The Enzyme-Enzyme Complex of Transaminase and Glutamate Dehydrogenase*

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SUMMARY

Glutamate dehydrogenase decreases the distribution coefficient of glutamate oxalacetate transaminase in Sephadex G-200. This is consistent with previous results which suggested that a complex is formed between these two enzymes. These gel filtration as well as kinetic experiments suggest that transaminase can react with monomeric but not polymer forms of glutamate dehydrogenase.

When the levels of both mitochondrial enzymes are too low to form a complex, there is little TPNH and NH₄⁺ generated by the combined aspartate aminotransferase and glutamate dehydrogenase reactions. This is because oxalacetate is a potent product inhibitor of transaminase, the reaction with glutamate and glutamate dehydrogenase is slow and α-ketoglutarate, oxalacetate, and aspartate all inhibit this latter reactions.

When the levels of both enzymes are sufficiently high to form a complex, aspartate can be dehydrogenated quite rapidly even in the absence of α-ketoglutarate. Furthermore, the aspartate dehydrogenase reaction catalyzed by the enzyme-enzyme complex is not markedly inhibited by oxalacetate, or activated by α-ketoglutarate, and can take place even in the presence of rather high levels of glutamate. Kinetic and gel filtration experiments suggest that the dissociation constant of the enzyme-enzyme complex is considerably lower than that of these substrates for the free enzymes.

These results suggest that an important physiological function of the enzyme-enzyme complex is to catalyze the aspartate dehydrogenase reaction in organs as brain, liver, and kidney, where the mitochondrial levels of these enzymes are sufficiently high to form a complex. An advantage of catalysis by the complex over transamination with aspartate followed by dehydrogenation of glutamate is that the complex is not markedly inhibited by low levels of oxalacetate.

When tyrosine is the substrate and the levels of these two enzymes are too low to form a significant amount of complex, the tyrosine aminotransferase and glutamate dehydrogenase reactions are coupled. This can occur with tyrosine but not aspartate because hydroxyphenylpyruvate is not a potent inhibitor. However, when the levels of both enzymes are sufficiently high to form a significant amount of the enzyme-enzyme complex, the tyrosine dehydrogenase reaction can be catalyzed by this complex and again α-ketoglutarate has no effect on the reaction. Similar relationships occur when phenylalanine is the substrate. Thus, the enzyme-enzyme complex can facilitate dehydrogenation of these amino acids which do not react with glutamate dehydrogenase.

Glutamate dehydrogenase [L-glutamate:NAD(P) oxireductase, EC 1.4.1.3] in the presence of reduced pyridine nucleotides and ammonium ions can react with the pyridoxal phosphate form of glutamate oxalacetate transaminase (L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.11) to produce the pyridoxamine form of that enzyme (1-4). Several experiments demonstrate that this reaction results from formation of an enzyme-enzyme complex and is not mediated by keto or amino acids bound to either enzyme (1-4) and that, in this reaction, glutamate dehydrogenase reacts with transaminase bound pyridoxal phosphate (1-4). That is, in this reaction the transaminase is not functioning as a catalyst but delivers the actual substrate, pyridoxal phosphate, to the active site of glutamate dehydrogenase.

This reaction can take place in the absence of keto or amino acid. However, if oxalacetate, a poor substrate of glutamate dehydrogenase, is added, this keto acid is reductively aminated by the combination of the enzyme-enzyme reaction plus the transaminase one-half reaction:

\[
\text{TPNH} + \text{H}^+ + \text{pyridoxal-P-transaminase} \rightarrow \text{TPN}^+ + \text{pyridoxamine-P-transaminase} + \text{H}_2\text{O}
\]

\[
\text{Pyridoxamine-P-transaminase} + \text{oxalacetate} \rightarrow \text{aspartate} + \text{pyridoxal-P-transaminase}
\]

This manuscript presents additional studies and evidence for the formation of the enzyme-enzyme complex. Also included are kinetic studies of amino acid dehydrogenase reactions catalyzed by the bovine liver mitochondrial glutamate dehydrogenase-glutamate-oxalacetate transaminase complex. Thus these experiments are performed with two mitochondrial enzymes both isolated from the same organ.

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Enzymes and Reagents—Pig heart cytoplasmic glutamate oxalacetate transaminase and glutamate pyruvate transaminase were obtained from Boehringer Mannheim Corporation. Bovine liver mitochondrial glutamate dehydrogenase and glutamate oxalacetate transaminase were prepared and crystallized according to previously described methods (5-7). Dinitrophenylhydrazine was obtained from Eastman Kodak. Uniformly labeled [1-14C] glutamic acid was obtained from New England Nuclear Corporation. Reagents for radioimmunoassays were obtained from Research Products International. Pyridine nucleotides, purine nucleotides, and substrates were obtained from P-L Laboratories. Solutions of coenzymes were prepared as sodium salts. Solutions of coenzymes were prepared fresh daily.

