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Isolation and Properties of Highly Purified Glutamine Transaminase*

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ABSTRACT: Glutamine transaminase has been obtained in highly purified form from rat liver. The isolated enzyme, which is apparently homogeneous by the criteria of ultracentrifugation ($s_{20,w}$ = 6.25 S) and polyacrylamide gel electrophoresis, has a molecular weight of about 110,000. The enzyme exhibits absorbance maxima at 278 and 415 nm and the data indicate that the enzyme contains two subunits (mol wt ~54,000). The enzyme is stabilized by α-keto acids and by 2-mercaptoethanol. Studies with 27 α-keto acids and 40 amino acids indicate that α-ketoglutaramate, α-keto-γ-methiolbutyrate, β-mercaptopyruvate, glyoxylate, pyruvate, and β-hydroxypyruvate are among the most active α-keto acid substrates, and that glutamine, glutamic acid γ-ethyl ester, γ-glutamylmethylamide, methionine, and ethionine are among the most active amino donors. The K_m values for a number of the α-keto acids and amino acids, and the equilibrium constants for the glutamine–pyruvate, –glyoxylate, –α-keto-γ-methiolbutyrate reactions were determined.

Rat liver contains two enzyme systems that catalyze the deamidation of glutamine; one of these "glutaminase I" is activated by phosphate and leads to the formation of glutamate and ammonia, while the other "glutaminase II" requires the presence of an α-keto acid (Greenstein and Carter, 1946; Carter and Greenstein, 1947; Greenstein, 1949; Errera, 1949; Errera and Greenstein, 1949; Greenstein and Price, 1949). The reaction catalyzed by "glutaminase II" was shown to involve the combined action of two enzymes, glutamine–α-keto acid transaminase and α-keto acid ω-amidase (Meister and Tice, 1950; Meister *et al.*, 1952; Meister, 1953, 1954a–c; Meister and Fraser, 1954; Meister, 1955a,b, 1962). Glutamine–α-keto acid transaminase catalyzes reaction 1 which leads to the formation of α-ketoglutaramate; this α-keto acid exists in solution in equilibrium with the corresponding cyclic ketolactam form (5-hydroxypyrrolidonecarboxylate) (Meister, 1953; Otani and Meister, 1957). α-Ketoglutaramate is hydrolyzed to α-ketoglutarate and ammonia by α-keto acid ω-amidase (reaction 2). Experiments with [^{15}N]amide-labeled glutamine established that the ammonia formed in the "glutaminase II" reaction is derived from the amide group of glutamine. In earlier studies it was shown that ammonia was not formed in the absence of an α-keto acid; furthermore, with most of the α-keto acids examined, replacement of glutamine by glutamate led to a decrease or loss of transamination. Preparations of glutamine transaminase were shown to catalyze transamination between γ-methylglutamine and α-keto acids; with this amino donor no ammonia was formed and α-keto-γ-methylglutaramate was demonstrated as a product of the reaction. It was also found that this α-keto acid is not a substrate of the amidase. In experiments with enzyme preparations obtained from *Neurospora crassa*, which exhibit very little ω-amidase activity, transamination between α-ketosuccinamate and glutamine was shown to yield asparagine and α-ketoglutaramate (Monder and Meister, 1958).

$$\text{glutamine} + \alpha\text{-keto acid} \xrightleftharpoons{\text{glutamine transaminase}} \alpha\text{-ketoglutaramate} + \text{amino acid} \quad (1)$$

$$\alpha\text{-ketoglutaramate} + H_2O \xrightarrow{\omega\text{-amidase}} \alpha\text{-ketoglutarate} + \text{ammonia} \quad (2)$$

Early studies on the deamidation of glutamine by the "glutaminase II" pathway were performed with rat liver preparations that contained both glutamine transaminase and the ω-amidase. A partially purified preparation of the ω-amidase which lacked glutamine transaminase activity was obtained from rat liver (Meister, 1953, 1955a,b; Meister *et al.*, 1955), and very recently a highly purified preparation of this enzyme has been obtained (Hersh, 1971). A partially purified preparation of rat liver glutamine transaminase was obtained which did not exhibit ω-amidase activity (Braunstein and T'ing-Sen,

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1960a,b); this preparation, about 75-fold purified, was free of glutamate–aspartate, asparagine–α-keto acid, and glutamate–phenylalanine transaminases, but it did exhibit glutamate–alanine transaminase activity and the possibility was considered that this activity and glutamine transaminase activity were properties of the same enzyme protein. A preparation of glutamine transaminase was later obtained from rat liver which was about 140-fold purified and which did not possess glutamate–alanine transaminase activity, but it did exhibit glutamate–aspartate transaminase activity (Yoshida, 1967).

In the present communication we describe the isolation of highly purified glutamine–α-keto acid transaminase from rat liver; a purification of about 900-fold has been achieved and the enzyme appears to be homogeneous by the criteria of polyacrylamide gel electrophoresis and ultracentrifugation. The purified enzyme does not exhibit ω-amidase, glutamate–aspartate transaminase, or glutamate–alanine transaminase activities. This paper also gives certain physical and catalytic properties of the purified enzyme including data on its molecular weight. In addition, some new data are presented on its amino acid and α-keto acid specificity, and the apparent equilibrium constants for several of the reactions catalyzed.

Experimental Section

Materials

Male Sprague-Dawley rats weighing about 350 g were used.

Generally labeled L-[^{14}C]glutamine (14.4 Ci/mole), L-[1-^{14}C]alanine (150 Ci/mole), L-[1-^{14}C]glutamic acid (0.25 Ci/mole), and [1-^{14}C]sodium pyruvate (10 Ci/mole) were obtained from Schwarz BioResearch. L-[^{35}S]Methionine-(*SR*)-sulfoximine (0.25 Ci/mole) was synthesized (Ronzio *et al.*, 1969) by Dr. S. L. N. Rao of this laboratory. [1-^{14}C]Sodium glyoxylate (3.2 Ci/mole) was obtained from Amersham-Searle. Ceric sulfate was obtained from Fisher Scientific Co. Malic dehydrogenase and pyridoxal 5′-phosphate were obtained from Sigma Chemical Corp. Phthalaldehyde was obtained from Aldrich Chemical Corp. Hydroxylapatite was obtained from Bio-Rad; silica gel thin-layer plates (Polygram SILN-MR) were obtained from Brinkmann, Inc., and DE-52 was obtained from Reeve Angel and Co.

Crystalline L-amino acid oxidase (isolated from *Crotalus adamanteus* venom by the method of Wellner and Meister (1960)) was kindly supplied by Mr. Edmund Hafner.

