ-dioxovalerate transaminase demonstrates many properties of classical transaminases, the apparent and unexpected irreversibility of the reaction remains unexplained. The in vivo reversibility of γ,δ-dioxovalerate transaminase remains to be examined. Thus the role of the enzyme in the succinate-glycine cycle (1, 6, 7) is unresolved.
The capacity of mammalian γ,δ-dioxovalerate transaminase to synthesize δ-aminolevulinic acid, as studied in vitro, appears to be far greater than the capacity of δ-aminolevulinic acid synthase from the same source. We were able to detect δ-aminolevulinic acid formation by the transamination reaction, even in our most crude preparations, with a simple colorimetric assay. When δ-aminolevulinic acid formation by the δ-aminolevulinic acid synthase reaction is studied, the yield of δ-aminolevulinic acid is so low that radiochemical assays are required to quantitate the reaction product (5). Evidence continues to accumulate that green plants synthesize δ-aminolevulinic acid exclusively via transamination reactions (12,13,41,42).

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