Treatment of Enzyme—The mitochondrial transaminase or glutamate dehydrogenase crystals were harvested by centrifugation and solubilized in 0.025 M sodium arsenate-0.1 mM EDTA, pH 7.8, at 4° and the 1-ml solutions were then dialyzed twice versus 2 liters of the arsenate buffer for 12 hours. To further insure that the dialyzed enzyme solutions were free of trace amounts of keto or amino acid impurities, these solutions were chromatographed on previously described columns of Sephadex G-200, Sephadex G-100, and DEAE-Sephadex A-25 and A-50 (1-3). The peak enzyme fractions from these columns were concentrated by dialyzing versus 80% saturated (NH4)2SO4, pH 7.0, and the ammonium sulfate precipitates then were centrifuged, solubilized, and dialyzed as described above. The above extensive treatment of the dialyzed crystalline enzymes did not alter their ability to interact. Similar results have been previously found with the cytoplasmic transaminase (3).

Gel Filtration—The cytoplasmic glutamate oxalacetate transaminase was resolved with previously described methods (8). After these treatments the enzyme was chromatographed on Sephadex G-25 and eluted with the arsenate buffer. Transaminases were prepared in the pyridoxamine phosphate form according to previously described methods followed by chromatography on Sephadex G-25 (9).

The term “dialyzed” refers to dialyzing 1-ml solutions of enzyme two times versus 2 liters of 0.025 M sodium arsenate-0.1 mM EDTA, pH 7.8, at 4° for 14 hours. This was done to all enzymes before use in these experiments.

Enzyme, Pyridoxal, and Pyridoxamine Concentration—The concentration of transaminase and glutamate dehydrogenase is expressed in terms of micromolar peptide chain. In the case of transaminase this was measured and calculated with previously described methods (1, 2, 10, 11). The concentration of glutamate dehydrogenase peptide chain was calculated from spectrophotometric measurements using 0.07 mg ml⁻¹ cm⁻¹ as the extinction coefficient (12) and 5.6 X 10⁻⁸ as the molecular weight of a peptide chain (13, 14).

Measurements of Products—The concentration of oxidized or reduced pyridine nucleotides and pyridoxamine or pyridoxal phosphate produced in reactions was measured as previously described (1-3). The rate of oxalacetate production was measured spectrophotometrically at 260 to 280 nm (15) or in a coupled assay with DPNH plus malate dehydrogenase (16). The rate and amount of hydroxyphenylpyruvate produced was measured with a colorimetric assay (17, 18). In the tyrosine amino transferase reaction with either α-ketoglutarate or oxalacetate as keto acids, the rate of production of hydroxyphenylpyruvate was linearly related to transaminase concentration and constant for at least 30 min. Most of these incubations were for 30 min. All assays were performed in 0.025 M sodium arsenate-0.1 mM EDTA, pH 7.8, at 25°.

Amino Acid Analysis and Assays for Radioactivity—Amino acid analyses were performed and solutions were deproteinized as described previously (3). Radioactivity assays were performed with previously described methods (3).

Reaction with 2,4-Dinitrophenylhydrazine—These were performed as previously described (10) except 1 ml of 4 N NaOH was added instead of 10 ml of 0.4 N NaOH.

Ole Filtration Equilibrium—These experiments were performed with previously described methods (20). Two-milliliter solutions of blue dextran 2000 plus transaminase alone, glutamate dehydrogenase alone, or both enzymes with or without substrates were incubated in 0.025 M sodium arsenate-0.1 mM EDTA, pH 7.8, for 30 min at 24° and then added to gels of Sephadex G-200 which were previously equilibrated with the sodium arsenate-EDTA buffer. The suspensions were stirred for 2 hours at 24°. At the end of this time the suspensions were centrifuged, aliquots were removed from the aqueous phase, and the volume of the aqueous phase (total blue dextran added divided by concentration of blue dextran in the aqueous phase), the penetration volume of transaminase (total units of transaminase added divided by units per ml of transaminase in the aqueous phase), and the penetration volume of glutamate dehydrogenase (which was equal to the volume of the aqueous phase) were measured. Control experiments revealed that blue dextran did not alter enzyme assays or the penetration volume of either enzyme. Also experiments were performed from 1/2 to 6 hours which revealed that as with previous experiments with this method (20) equilibrium was reached in 1 hour. The latter experiments also revealed that neither enzyme was denatured in 2 hours.