Keto Acids. The sodium salts of α-ketoisovaleric, α-ketobutyric, α-keto-γ-methiolbutyric, α-keto-*n*-caproic, D- and L-α-keto-β-methylvaleric, α-ketoisocaproic, *S*-methyl-β-mercaptopyruvate, α-keto-γ-hydroxybutyric, and α-keto-γ-methylsulfonylbutyric acids, α-keto-δ-guanidinovaleric acid, and the barium salts of α-keto-δ-carbamidovaleric, β-sulfopyruvic, α-keto-γ-methylsulfinylbutyric, α-ketoglutaric-γ-ethyl ester, and α-keto-γ-ethiolbutyric acids were prepared by enzymatic oxidation of the corresponding L-amino acids with L-amino acid oxidase in the presence of catalase as described by Meister (1951, 1952, 1954d, 1957). Solutions of the barium salts were treated with a slight excess of sodium sulfate and centrifuged to remove the barium sulfate just prior to use. β-Hydroxypyruvate was obtained from β-bromopyruvic acid (Calbiochem) as described by Sprinson and Chargaff (1947). β-Mercaptopyruvic acid was prepared from β-bromopyruvic acid (Parrod, 1942); the sodium salt was obtained from the ammonium salt (Meister *et al.*, 1954). Sodium trimethylpyruvate was prepared according to Glucksmann (1889). α-Ketoglutaramic, α-keto-*N*-methylglutaramic, and α-ketosuccinamic acids were prepared as described by Meister (1953). α-Ketomalonic acid (mesoxalic acid), oxaloacetic acid, α-ketoglutaric acid, and the sodium salts of glyoxylic, pyruvic, *p*-hydroxyphenylpyruvic, and phenylpyruvic acids were obtained from Calbiochem. A solution of sodium α-keto-β-hydroxybutyrate was prepared by enzymatic oxidation of L-threonine; this amino acid is a poor substrate for L-amino acid oxidase, but it is converted to the corresponding α-keto acid at a significant rate in the presence of relatively large amounts of enzyme (Lichtenberg and Wellner, 1967). L-Threonine (200 μmoles) was dissolved in 2 ml of Tris-HCl buffer (0.1 M; pH 7.5) containing *Crotalus adamanteus* L-amino acid oxidase (1 mg) and catalase (800 units). After incubation with shaking at 37° for 24 hr the protein was removed by passing the mixture through a Diaflow XM 50 membrane, and the solution was worked up (Meister, 1952); 0.5 ml of solution containing 35 μmoles of α-keto acid was obtained, as determined with lactate dehydrogenase and DPNH (Meister,1950).

Amino Acids. L-Glutamic acid, L-valine, and L-cysteine were obtained from Mann Research. L-Alanine and L-asparagine were Eastman products. L-Leucine and L-lysine hydrochloride were obtained from Schwarz BioResearch, L-arginine hydrochloride from Nutritional Biochemicals Corp; and β-alanine from Fisher Scientific Co. L-γ-Glutamylmethylamide was prepared as described (Meister, 1953). L-Methionine-(*SR*)-sulfoximine phosphate (Rowe *et al.*, 1969) was prepared by Dr. W. B. Rowe, aminomalonic acid (Matthew and Neuberger, 1963) was synthesized by Dr. S. S. Tate, and L-2-amino-4-oxo-5-chloropentanoic acid (Khedouri *et al.*,1966) by Mr. L. Pinkus in this laboratory. L-Homoglutamine and L-α-amino-*N*-methyladipamic acid were prepared as described (Meister, 1954b). The γ-methyl and γ-benzyl esters of L-glutamic acid were obtained from Fox Chemical Co. The remainder of the amino acids were purchased from Calbiochem.

Methods

Determination of Glutamine Transaminase Activity. The usual assay system contained (in a final volume of 0.1 ml) 0.02 M sodium glyoxylate, 0.02 M L-glutamine, 0.05 M Tris-HCl buffer (pH 8.4), and enzyme. After incubation for 3–60 min at 37° (during the linear part of the time course), the reaction was stopped and the formation of α-ketoglutaramate or glycine was determined by one of the methods described below. A unit of enzyme activity is defined as the amount that catalyzes the formation of 1 μmole of α-ketoglutaramate/hr under these conditions. Specific activity is defined in terms of units per milligram of protein. Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

PROCEDURE 1. The reaction mixture contained generally labeled L-[^{14}C]glutamine (approximately 100,000 cpm). The reaction was stopped by addition of 0.05 ml of 1 N H_2SO_4, and the mixture was then transferred quantitatively with a pipet and a few drops of water and 1 N H_2SO_4 to the bottom of a small bottle. Bottles measuring 3 cm in diameter, 3 cm in height, with a neck 1 cm in diameter and 1 cm in height were used. The walls of the bottle curve inward so that when placed horizontally two compartments are formed. The compartment at the bottom of the bottle contains the reaction mixture, and 0.1 ml of 2 N $Ce(SO_4)_2$ in 2 N H_2SO_4 is introduced into the compartment at the top of the bottle. The bottle is then closed by introduction of a rubber stopper to which is attached a glass rod on which is previously layered 0.05 ml of freshly prepared 5 N NaOH. The contents of the two compartments are mixed by tipping and the bottle is then clamped to a rotating wheel (42 cm in diameter) and allowed to rotate for 30

min at 20 rpm. The stoppers are then removed and the sodium hydroxide is carefully washed into 10 ml of liquid scintillation fluid (Jeffay and Alvarez, 1961). Radioactivity is determined with a liquid scintillation counter. Under these conditions, 20% of the radioactivity of the [^{14}C]α-ketoglutaramate (and/or α-ketoglutarate) is converted to [^{14}C]carbon dioxide. It is important to note that the procedure as described above is satisfactory only if the solution to be analyzed contains no more than about 0.08 M phosphate; when higher concentrations of phosphate are present more than 0.1 ml of cerric sulfate solution must be used because phosphate precipitates ceric ions as an insoluble complex. Valid results are obtained only when the solution remains yellow at the end of the decarboxylation reaction.

This procedure and procedure 2 (below) were also used in experiments with α-keto acids other than glyoxylate.

PROCEDURE 2. In this procedure [^{14}C]glutamine was used and the [^{14}C]α-ketoglutaramate formed was determined as follows. After incubation, the assay mixture was quantitatively applied to the top of a Dowex 50 column (H^+; 0.5 $\times$ 1 cm) and the column was eluted with 1.0 ml of water. An aliquot (usually 0.2 ml) of the effluent was counted in a liquid scintillation counter. A small but very constant amount of the label (1% of the total ^{14}C present) passed through the column in blanks that lacked enzyme. The values for the enzyme experiments were corrected by subtraction of the blank. Procedures 1 and 2 gave results which were in close agreement. The transamination of [^{35}S]methionine sulfoximine with glyoxylate was carried out in an analogous manner.

PROCEDURE 3: DETERMINATION OF GLYCINE. This method is based on the determination of glycine described by Patton (1935). After incubation, the standard assay mixture (0.1 ml) was treated with 0.1 ml of a solution containing 0.05 M phthalaldehyde and 0.01 M potassium phosphate buffer (pH 7.8) in 50% (v/v) ethanol. After incubation at 37° for 10 min, 0.8 ml of 50% (v/v) ethanol was added and the solution was shaken vigorously. The absorbance was read at 400 nm against a control reaction mixture treated in the same fashion but containing heat-inactivated enzyme or no enzyme. Glutamine (as well as glycine) forms a complex with phthalaldehyde under these conditions. The absorbance maximum of the glycine complex is at 380 nm, while that of the glutamine complex is at 330 nm. Although the glutamine complex exhibits negligible absorbance at 400 nm under these conditions, the presence of glutamine decreases the absorbance at 400 nm due to the glycine complex. A standard curve (based on various mixtures of glycine and glutamine) was constructed for use in the determination of glycine formed in the glutamine–glyoxylate transamination system used here. The curve, although nonlinear, is reproducible; values obtained by this procedure agreed with determinations carried out by other methods. The phthalaldehyde method was applied in an entirely analogous way to the determination of glycine formed by transamination between glyoxylate and γ-glutamylmethylamide, ethionine, methionine sulfone, methionine sulfoximine phosphate, phenylalanine, and alanine.

PROCEDURE 4: DETERMINATION OF [^{14}C]AMINO ACIDS. [^{14}C]-Glyoxylate and [^{14}C]pyruvate (200,000 cpm/0.1 ml, final volume) were used in some experiments. After incubation, the reaction mixtures were quantitatively transferred to the top of a column (0.5 $\times$ 1 cm) of a strong anion exchanger (Dowex 1; acetate form), and the column was eluted with 2 ml of water. Aliquots (0.2 ml) of the effluent were counted in a scintillation counter.

PROCEDURE 5: DETERMINATION OF VARIOUS α-KETO ACIDS.