The distribution coefficient of transaminase was defined as

\[ K_D = \frac{V_f - V_s}{V_t - V_s} \]

where \( V_f \) is the penetration volume of transaminase, \( V_s \) is the volume of the aqueous phase, and \( V_t \) is the total volume of the system.

RESULTS

Aspartate is a poor substrate of glutamate dehydrogenase; however, if either mitochondrial (Fig. 1) or cytoplasmic (not shown) glutamate oxalacetate is added, aspartate dehydrogenase activity is increased severalfold. Under conditions where alanine, tyrosine, and phenylalanine do not react at all with glutamate dehydrogenase, the addition of glutamate pyruvate transaminase (not shown) results in alanine dehydrogenase ac-
tivity or the addition of mitochondrial glutamate oxalacetate transaminase results in tyrosine or phenylalanine dehydrogenase activity (Fig. 1). These transaminases do not have amino acid dehydrogenase activity. The effect of these transaminases is not due to protection of glutamate dehydrogenase against inactivation since this enzyme is stable in these experiments.

These amino acid dehydrogenase reactions take place with low levels of transaminase (Fig. 1), do not require added keto acids, and, therefore, appear to be mediated by the previously proposed enzyme-enzyme complex between transaminase and glutamate dehydrogenase. An alternative explanation is that these reactions are mediated by α-ketoglutarate or glutamate impurities in the system. These are the only known substrates sufficiently reactive with all four of these enzymes to conceivably play this role. Furthermore, low (micromolar) levels of glutamate-oxalacetate transaminase can increase the rate of reductive amination of oxalacetate or dehydrogenation of aspartate when the level of these substrates are high (1 to 10 mM). Similarly, low levels of glutamate pyruvate transaminase can increase the rate of reductive amination of pyruvate or dehydrogenation of alanine when the levels of these substrates are high (1 to 10 mM). To explain such increases on the basis of substrate contamination would require that micromolar levels of these transaminases would contain millimolar amounts of these substrates, and this is not the case (Fig. 1 and Refs. 1 and 2).

Estimates of Levels of Substrate—If the two enzymes are assumed to be coupled through the presence of contaminant α-ketoglutarate or glutamate in the experiments of Fig. 1, how much of these would be required to explain the observed rate? To estimate this, assays were performed with a range of these substrates (10 to 100 μM) and with aspartate (10 mM) plus cytoplasmic glutamate oxalacetate transaminase alone or with TPN (1.0 mM) plus glutamate dehydrogenase alone. The initial velocity in the presence of either of these enzymes alone with very low levels of these substrates then was calculated by extrapolation from the standard curve that was obtained. These calculations are apparently valid since the observed velocities were proportional to enzyme concentration; that is, the dissociation constants of both substrates for both enzymes are quite high. Furthermore, the measured velocities were observed to decrease linearly as the concentration of substrate remaining in the reaction decreased (because the concentration of substrate was much lower than the K_m).

The rate of TPNH production observed in the presence of TPN, aspartate, cytoplasmic glutamate oxalacetate transaminase (0.2 μM), and glutamate dehydrogenase (7.2 μM), conditions similar to those used in Fig. 1B, is (after correcting for the slow rate of aspartate oxidation catalyzed by glutamate dehydrogenase in the absence of transaminase) equal to that expected in the transaminase reaction with aspartate (as assayed with the coupled malate dehydrogenase system) and 0.13 μM α-ketoglutarate or the glutamate dehydrogenase reaction with TPN and 4 μM glutamate. Therefore, if the enzyme-enzyme reaction is actually due to recycling of these substrates between the two enzymes, then the level of either of these substrates would have to be at least 4 μM or the molar ratio of these substrates to transaminase or glutamate dehydrogenase peptide chains would have to be respectively 20 or 0.8. This is not consistent with experiments performed with the reverse reaction which revealed that the molar ratio of these substrates to transaminase or glutamate dehydrogenase could not be higher than 0.3 or 0.08. This is, respectively, 66- and 7-fold lower than that required to explain the rate observed with TPN, aspartate, and both enzymes.