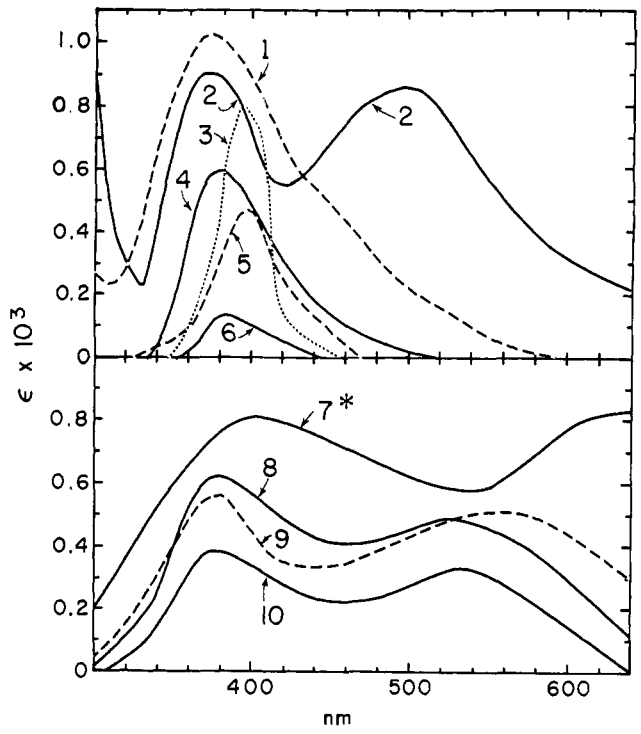

FIGURE 1: Spectra of ferric–α-keto acid complexes. The α-keto acid solution (0.1 ml) was treated with 0.7 ml of 3.94 mM ferric chloride; the spectra were determined with a Cary Model 14 recording spectrophotometer. Curve 1, oxaloacetic acid; curve 2, α-ketosuccinamic acid; curve 3, α-ketoglutaramic acid; curve 4, pyruvic acid; curve 5, glyoxylic acid; curve 6, α-ketosuccinamic acid dimer; curve 7, phenylpyruvic acid; curve 8, α-keto-γ-methylsulfonylbutyric acid; curve 9, α-keto-γ-methiolbutyric acid; curve 10, α-keto-γ-methylsulfinylbutyric acid. In curve 7, the ordinate should be multiplied by 2 to give the correct extinction.

In the experiments in which transamination between pyruvate (or glyoxylate) and various amino acids (asparagine, methionine, methionine sulfoxide, methionine sulfone, and phenylalanine) was studied, the reactions were followed by determining the rate of formation of the corresponding α-keto acids. These were determined from the absorbance of the complexes formed on addition of ferric chloride. In these studies the reaction mixtures (0.1 ml) were treated with 0.1 ml of 3.44 mM $FeCl_3$ in 0.001 M HCl (final pH, 2.0–2.3). Maximum color development was observed in 10 min for the complexes with phenylpyruvate and ketosuccinamic acid, and in 30 min for the α-keto analogs of methionine and its derivatives. The colors were stable for about 15 min except that produced with phenylpyruvate, which disappeared rapidly after reaching its maximum. The spectra of the various Fe^{3+}–α-keto acid complexes are given in Figure 1. α-Ketosuccinamate, phenylpyruvate, and the α-keto acid analogs of methionine, methionine sulfoxide, and methionine sulfone were determined from the absorbance at 495, 630, 550, 515, and 515 nm, respectively; the corresponding molar extinction coefficients are 8.1×10^3, 16.2×10^3, 5.05×10^3, 3.02×10^3, and 4.8×10^3. It was established that the relationship between α-keto acid concentration and absorbance was linear over the range used (up to 2×10^{-3} M).

PROCEDURE 6: DETERMINATION OF α-KETO ACIDS FORMED BY TRANSAMINATION BETWEEN α-KETOGLUTARAMIC ACID AND VARIOUS AMINO ACIDS. α-Ketoglutaramic acid exists mainly in the cyclic ketolactam form at pH 8.4 (Meister, 1953; Otani and Meister, 1957), and therefore does not readily form a

phenylhydrazone. This makes it possible to determine the formation of a new α-keto acid in the presence of α-ketoglutaramate by measuring the appearance of a 2,4-dinitrophenylhydrazone. The assay mixture (0.1 ml) containing amino acid, α-ketoglutaramic acid, buffer, and enzyme was incubated at 37° (for 5–20 min) and then mixed with 0.1 ml of 0.1% 2,4-dinitrophenylhydrazine in 2 N HCl. This mixture was incubated at 37° for 20 min; then 0.8 ml of 1.0 M KOH was added. After standing at 26° for 20 min, the absorbance at 460 nm was read against a control lacking enzyme. α-Ketoglutaramic acid exhibits negligible absorbance under these conditions. The validity of this procedure was established by studies in which known amounts of the various α-keto acids were added to the reaction mixtures used in these experiments.

Glyoxylate was determined by the method of McFadden and Howes (1960). In the studies in which aspartate was used as an amino donor, oxaloacetate was determined with malate dehydrogenase and DPNH (Bergmeyer and Bernt, 1965).

Polyacrylamide Gel Electrophoresis. METHOD A. This is based on the procedure of Ornstein (1964), as modified by Dr. George W. Dietz, Jr., of this Department. The upper buffer was 0.5 M Tris-glycine (pH 8.9) and the lower buffer was 0.10 M Tris-HCl (pH 8.1); 7% gels in 0.37 M Tris-HCl (pH 8.9) were used.

METHOD B. Tris-acetate (0.1 M, pH 8.0) was used throughout and the concentration of the gel was 6% (Chrambach *et al.*, 1967). The current was applied to the gels for 30 min before the protein was added.

Both systems were run at 25° and 1 μl of Temed/gel was used as an initiator and ammonium persulfate was used as the catalyst; 0.001% riboflavin was used as the catalyst in some runs. The gels were 0.5 × 6 cm and were stained in 0.1% coomassie blue in 7.0% acetic acid for 1 hr; destaining was carried out in 7.0% acetic acid. Bromothymol blue was used as a marker.

Polyacrylamide gel electrophoresis in sodium dodecyl sulfate was carried out by the procedure of Weber and Osborn (1969) using several modifications developed by Dr. Paul Trotta of this laboratory. The enzyme (100 μg), in 0.1 ml of 0.05 M Tris-acetate buffer (pH 8.0) containing 1% 2-mercaptoethanol and 1% sodium dodecyl sulfate, was incubated for 3 hr at 37°; standard proteins (bovine hemoglobin, egg albumin, and bovine serum albumin) were treated in the same manner. Samples (50 μl) of these solutions were applied to 6% acrylamide gels in 0.05 M Tris-acetate (pH 8.4) containing 0.1 % 2-mercaptoethanol and 0.1% sodium dodecyl sulfate; the upper and lower buffers contained the same solution. A current of 3–5 mA was applied to each tube. The gels were stained in 0.1% coomassie blue in a solution containing 5% ethanol and 7.5% glacial acetic acid (v/v) for 2 hr; destaining was carried out with the same ethanol–acetic acid solution.

Paper and Thin-Layer Chromatography. Paper chromatography was carried out by ascending technique using solvents consisting of *tert*-butyl alcohol–methyl ethyl ketone–formic acid–water (40:30:15:15, v/v) (solvent I) and 80% phenol (solvent II). Thin-layer chromatography was carried out with a solvent (III) consisting of methyl ethyl ketone–acetone–ethanol–water (20:5:5:6, v/v).