Similarly, it was found that, in the presence of the mitochondrial transaminase (0.25 μM), TPN (1.0 mM), aspartate (10 mM), and glutamate dehydrogenase (10 μM), the rate of TPNH production (after subtracting the slow rate in the absence of transaminase (see Fig. 1)) was equivalent to that expected in this transaminase reaction (as assayed with the coupled malate dehydrogenase system) with aspartate and 1.0 μM α-ketoglutarate or the glutamate dehydrogenase reaction with TPN and 4 μM glutamate. Thus, if this reaction is mediated by α-ketoglutarate or glutamate associated with this transaminase, the molar ratio of these substrates to transaminase peptide chains would have to be 16. Again, this is not consistent with experiments performed with the reverse reaction which revealed that the molar ratio of these substrates transaminase could not be higher than about 0.03 or about 500-fold lower than the value required to explain the reaction with TPN and aspartate. Thus, the reductiveamination of oxalacetate and dehydrogenation of aspartate cannot both be explained on the basis of a constant ratio of these substrates to those enzymes. Also, a high level of either of these substrates would be required to explain the rate observed with TPN, aspartate, and these enzymes. Previous experiments have ruled out the possibility that the ratio of α-ketoglutarate or glutamate to the cytoplasmic transaminase is 20 (3). Similar experiments were performed which ruled out the possibility that this ratio is 16 in the case of the mitochondrial transaminase. In addition, neither α-ketoglutarate nor glutamate are detected when 30 to 60 nmoles of either of the transaminases or glutamate dehydrogenase are deproteinized and the centrifuged protein-free supernatant solution is assayed with the amino acid analyzer, with DPNH, and NH_4^+ plus native glutamate dehydrogenase, or with 2,4-dinitrophenylhydrazine. If these substrates were associated with these enzymes in levels even considerably lower than required to explain the rate observed with TPN and aspartate, they would have been detected in these assays. Also when tyrosine was incubated with the pyridoxal phosphate form of the mitochondrial enzyme it was found that pyridoxamine phosphate and hydroxyphenylpyruvate were produced in equal amounts, and glutamate was not detected when 20 nmoles of this tyrosine treated transaminase was deproteinized and applied to the amino acid analyzer. Therefore, there is no evidence that tyrosine is transaminating with endogenous α-ketoglutarate.

The possibility that these substrates are associated with glutamate dehydrogenase was also eliminated by finding that high levels of this enzyme do not oxidize TPNH (in the presence of NH_4^+) or reduce TPN. Also incubating this enzyme with 80 μM phenylhydrazine for 1 hour followed by dialysis does not alter its ability to react with transaminase.

The above and other experiments (1-4) rather conclusively rule out the possibility that these reactions are mediated by contaminating amounts of α-ketoglutarate or glutamate associated with the enzymes. It is also unlikely that these substrates are contaminants of the reagents. First they would have to be rather ubiquitous contaminants since enzyme-enzyme interaction occurs with TPNH, DPNH, DPN, and TPN with or without several different keto or amino acids (1-4) and in all buffers tested (1). Also it would be difficult to explain why the reaction with TPN plus aspartate would be saturated with such low levels of transaminase (0.2 μM, see Fig. 1) on the basis of impurities in the reagents. Furthermore, assays performed
transaminase is labile for 2 hours and stable for the remaining phase was constant for 2 to 8 hours. Thus it is unlikely that the substrates or coenzymes used increased the distribution coefficient to be increased in the presence of these compounds and absence of glutamate dehydrogenase. If these compounds were inactivating transaminase, binding to glutamate dehydrogenase is so low that the only possible conclusion is that glutamate dehydrogenase does not alter transaminase activity. Furthermore, none of the enzymes tested, decreases the distribution coefficient in the absence of glutamate dehydrogenase. Also, the amount of transaminase in the aqueous phase was constant for 2 hours and stable for the remaining 6 hours. In addition at the end of these gel filtration experiments gels containing transaminase were transferred (by washing and stirring with buffer) into small glass columns. The gels then were eluted until transaminase was no longer recovered in the eluate. About 80% of the transaminase units were recovered. This would be the expected recovery (within experimental error) if there were no loss of transaminase activity during the course of these experiments.

The distribution coefficient of transaminase in the presence of DPNH plus aspartate or DPN plus NH$_4^+$ and glutamate dehydrogenase is 10$^{-5}$ (13, 14, 21).

Equation 1:

$$E = E_T \frac{X}{V_s + K_D(V_1 - V_s)} + V_s$$

where $E$ is the concentration of transaminase in the aqueous phase, $E_T$ is the total amount of transaminase added, $V_s$ is the volume of the aqueous phase, $V_1$ is the total volume, and $K_D$ is the distribution coefficient of transaminase in the absence of glutamate dehydrogenase (see “Methods and Materials”). Since the concentration of glutamate dehydrogenase used was not the same in all experiments, the results are also expressed (Table 1) as an apparent equilibrium constant, $K$, which is the concentration of glutamate dehydrogenase in the aqueous phase (glutamate dehydrogenase does not penetrate Sephadex G-200) times the ratio of the fraction of transaminase free to the fraction of transaminase bound.

Binding of transaminase to glutamate dehydrogenase is maximal when either DPNH plus NH$_4^+$ or DPN plus aspartate are present (Table 1). There is much less binding in the presence of DPNH plus NH$_4^+$ than with DPN plus aspartate. In experiments with DPNH, NH$_4^+$, and glutamate dehydrogenase binding of transaminase reaches a maximum when the level of glutamate dehydrogenase is 10 $\mu$M (Fig. 2). That is, there is no additional binding when higher levels of glutamate dehydrogenase are added.

Reaction with Aspartate—A concept of the physiological role of these enzymes in mitochondria is that aspartate reacts with oxaloacetate to produce oxalacetate plus glutamate. Glu-

![Fig. 2. Plot of per cent cytoplasmic glutamate oxalacetate transaminase bound (right ordinate, $\Delta$) or initial velocity of pyridoxamine phosphate production (left ordinate, $C$) versus the concentration of glutamate dehydrogenase. Binding experiments were performed with the gel filtration equilibrium method. In these experiments 2 ml solutions of DPNH (100 $\mu$M), NH$_4$Cl (50 mM), transaminase (0.25 $\mu$M), and glutamate dehydrogenase were added to Sephadex G-200 gels (see “Methods and Materials” and legend to Table 1). The concentration of glutamate dehydrogenase refers to the amount added divided by the volume of aqueous phase. Methods of calculating per cent bound and performing these experiments are described in the text. Kinetic experiments were performed with TPNH (100 $\mu$M), NH$_4$Cl (50 mM) and transaminase (30 $\mu$M). All experiments were performed in the presence of 0.025 $\mu$M sodium arsenate-0.1 mM EDTA, pH 7.8, at 24°.](http://www.jbc.org/content/2699/9/2699/F2)

**TABLE I**

Effect of glutamate dehydrogenase on penetration of glutamate oxalacetate transaminase in Sephadex G-200

<table>
<thead>
<tr>
<th>Additions</th>
<th>GDI</th>
<th>$K_D$ (mM)</th>
<th>Transaminase bound</th>
<th>$K$ (mM)</th>
</tr>
</thead>
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<tr>
<td>GT-PLP or GT-PMP</td>
<td>0.50</td>
<td>0.50</td>
<td>15</td>
<td>67</td>
</tr>
<tr>
<td>GT-PLP</td>
<td>0.39</td>
<td>0.39</td>
<td>15</td>
<td>57</td>
</tr>
<tr>
<td>GT-PLP + DPNH</td>
<td>0.40</td>
<td>9.6</td>
<td>51</td>
<td>22</td>
</tr>
<tr>
<td>GT-PLP + DPNH + NH$_4^+$</td>
<td>0.36</td>
<td>15</td>
<td>19</td>
<td>64</td>
</tr>
<tr>
<td>GT-PMP + DPNH</td>
<td>0.29</td>
<td>8.4</td>
<td>27</td>
<td>23</td>
</tr>
<tr>
<td>GT-PMP + DPNH + NH$_4^+$</td>
<td>0.17</td>
<td>20</td>
<td>56</td>
<td>16</td>
</tr>
</tbody>
</table>
were measured with the coupled DPNH plus malate dehydrogenase is shown in Curves A and B. The results shown in Curve A were measured with the coupled DPNH plus malate dehydrogenase assay while those shown in Curve B were measured directly in the absence of DPNH plus malate dehydrogenase. The rate of TPNH production in the presence of 10 mM aspartate, 8 μM glutamate dehydrogenase, 16 μM TPN, and presence or absence of 100 μM α-ketoglutarate is shown, respectively, in Curves C and D. The other experimental conditions are described in the legend to Fig. 1.

Fig. 3 (left). Plot of velocity (in nanomoles per ml per min) versus transaminase concentration. The rate of oxalacetate formation in the presence of 10 mM aspartate and 100 μM α-ketoglutarate is shown in Curves A and B. The results shown in Curve A were measured with the coupled DPNH plus malate dehydrogenase assay while those shown in Curve B were measured directly in the absence of DPNH plus malate dehydrogenase. The rate of TPNH production in the presence of 10 mM aspartate, 8 μM glutamate dehydrogenase, 10 mM TPN, and presence or absence of 100 μM α-ketoglutarate is shown, respectively, in Curves C and D. Remaining experimental conditions are given in the legend to Fig. 1.

Fig. 4 (center). Plot of velocity (in nanomoles per min per ml) versus keto acid concentration. The effect of α-ketoglutarate on the rate of TPNH production in the presence of 1.0 mM TPN, 10 mM aspartate, 8 μM glutamate dehydrogenase, and 0.2 or 0.012 μM transaminase is shown, respectively, in Curves A and C. The effect of oxalacetate on this reaction in the presence of glutamate dehydrogenase (0 μM) and mitochondrial transaminase (0.25 μM) is shown in Curve B. Remaining experimental conditions are given in the legend to Fig. 1.