Pyridoxal 5′-Phosphate Determination. The presence of pyridoxal 5′-phosphate in glutamine transaminase has not yet been unequivocally established. In earlier studies on vitamin B_6 deficient rats, it was found that liver homogenates from the deficient animals exhibited markedly reduced glutamate–pyruvate transaminase and cysteine desulfhydrase activities, while the glutamine–pyruvate and glutamine phenylpyruvate transaminase activities were not reduced (Meister *et al.*, 1953). In later work, liver homogenates prepared from rats given large doses of isonicotinic acid hydrazide were found to exhibit reduced glutamine–pyruvate transaminase activity, and purified enzyme preparations obtained from such livers catalyzed the glutamine–pyruvate reaction at markedly reduced rates as compared to controls. Addition of pyridoxal 5′-phosphate or pyridoxamine 5′-phosphate produced a considerable increase in the activity of preparations obtained from the livers of treated rats but had no effect on preparations from untreated controls (Meister and Downey, 1956). Beaton and Ozawa (1955) reported that liver extracts from vitamin B_6 deficient rats exhibited decreased ability to catalyze the glutamine–pyruvate reaction, but that addition of pyridoxal 5′-phosphate to the extracts produced only very small and erratic activation. In the present work we found no consistent significant activation of the enzyme at any stage of purification on addition of pyridoxal 5′-phosphate; however, addition of pyridoxal 5′-phosphate occasionally activated preparations of the enzyme which had become inactive during storage at 4°. When the purified enzyme was dialyzed against 20 mM L-glutamine, L-alanine, or L-cysteine, there was no loss of activity. When the enzyme (1 mg/ml) was stored at 0° for 2 days in 4 M urea containing 0.1 M L-cysteine and 50 mM potassium phosphate buffer (pH 7.2), no loss of activity was observed. Application of the procedure for the determination of pyridoxal 5′-phosphate described by Wada and Snell (1963) after denaturation of the enzyme by heating or by treatment with 1 N perchloric acid failed to reveal the presence of pyridoxal 5′-phosphate. However, when the enzyme (0.5 mg) was digested with Pronase (0.5 mg, Calbiochem; 45,000 PUK/g) in 0.5 ml of 10 mM potassium phosphate buffer (pH 7.2) at 37° for 48 hr and then deproteinized with 1 M perchloric acid, analysis by the procedure of Wada and Snell gave a value of 0.0195 μmole of pyridoxal 5′-phosphate; this leads to a value of 2.4 moles of pyridoxal 5′-phosphate/110,000 g of enzyme. An attempt was also made to determine the pyridoxal 5′-phosphate content of the enzyme with treatment by 2,4-dinitrophenylhydrazine. The enzyme (1.1 mg in 0.1 ml of 10 mM potassium phosphate buffer, pH 7.2) was mixed with 0.1 ml of 0.1% 2,4-dinitrophenylhydrazine in 2 N HCl, and the mixture was allowed to stand at 37° for 10 min. It was then diluted to 1 ml with 1.25 N sodium hydroxide and the absorbance was determined at 480 nm; a value of 0.65 was obtained compared to one of 0.05 for a control sample of the enzyme that had been reduced by treatments with sodium borohydride prior to mixing with 2,4-dinitrophenylhydrazine. When pyridoxal 5′-phosphate was treated with 2,4-dinitrophenylhydrazine under the same conditions, a molar extinction coefficient of 26,000 was observed. These data lead to a value of 2.3 moles of pyridoxal 5′-phosphate/110,000 g of enzyme protein.

Additional evidence consistent with the presence of pyridoxal 5′-phosphate includes the spectrum of the enzyme (see below) and also the observation that the enzyme is inactivated by reduction with sodium borohydride and markedly inhibited by carbonyl reagents. Thus, the enzyme (1 mg/ml) was inactivated after 60 min at 0° in the presence of 0.1 M hydroxylamine or isonicotinic acid hydrazide in 0.01 M potassium phosphate buffer (pH 7.2). While the findings are consistent with the presence of pyridoxal 5′-phosphate, they also indicate that the cofactor is more tightly bound by glutamine transaminase than by a number of other vitamin B_6 enzymes; further studies on the cofactor of glutamine transamination are in progress and will be reported subsequently.

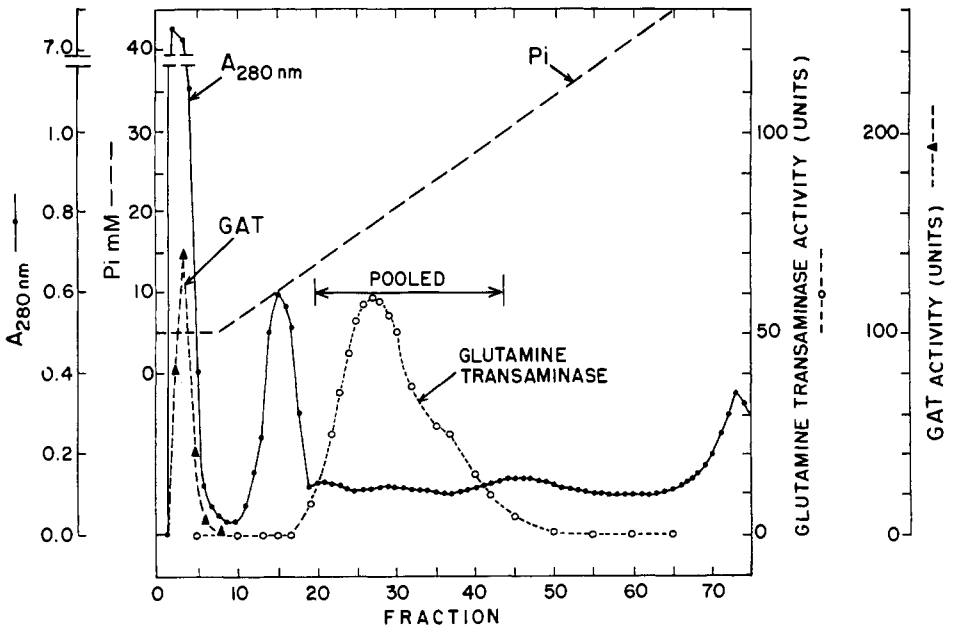

FIGURE 2: Chromatography of the enzyme on DE-52 (see the text; step 5 of the purification procedure).

Results

Purification of the Enzyme. The method given below is suitable for isolation of the enzyme from 200 g of fresh or fresh-frozen rat liver. All steps were carried out at 4° unless otherwise stated and all centrifugations were carried out at 45,000*g* for 10 min.

STEP 1. The rats were decapitated and exsanguinated. The livers were removed and homogenized at full speed for 30 sec in a Waring Blendor in three volumes of 0.05 M Tris-HCl buffer (pH 8.4). The homogenate was centrifuged and the sediment was discarded.

STEP 2. The supernatant solution from step 1 was treated with sodium pyruvate to yield a final concentration of 0.01 M. This solution was then heated with constant stirring to 63–65° at which temperature it was kept for 20 min. The solution was then cooled in ice and centrifuged. The pellet was discarded. No ω-amidase activity was found in the supernatant solution.[1]

STEP 3. The supernatant solution from step 2 was adjusted to pH 5.0 by addition of 2 N acetic acid. Solid ammonium sulfate (25 g/100 ml) was slowly added with constant stirring and after standing for 20 min the precipitate which formed was removed by centrifugation and discarded. The supernatant solution (fraction 1) was saved.

STEP 4. The supernatant solution from step 3 was treated with solid ammonium sulfate (18 g/100 ml) and after standing for 20 min the precipitate which formed was collected by centrifugation and dissolved in the minimal amount (approximately 20 ml) of 0.005 M potassium phosphate buffer (pH 7.2). The solution was dialyzed against two changes of 5 l. each of 0.005 M potassium phosphate (pH 7.2). An inactive precipitate formed which was removed by centrifugation.