Fig. 5 (right). Plot of velocity (in nanomoles per ml per min) (left ordinate, v) or the ratio of velocity in the presence to absence of α-ketoglutarate (right ordinate, v') versus glutamate dehydrogenase concentration. The velocity of TPNH production in the presence of 1.0 mM TPN, 10 mM aspartate, 0.2 μM transaminase, and presence or absence of 100 μM α-ketoglutarate is shown, respectively, in Curves A and B. The ratio of Curve A to B is shown in Curve C. The ratio of velocity in the presence to absence of 10 μM α-ketoglutarate and presence of 5.0 mM tyrosine, 1.0 mM DPN, and 2.5 μM transaminase is shown in Curve D. Remaining experimental conditions are given in the legend to Fig. 1.

The equilibrium state of the glutamate dehydrogenase reaction is essentially equal to the amount of TPNH produced in the absence of glutamate (Fig. 6).

Reaction with Tyrosine—When the concentrations of glutamate dehydrogenase and transaminase are low, DPN plus glutamate dehydrogenase can serve as a coupled assay of the tyrosine-α-ketoglutarate amino transferase reaction (Fig. 1, Curve A). Thus the rate of this reaction is: (a) the same in either the presence or absence of DPN plus glutamate dehydrogenase; (b) directly proportional to transaminase concentration (Fig. 1, Curve A); and (c) independent of glutamate dehydrogenase concentration over a range from 1 to 14 μM (Fig. 7, Curve A). Furthermore, the apparent $K_m$ of α-ketoglutarate is in the same range whether this reaction is measured directly or in the coupled assay with DPN plus glutamate dehydrogenase. Apparently, glutamate dehydrogenase can assay the tyrosine but not the aspartate amino transferase reaction because hydroxyphenylpyruvate unlike oxalacetate is not a potent inhibitor of transaminase.

When the level of glutamate dehydrogenase is high so that DPNH and hydroxyphenylpyruvate can be produced via the enzyme-enzyme complex, then α-ketoglutarate has no effect.

1 The $K_m$ of α-ketoglutarate or oxalacetate cannot be measured accurately in the absence of DPN plus glutamate dehydrogenase. However, it can be stated that these values are quite low since in the tyrosine transferase reaction velocity is independent of α-ketoglutarate or oxalacetate concentration over a range of these substrates from 0.1 to 1.0 mM.
Fig. 6 (left). Time course of TPNH production in the presence of glutamate plus aspartate or either amino acid alone. The reactions are those in the presence of: Curve A, mitochondrial transaminase (GOT), aspartate, and glutamate; Curve B, glutamate plus transaminase; Curve C, glutamate; Curve D, glutamate plus aspartate; Curve E, transaminase plus aspartate; and Curve F, glutamate minus that produced in the presence of transaminase, glutamate, and aspartate minus that produced in the presence of transaminase and aspartate (Curve A - Curve E) is shown in Curve F. These experiments were performed in the presence of TPN (1.0 mM), glutamate dehydrogenase (10 μM), mitochondrial glutamate oxaloacetate transaminase (0.5 μM), and (where indicated) aspartate (10 mM) or glutamate (1.0 mM). Remaining experimental conditions are given in the legend to Fig. 1.

Fig. 7 (center). Plot of velocity of reduced pyridine nucleotide production (in nanomoles per min per ml) versus glutamate dehydrogenase concentration (peptide chains). The results obtained on the reaction rate (Figs. 5 and 7, Curves A and B) and the velocity: (a) reaches a plateau when the level of transaminase is 8 μM (Fig. 1, Curve B), (b) is proportional to glutamate dehydrogenase concentration over the range from 1 to 10 μM (Fig. 7, Curve B), (c) is inhibited by ADP (Fig. 8, Curve B), and (d) is activated by GTP (Table II). When the level of glutamate dehydrogenase is low and this enzyme is reacting with glutamate rather than with the pyridoxamine phosphate form of the transaminase, GTP is an inhibitor (Table II).

Reaction with Phenylalanine—Reactions with phenylalanine are similar but slower than those with tyrosine (Figs. 1 and 7). Again, α-ketoglutarate has no effect on the dehydrogenase reaction catalyzed by the enzyme-enzyme complex.

Reaction with Pyridoxal Phosphate and Pyridoxamine Phosphate—These coenzymes are such poor substrates of glutamate dehydrogenase that a kinetic study is hardly feasible. For example, if pyridoxamine phosphate (0.1 mM) is incubated with DPN or TPN (1.0 mM) and glutamate dehydrogenase (0.85 mg per ml), the initial velocity is hardly detectable. However, the fact that both pyridoxal phosphate and pyridoxamine phosphate are substrates for glutamate dehydrogenase can be confirmed by carrying out the incubation with higher levels of these coenzymes. Fractional chromatography of the incubation on DEAE-Sephadex gave fractions which eluted as and had an absorption spectrum like the suspected product. The identity of the product was confirmed in each case by finding that it could reconstitute the transaminase apoenzyme.