STEP 5. The dialyzed solution from step 4 was applied to the top of a column (3.5 × 20 cm) of DE-52 previously degassed (by shaking a suspension of it in water in a side-arm flask attached to a vacuum line) and then equilibrated with 0.005 M potassium phosphate buffer (pH 7.2). The column was eluted with a linear gradient established between 1 l. of 0.005 M and 1 l. of 0.07 M potassium phosphate (pH 7.2). Both buffers also contained 1 mM 2-mercaptoethanol (Figure 2). The flow rate was 20 ml/hr.

STEP 6. Tubes 20–43 were pooled, concentrated by ultrafiltration (with a Diaflow XM 50 membrane) to 10 ml, and dialyzed against 0.015 M potassium phosphate buffer (pH 7.2). The dialyzed solution was then applied to the top of a column (1.0 × 10 cm) of hydroxylapatite previously equilibrated with the same buffer used for dialysis. The column was eluted with this buffer until very little protein appeared (*i.e.*, A_{280} <0.01) in the effluent; it was then further eluted with a linear gradient established between 250 ml of 0.015 M potassium phosphate buffer (pH 7.2) and 250 ml of 0.080 M potassium phosphate buffer (pH 7.2), both containing 1 mM 2-mercaptoethanol. The flow rate was 20 ml/hr. A single protein peak containing the enzyme was eluted at about 30 mM phosphate. The enzyme solution was concentrated with a Diaflow XM 50 membrane to 10 ml.

Comments on the Purification Procedure. The isolation procedure yields a preparation of glutamine transaminase which is completely devoid of glutamate–aspartate transaminase, glutamate–alanine transaminase, and ω-amidase activities (Table I). Valid assays of the glutamine transaminase activity of the crude extract were obtained either by the use of the assay procedure 1, which determines the formation of both α-ketoglutaramate and α-ketoglutarate, or by determinations of glycine. Although step 2 of the isolation procedure, in which the enzyme is heated at 63–65° in the presence of 0.01 M pyruvate, gives only about a twofold purification of glutamine transaminase, it is particularly useful since it removed all of the ω-amidase activity. It is notable that the enzyme is stabilized by α-keto acids. Thus, when crude liver extracts were heated at 50°, about half of the activity was lost in 10 min, while at 60° almost all of the activity was lost in 10 min. In the presence of 0.01 M sodium pyruvate, sodium glyoxylate, or sodium phenylpyruvate the crude extract could be heated at 60° for

[1] Heat treatment of the homogenate in the presence of 0.01 M sodium pyruvate also effectively destroys the ω-amidase without affecting glutamine transaminase activity. This has been carried out three times, and on each occasion satisfactory purifications of the enzyme were obtained.

TABLE I: Purification of the Enzyme.

Step	Vol (ml)	Protein (mg)	Glutamine Transaminase				Other Transaminases	
			Total Units[a]	Sp Act. (Units/mg)	Yield (%)	Purificn Factor	Glu-Asp Units[b]	Glu-Ala Units[c]
1. Crude extract from 200 g of liver	600	47,800	16,500	0.345	100	1	165,000	80,000
2. After heat treatment[d]	580	19,800	15,200	0.768	92	2.2	120,000	60,000
3. $(NH_4)_2SO_4$ fraction I	590	5,400	10,800	2.00	65	5.8	40,000	20
4. $(NH_4)_2SO_4$ fraction II	25	906	8,250	9.10	50	26.4	37,000	20
5. DE-52 chromatography	500	47.3	7,250	153	44	443	0	0
6. Hydroxylapatite chromatography	120	22.0	6,600	300	40	870	0	0

[a] μmoles/hr (37°). [b] μmoles/min (25°); Bergmeyer and Bernt (1965). [c] μmoles/min (25°); Segal *et al.* (1962). [d] ω-Amidase activity was not detected after the heating procedure.

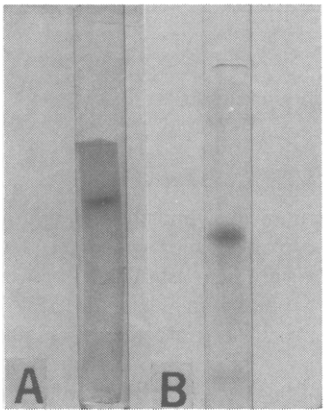

FIGURE 3: Polyacrylamide gel electrophoresis of purified glutamine transaminase. A sample (50 μg) of enzyme was applied to each gel. (A) Disc gel electrophoresis, method A. (B) Sodium dodecyl sulfate gel electrophoresis. The migration of the protein was downward toward the anode. In part B the lower band is a bromothymol blue marker. For details, see Methods.

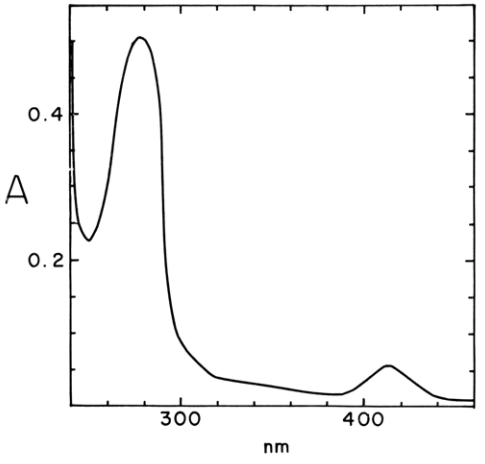

FIGURE 4: Spectrum of purified glutamine transaminase (enzyme concentration, 0.79 mg/ml in potassium phosphate buffer (5 mM; pH 7.2); 25°).

1 hr without loss of activity; after 3 hr at 60° only 25% of the initial activity was lost.

The purified enzyme (in 0.03–0.05 M potassium phosphate buffer (pH 7.2) containing 1 mM 2-mercaptoethanol) may be stored frozen at −20° for at least 7 weeks without loss of activity. Little or no activity is lost when the enzyme is stored in such a buffer at 0–5° for 1–2 weeks, and when kept at 24–26° for 3 days, the purified enzyme lost only 20% of its initial activity. However, the enzyme is much less stable in phosphate buffer in the absence of 2-mercaptoethanol; thus, under these conditions most of its activity is lost at 0–4° within 2 days. We have found that a very satisfactory way to store the enzyme is to keep it at 4° in 0.01 M potassium phosphate buffer (pH 7.2) containing 10% glycerol, 1 mM 2-mercaptoethanol, and 10^{-5} M pyridoxal 5′-phosphate; under these conditions, it is stable for at least 3 months.

Physical Properties of the Purified Enzyme. The purified enzyme moved as a single band on polyacrylamide gel electrophoresis at pH 8.0 using system B (see Methods). However, at pH 8.9 using system A, the enzyme moved as two very closely spaced bands. When 0.001% riboflavin was used in place of ammonium persulfate as the catalyst, only one band was observed (Figure 3A). It thus appears that the enzyme is partially modified by persulfate; similar findings have been noted with other enzymes that are susceptible to oxidation (Brewer, 1967; Mitchell, 1967; Yue *et al.*, 1968).

The enzyme sedimented as an apparently homogeneous component in the analytical ultracentrifuge; it exhibited a sedimentation coefficient ($s_{20,w}$) of 6.25 S (24°; 5 mM potassium phosphate (pH 7.2); protein, 1 mg/ml).[2]

Glutamine transaminase cannot be separated from glutamate–alanine or glutamate–aspartate transaminases by gel filtration on Sephadex G-150 or Sephadex G-200; the molecular weight of glutamate–alanine transaminase has been reported to be 110,000 (Segal *et al.*, 1968), and that of glutamate–aspartate transaminase is close to 100,000 (Bertland and Kaplan, 1968). In the present studies, polyacrylamide gel electrophoresis in sodium dodecyl sulfate gave a single protein component that had an estimated molecular weight of 54,000; (Figure 3B) with most preparations of the enzyme faint bands were observed that corresponded to proteins having molecular weights of 103,000 and 150,000. The data available thus indicate that the isolated enzyme has a molecular weight of about 110,000 and that it probably consists of two subunits of molecular weight about 54,000.

[2] We are indebted to Dr. Paul Trotta for carrying out this determination.

TABLE II: Relative Rates of Transamination between Glutamine and Various α-Keto Acids.[a]

α-Keto Acid	Rel Rates[b]	α-Keto Acid	Rel Rates[b]
Glyoxylate	[100]	*S*-Methyl-β-mercapto-pyruvate	80
Pyruvate	28	*S*-Methyl-β-mercapto-pyruvate (5 mM)	60
α-Ketobutyrate	24	α-Keto-γ-ethiolbutyrate	66
α-Keto-*n*-caproate	24	α-Keto-γ-ethiolbutyrate (5 mM)	250
α-Ketoisocaproate	3	α-Ketoglutarate γ-ethyl ester	77
(D- and L)-α-Keto-β-methylvaleric	0.2	β-Sulfopyruvate	6
α-Ketoisovalerate	0	Mesoxalate	16[d]
Trimethylpyruvate	0	Oxaloacetate	4
β-Hydroxypyruvate	50	α-Ketoglutarate	3
α-Keto-γ-hydroxy-butyrate	24	Phenylpyruvate	17
α-Keto-β-hydroxy-butyrate	0	*p*-Hydroxyphenyl-pyruvate (2 mM)	0
α-Keto-γ-methiol-butyrate	50	α-Keto-δ-carbamido-valerate	10
α-Keto-γ-methiol-butyrate (5 mM)	240	α-Keto-δ-guanidino-valerate	6
β-Mercaptopyruvate[c]	195	α-Ketosuccinamate	62

[a] The reaction mixtures contained 0.02 M L-[^{14}C]glutamine, 0.02 M α-keto acid (except where indicated), and enzyme (0.93 unit) in a final volume of 0.1 ml. After incubation at 37° for 3–60 min, the rate of transamination was determined by measuring the formation of [^{14}C]α-ketoglutaramate by procedure 1 or 2 (see Methods). [b] The values given are initial rates and expressed relative to that for the glutamine–glyoxylate reaction, *i.e.*, 300 units/mg of purified enzyme. [c] Based on a 3-min experiment; the reaction was linear with time for only 5 min. [d] Only traces of aminomalonate were formed as determined by paper chromatography. The relatively high rate of α-ketoglutaramate formation recorded in the table reflects nonenzymatic conversion of mesoxalate to glyoxylate; we found 5–10% conversion as determined by the method of McFadden and Howes (1960) and by determinations with lactate dehydrogenase (Meister, 1950).

The enzyme exhibits absorbance maxima at 278 and 415 nm in 0.005 M potassium phosphate buffer (pH 7.2). The ratio of absorbance at 280 nm to that at 260 nm is 1.73; the ratio of absorbance at 415 nm to that at 280 nm is 0.12 (Figure 4). The absorbance of a solution of the enzyme containing 1 g/l. (as measured by the method of Lowry *et al.*, 1951) at 280 nm is 0.65.

pH Dependence of the Enzyme. The activity of the enzyme was examined in a series of Tris and borate buffers over the pH range 7.0–8.8. Maximum activity was observed in the range 8.0–8.7; the optimal pH value varied slightly from about 8.2 to 8.5 depending on the conditions employed (Figure 5).

Specificity of the Enzyme for α-Keto Acids. Table II shows the relative rates of transamination of glutamine with a number of α-keto acids. The values for transamination of glutamine with various α-keto acids are in general accord with

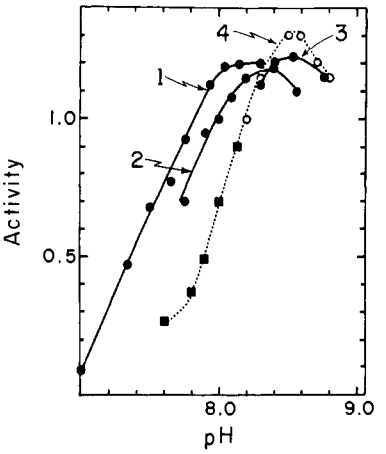

FIGURE 5: pH dependence of the glutamine–glyoxylate transaminase reaction. Activity was determined by procedure 1 (see Methods). Curve 1, 0.05 M Tris-HCl buffers; curve 2, 0.125 M Tris-HCl buffers; curve 3, 0.25 M Tris-HCl buffers; curve 4, 0.025 M sodium borate (NaOH, open circles; HCl, closed squares) buffers. Activity is expressed in terms of μmoles/hr; 1.18 units of enzyme was used.

earlier findings obtained with less purified preparations of glutamine transaminase (Meister and Tice, 1950; Meister *et al.*, 1952; 1954; Meister, 1954a–c); however, the high values with β-mercaptopyruvate and α-keto-γ-methiolbutyrate were unexpected. The initial reaction rate with β-mercaptopyruvate was about twice that with glyoxylate; however, it was observed that the reaction was linear with time for only about 5 min under the conditions employed. Substantial rates of reaction were also observed with α-ketosuccinamate, β-hydroxypyruvate, pyruvate, and several other α-keto acids. The effect of

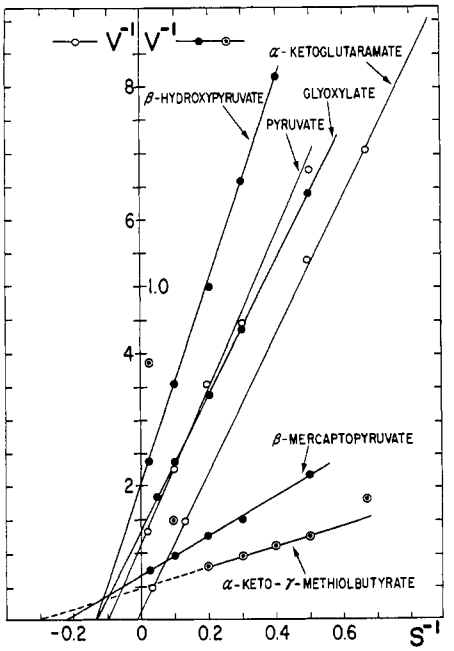

FIGURE 6: The effect of α-keto acid concentration on the rate of transamination. The reaction mixtures contained enzyme (2.7 units), L-[^{14}C]glutamine (0.02 M; 200,000 cpm), Tris-HCl buffer (0.05 M, pH 8.4) and α-keto acid (final volume, 0.1 ml); incubated at 37° for 3–10 min. [^{14}C]α-Ketoglutaramate was determined by procedure 2 (see Methods). $1/S$ *vs.* $1/V$, where V = μmoles/hr and S = α-keto acid concentration (mM).