**DISCUSSION**

The above and previous experiments (1–4): (a) rule out the possibility that reactions catalyzed by the proposed enzyme-complex are actually mediated by trace amounts of keto or amino acids, (b) demonstrate that pyridoxal or pyridoxamine phosphate are required for these enzyme-enzyme complex reactions, (c) show that pyridoxal and pyridoxamine phosphate are not transferred from transaminase to glutamate dehydrogenase in these reactions, (d) demonstrate that the two enzymes do not react if separated by a semipermeable membrane, and (e) demonstrate that glutamate dehydrogenase can decrease the distribution coefficient of transaminase in Sephadex G-200. Consequently, if can be concluded that these two enzymes can form a complex and in this complex glutamate dehydrogenase

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**TABLE II**

**Effect of GTP on reactions with glutamate or tyrosine**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Specific activity</th>
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<tr>
<td>Glutamate plus GDH</td>
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</tr>
<tr>
<td>Tyrosine, GOT, plus GDH</td>
<td>0.25</td>
</tr>
<tr>
<td>Tyrosine, α-KG, GOT, plus GDH</td>
<td>1.2</td>
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<table>
<thead>
<tr>
<th>Specific activity</th>
</tr>
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<tbody>
<tr>
<td>−GTP</td>
</tr>
<tr>
<td>+GTP</td>
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</table>

Tyrosine, α-KG, GOT, plus GDH

Glutamate dehydrogenase reactions are performed in the presence of tyrosine (5 mM), DPN (1.0 mM), transaminase (2.3 μM), and presence or absence of 10 μM α-ketoglutarate are shown, respectively, in Curves A and B. The results obtained in the presence of aspartate (10 mM), TPN (1.0 mM), and presence or absence of 0.58 μM transaminase are shown, respectively, in Curves C and E. Curve D shows the results obtained in the presence of 5.0 mM phenylalanine, 1.0 mM DPN, and 1.7 μM transaminase. All of these experiments were performed in 0.025 mM sodium arsenate-0.1 mM EDTA, pH 7.8, at 24°C.

Fig. 8 (right). Plot of velocity (in nanomoles per min per ml) versus concentration of ADP. The results obtained in the presence of TPN (1.0 mM), aspartate (10 mM), transaminase (0.2 mM), and glutamate dehydrogenase (8 μM) are shown in Curve A. The results obtained in the presence of tyrosine (5 mM), α-ketoglutarate dehydrogenase (8 μM), and transaminase (2 μM) are shown in Curve B. Remaining experimental conditions are given in the legend to Fig. 1.
can react with transaminase bound pyridoxal or pyridoxamine phosphate. In addition the fact that GTP activates and ADP inhibits the tyrosine dehydrogenase reaction catalyzed by the enzyme-enzyme system also essentially rules out the possibility that this reaction is mediated by recycling of α-ketoglutarate and glutamate between the two enzymes. If this were the case then GTP would inhibit and ADP would activate (24).

While these transaminases apparently possess a keto group which can oxidize DPNH (but not TPNH) (3), this group does not react with glutamate dehydrogenase since reducing it with NaBH₄ or reacting it with phenylhydrazine does not inhibit the ability of transaminase to react with glutamate dehydrogenase (1).

Several experiments suggest that, when the level of glutamate dehydrogenase is high (8 μM), transaminase does not react with all of the glutamate dehydrogenase present. For example, under these conditions and in the presence of aspartate (which is the only amino acid used in a concentration sufficiently high to maintain the transaminase in the pyridoxamine phosphate form), the system is saturated by 0.2 μM transaminase. It is not conceivable that 0.2 μM transaminase could completely bind 8 μM glutamate dehydrogenase. However, these results are consistent with the concept that transaminase only reacts with the small fraction of glutamate dehydrogenase molecules which are monomeric when the total level of glutamate dehydrogenase is 8 μM (14, 25). This would suggest that glutamate dehydrogenase polymers do not have sites available for binding of transaminase. This is consistent with the fact that GTP which dissociates glutamate dehydrogenase (24) activates reactions with tyrosine and the enzyme-enzyme complex while ADP which facilitates association inhibits both the tyrosine and aspartate dehydrogenase reactions catalyzed by the complex. The activating effect of GTP is not large. However, if GTP (0.1 mM) is added to the assays performed with aspartate described in the legend to Fig. 1, the weight average molecular weight is decreased only from about 1.5 to 1.1 × 10⁶ (as estimated by light scattering). Thus, under these conditions GTP does not markedly dissociate glutamate dehydrogenase. Furthermore, it is conceivable that GTP is not a potent activator of the tyrosine dehydrogenase reaction because, while it increases the concentrations of monomeric forms of glutamate dehydrogenase, it might also inhibit the reaction catalyzed by these forms. Thus, the slight activation could be the net result of two opposing effects.