TABLE III: Relative Rates of Transamination with Various Amino Acids.[a]

Amino Acid[b]	Relative Rates: Glyoxylate	Pyruvate	α-Ketoglutaramate[j]
L-Glutamine	[100][c,h,i]	28[d]	134
L-γ-Glutamylmethylamide	63[h]	17[d]	
L-Glutamic γ-methyl ester	57[i]		20
L-Glutamic γ-benzyl ester			6
L-Methionine	20[g]	3[g]	18
L-Ethionine	30[h]		11
L-Methionine (*SR*)-sulfoxide	36[h], 34[g,i]	5[g]	17
L-Methionine sulfone	10[g,h], 8[i]	2[g]	5
L-Methionine (*SR*)-sulfoximine	12[e,i]	3[g]	
L-Methionine (*SR*)-sulfoximine phosphate	6[h]		
L-Homoserine	10[i]		14
DL-Homocysteine (40 mM)	8[i]		
L-Phenylalanine	8[h], 7[g]	1[g]	4
L-2-Amino-4-oxo-5-chloropentanoic acid	4[i]		
L-Asparagine	2[g]		5
L-Cysteine	2[i]		6
L-Alanine	2[h,i] 1[d]		2
L-Serine	2[i]		4
Glycine	0.05[i]	0.05[f]	1
L-Norleucine	<1.0[i]		
L-α-Aminobutyric acid	<1.0[i]		
S-Methyl-L-cysteine	10[i]		

[a] The reaction mixtures contained the sodium salt of the α-keto acid (pyruvate, 0.02 M; glyoxylate, 0.02 M; α-ketoglutaramate, 0.09 M), the amino acid (0.02 M, except as indicated), 0.05 M Tris-HCl buffer (pH 8.4) and enzyme in a final volume of 0.1 ml. After incubation for 3–10 min (in most of the experiments with glyoxylate and α-ketoglutaramate) or 1–3 hr (with pyruvate), the initial rates of transamination were determined. The values are expressed relative to that for the glutamine–glyoxylate reaction, *i.e.*, 300 units/mg of enzyme. [b] The following amino acids gave values that were less than 1.0 (with glyoxylate): L-glutamate, L-aspartate, L-ornithine, L-tyrosine (0.002 M), L-cystine (0.002 M), L-leucine, L-threonine, L-valine, L-isoleucine, L-arginine, L-cysteate, DL-2-amino-4-phosphonobutyrate, L-tryptophan, L-histidine, L-lysine, β-alanine, L-2,4-diaminobutyrate, and aminomalonate. The following amino acids did not exhibit detectable transamination (by the phenylhydrazine procedure) with α-ketoglutaramate: L-glutamate, L-aspartate, L-leucine, L-threonine, L-valine, L-isoleucine, L-arginine, L-cysteate, L-ornithine, L-histidine, L-lysine, L-tryptophan, L-homoglutamine, L-α-amino-*N*-methyladipamic acid, L-2-amino-4-hydroxyvaleric acid, and L-citrulline. [c] α-Ketoglutaramate was determined by procedure 2. [d] [^{14}C]Pyruvate was used; [^{14}C]alanine was determined (procedure 4). [e] [^{35}S]-Amino acid was used; [^{35}S]α-keto acid was determined (procedure 2). [f] Glyoxylate was determined by the method of McFadden and Howes (1960). [g] α-Keto acid was determined by the Fe^{3+} complex procedure (procedure 5). [h] Glycine was determined with phthalaldehyde (procedure 3). [i] [^{14}C]Glyoxylate was used; [^{14}C]glycine was determined (procedure 4). [j] α-Keto acid was determined by the phenylhydrazone procedure (procedure 6), except in the experiments with L-glutamine, where it was determined by procedure 2.

α-keto acid concentration on initial velocity was determined for glyoxylate, pyruvate, β-hydroxypyruvate, β-mercaptopyruvate, α-keto-γ-methiolbutyrate, and α-ketoglutaramate; the double-reciprocal plots of the data obtained are given in Figure 6. The respective K_m values are 8.0, 10.5, 8.0, 4.8, 3.2, and 50 mM; the corresponding V_{max} values estimated from these plots are 410, 100, 270, 860, 1120, and 1500 ± 300 μmoles per hr per mg of enzyme. The K_m value for α-keto-γ-methiolbutyrate was extrapolated (dashed line, Figure 6 from the data obtained between 5 and 20 mM); the explanation for the substantial substrate inhibition observed at concentrations of this α-keto acid higher than 5 mM is not yet clear. When the enzyme was incubated with 9 mM α-keto-γ-methiolbutyrate for various periods and then diluted 20-fold and assayed for glutamine–glyoxylate activity, substantial inhibition was noted; under these conditions, 25 and 45% inhibition was found after 10 and 60 min, respectively. In a similar study in which the enzyme was preincubated with 20 mM β-mercaptopyruvate, 11 and 25% inhibition was observed after 20 and 60 min, respectively. Control studies with other keto acids (pyruvate, glyoxylate) at concentrations of 20 mM did not show inhibition. The findings suggest that β-mercaptopyruvate and α-keto-γ-methiolbutyrate inhibit the enzyme irreversibly, but the mechanism of inhibition requires further study.

Transamination between [^{14}C]glutamine and α-ketoglutaramate also takes place at a relatively rapid rate; the true K_m value for α-ketoglutaramate is much lower than the apparent K_m value (50 mM), perhaps about 0.2 mM, if calculated on the basis of the concentration of α-ketoglutaramate in the open-chain form.[3]

Specificity of the Enzyme for Amino Acids. The amino acid specificity of the enzyme was examined with glyoxylate, pyruvate, and α-ketoglutaramate (Table III). Glutamine is the most active substrate, but substantial activity was also observed with γ-glutamylmethylamide and several other amino acids, notably glutamic acid γ-methyl ester, methionine, methionine sulfoxide, and ethionine. The effect of amino acid concentration on the rate of transamination with α-ketoglutaramate was determined with L-glutamine, L-methionine, L-methionine (*SR*)-sulfoxide, and L-glutamic acid γ-methyl ester (Figure 7). The respective V_{max} values derived from these data were 1800, 67, 61, and 70 μmoles per hr per mg of enzyme; the corresponding K_m values were 2.0, 1.9, 2.0, and 4.4 mM.

The formation of glutamine was demonstrated in the reaction mixtures (Table III) containing α-ketoglutaramate and methionine (as well as methionine sulfone, methionine sulfoxide, and ethionine) by paper chromatography in solvents I and II (see Methods). The formation of the corresponding α-keto acids was also demonstrated; in these studies the reaction mixtures (0.1 ml) were treated with 0.1 ml of 0.1% 2,4-dinitrophenylhydrazine in 2 N HCl, and after 20 min (at 37°), the hydrazones were extracted with ethyl acetate and chromatographed on silica gel thin-layer plates in solvent III (see Methods). The hydrazones isolated from the reaction mixtures exhibited R_F values that were identical with those of the respective authentic compounds.

Equilibrium Constants. The apparent equilibrium constants

[3] It has been estimated that less than 1% of α-ketoglutaramate is present in the open chain form at pH 7.5 and 37° (Meister, 1953). Hersh (1971) has reported data that indicate that the concentration of the open chain form is close to 0.3% of the total α-ketoglutaramate concentration; this value (obtained at 30° between pH 6.3 and 7.5) is assumed in the present work.

TABLE IV: Equilibrium Constants for Several Glutamine–α-Keto Acid Reactions.

Expt	Reaction[a]	Equilibrium Constants K_{app}	K' [b]
1	Glutamine + glyoxylate	607 ± 100	1.82 ± 0.30
2	Glutamine + pyruvate	340 ± 50	1.02 ± 0.15
3	Glutamine + α-keto-γ-methiolbutyrate	69 ± 20	0.21 ± 0.06

[a] The reaction mixtures (final volume, 0.1 ml) initially contained enzyme (10.8 units), Tris-HCl buffer (50 mM, pH 8.4), and other components as follows: expt 1, L-glutamine, sodium glyoxylate, and glycine in the following combinations, 12, 20, 560 and 20, 20, 400 mM; expt 2, L-glutamine, sodium pyruvate, and L-alanine in the following combinations, 20, 10, 140, 20, 10, 80, and 20, 20, 130 mM; expt 3, L-glutamine, sodium α-keto-γ-methiolbutyrate, and L-methionine in the following combinations, 10, 8.5, 60 and 20, 3.4, 0 mM; incubated at 37°. Equilibrium was achieved within 24 hr. The initial and final concentrations of the α-keto acids were determined by the 2,4-dinitrophenylhydrazone procedure (Methods, procedure 5). The values for glutamine were determined by inclusion of L-[^{14}C]glutamine (200,000 cpm) in each experiment. [^{14}C]Glutamine was determined by scintillation counting after paper chromatography in solvent 1 (see Methods); a small correction was applied to account for the nonenzymatic conversion of glutamine to pyrrolidonecarboxylate, which was determined in a separate control. [b] See the text.