Other experiments also suggest that only monomeric forms of glutamate dehydrogenase react with transaminase. For example, in the presence of reduced pyridine nucleotides, plus NH₄Cl, a level of glutamate dehydrogenase is reached where adding more enzyme neither increases the rate of conversion of transaminase from the pyridoxal to pyridoxamine phosphate form nor the amount of transaminase bound. This suggests that when glutamate dehydrogenase reaches a certain level it is mainly polymerized and adding more enzyme does not proportionally increase the concentration of monomers. Furthermore, a complex between these two enzymes has not been detected in sedimentation experiments where the levels of both enzymes must be high for visualization. The explanation for this may be that in these experiments glutamate dehydrogenase is mainly polymerized and consequently only a small fraction of this enzyme can form a complex with transaminase. Also, since transaminase only forms a complex with the small fraction of glutamate dehydrogenase molecules which are monomeric, most of the transaminase molecules are not bound. Consequently, the two enzymes are present primarily as free enzymes in the presence of a small, undetectable amount of enzyme-enzyme complex. Gel filtration experiments are performed with low levels of transaminase. Therefore, in these experiments the amount of enzyme-enzyme complex is quite significant compared with the total amount of transaminase added and thus the complex can be detected.

Free pyridoxal or pyridoxamine phosphate are not very reactive substrates with glutamate dehydrogenase and pyridine nucleotides. This is in spite of the fact that pyridoxal phosphate can be reduced nonenzymatically by various 1,4-dihydropyridines (26). It is possible, as suggested by unpublished experiments, that the transaminase molecule is necessary for significant binding of pyridoxal or pyridoxamine phosphate to the active site of glutamate dehydrogenase. That is, certain chemical modifications of transaminase enhance its reactivity with glutamate dehydrogenase. This suggests that groups on the transaminase in addition to pyridoxal phosphate play a role in binding of transaminase to glutamate dehydrogenase.

The enzyme-enzyme complex can be formed in the presence of high levels of α-ketoglutarate, oxaloacetate, or glutamine. On the basis of gel filtration experiments, the dissociation constant of the enzyme-enzyme complex is apparently lower than that of these substrates for either of the free enzymes (16, 22). A possible physiological role of the enzyme-enzyme complex would be to catalyze amino acid dehydrogenase reactions with amino acids which are not very reactive with glutamate dehydrogenase but are reactive with a transaminase. The advantage of catalyzing the aspartate dehydrogenase reaction by the enzyme-enzyme complex over recycling glutamate and α-keto-glutarate between the two enzymes is that the former is not markedly inhibited by low levels of oxaloacetate.

The enzyme-enzyme complex could be important in tyrosine metabolism because: (a) the rate of conversion of tyrosine to hydroxyphenylpyruvate is in the same range regardless of whether DPN plus glutamate dehydrogenase or saturating levels of oxaloacetate or α-ketoglutarate recycle the pyridoxamine phosphate form of the transaminase; (b) both enzymes are present in high levels in brain and liver mitochondria (27-29); (c) glutamate oxaloacetate transaminase is apparently the only bovine liver mitochondrial protein that can transaminate tyrosine (since the ratio of aspartate to tyrosine aminotransferase activity is constant through purification). Thus it seems possible that, in brain, for example, these reactions could be rapidly compared with known slow steps in tyrosine metabolism as tyrosine hydroxylase (30). This could be related to the fact that some tranquilizers are potent in vivo and in vitro inhibitors of glutamate dehydrogenase (29, 31, 32) but do not inhibit reactions catalyzed by the enzyme-enzyme complex (33).

The levels of these two enzymes are sufficiently high in vivo to interact. Both enzymes are rather uniformly distributed in mitochondria (34, 35). It has been estimated that the level of glutamate dehydrogenase in bovine liver mitochondria is 1 to 1.25 mg per ml or 20 μM (36). On the basis of the ratio of specific activity in our mitochondrial extracts to that of the pure enzymes, the level of glutamate oxaloacetate transaminase is about 3-fold lower than that of glutamate dehydrogenase (5, 16). Thus, the level of transaminase would be about 6 μM.
These levels are even higher than required for interaction in in vitro experiments.

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