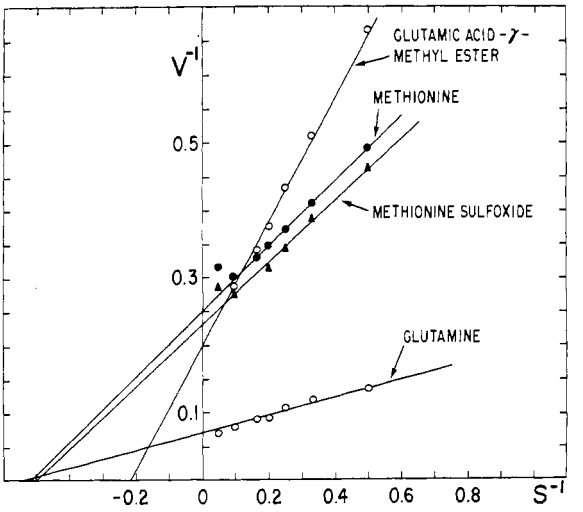

FIGURE 7: The effect of amino acid concentration on the rate of transamination. The reaction mixtures contained enzyme (2.7 units), sodium α-ketoglutaramate (0.08 M), Tris-HCl buffer (0.05 M, pH 8.4), and amino acid; incubated at 37° for 5–10 min. In the experiment with glutamine, α-ketoglutaramate was determined by procedure 2. The other reactions were followed by the phenylhydrazone method (procedure 6). $1/S$ *vs.* $1/V$, where V = moles × 10^{-8}/5 min except for glutamine, where V = moles × 10^{-7}/5 min and S = amino acid concentration (mM).

were determined for the glutamine–glyoxylate, glutamine–pyruvate, and glutamine–α-keto-γ-methiolbutyrate reactions (Table IV). The equilibrium constants designated K' were obtained using the value reported by Hersh (1971) for the concentration of the open-chain form of α-ketoglutaramate.[8]

Discussion

Glutamine transaminase has been purified to a much greater extent than previously reported; the present preparation appears to be homogeneous and does not exhibit glutamate–alanine, glutamate–aspartate, and ω-amidase activities. The data indicate that glutamine transaminase has a molecular weight of about 110,000 and that it is composed of two subunits. The pH optimum is similar to that reported for less purified preparations (Meister and Tice, 1950; Yoshida, 1960).

Removal of contaminating enzymes has made it possible to characterize the specificity of the enzyme more extensively than previously. The present studies confirm previous findings (Meister, 1954a, 1955, 1962) on the relative activities of certain α-keto acids. Thus, glyoxylate, pyruvate, β-hydroxypyruvate, and α-ketosuccinamate are active substrates; however, the very high activities exhibited by β-mercaptopyruvate, α-keto-γ-methiolbutyrate, and related α-keto acids were not previously recognized. As summarized in Table V, glutamine is by far the most active amino acid substrate; however, substantial activity was also found with methionine and closely related compounds. The α-keto analogs of methionine, ethionine, and glutamic acid γ-ethyl ester are also very active. *S*-Methylcysteine and homoserine, as well as their α-keto analogs exhibit moderate activity. However, it is curious that although the α-keto analogs of cysteine, glycine, asparagine, serine, alanine, α-aminobutyrate, and norleucine are moderate-to-good substrates, the corresponding amino acids are much less active; indeed, they are no more than 2% as active as glutamine when examined at a concentration of 0.02 M. The K_m values for alanine and glycine are very much higher than those for the other amino and α-keto acids studied (Table V). It thus appears that α-amino and α-keto acid substrates that have side chains of the type, CH_2CH_2X, where X = $CONH_2$, $CONHCH_3$, COOR, $S(O)CH_3$, SCH_3, and SC_2H_5, effectively interact with the enzyme. On the other hand, a number of compounds that do not have this type of side chain are substantially active, but only when an α-keto group is present in the molecule. The findings suggest that binding to the enzyme of glutamine, methionine, related amino acids and their α-keto analogs may involve the carboxyl group as well as the moiety attached to C-4 of these molecules; however, this moiety is evidently not required for the binding of certain α-keto acid substrates (*e.g.*, 9–15, Table V). The more effective binding of certain α-keto acids as compared to the analogous α-amino acids may possibly be ascribed to a more favorable geometry (planar rather than tetrahedral) about the α-carbon atom, or perhaps to the greater acidity of the α-keto acids. It may also be noted that (with the exception of glyoxylate) all of the active substrates possess two β-hydrogen atoms.

The availability of a highly purified preparation of the enzyme has also made it possible to study the interaction of the enzyme with α-ketoglutaramate. Evidence suggesting that the transamination reaction between glutamine and α-keto acids is reversible was reported previously in studies with transaminase preparations that contained ω-amidase; thus, α-keto-γ-methylglutaramate (which is not a substrate of the ω-amidase) was shown to transaminate with alanine to form γ-methylglutamine (Meister, 1954b). In the present work, transamination of α-ketoglutaramate with glutamine as well as with a number of other amino acids was clearly demonstrated and α-ketoglutaramate was found to be an excellent substrate.

TABLE V: Summary of Data on the Specificity of Glutamine Transaminase.

No.	R = $^{-}$OOCCO or R = $^{-}$OOCCHN^{+}H$_3$	Relative Rates: Amino Acid + Glyoxylate[a]	Relative Rates: Glutamine + α-Keto Acid[b]	Approx K_m Value (mM): Amino Acid	Approx K_m Value (mM): Keto Acid
1	$RCH_2CH_2CONH_2$	(100)	54	2	0.2
2	$RCH_2CH_2CONHCH_3$	63			
3	$RCH_2CH_2COOCH_3$ (or OC_2H_5)	57	[31]	4	
4	$RCH_2CH_2SOCH_3$	36		2	
5	$RCH_2CH_2SCH_2CH_3$	30	(100)		
6	$RCH_2CH_2SCH_3$	20	96	2	3
7	RCH_2CH_2OH	10	10		
8	RCH_2SCH_3	10	32		
9	RCH_2SH	2	78		5
10	RH	0.05	40	>1000	8
11	RCH_2CONH_2	2	25		
12	RCH_2OH	2	20		8
13	RCH_3	2	11	>1000	11
14	RCH_2CH_3	<1	10		
15	$RCH_2CH_2CH_2CH_3$	<1	10		

[a] From Table II. [b] Calculated from data of Table III.

Although the data indicate that the reactions catalyzed by glutamine transaminase are, like other transaminase reactions, freely reversible and have equilibrium constants fairly close to unity, the rapid nonenzymatic cyclization of α-ketoglutaramate to 5-hydroxypyrrolidone carboxylate effectively drives the reaction in the direction of glutamine utilization. Under physiological conditions (in the presence of ω-amidase) the open-chain form of α-ketoglutaramate produced by transamination of glutamine probably undergoes enzyme-catalyzed hydrolysis rather than cyclization. Transamination of glutamine is therefore essentially irreversible under physiological conditions and leads to the production of ammonia and to the formation of new amino acids from α-keto acids. Although the transaminase can effectively synthesize glutamine from its α-keto analog, this pathway does not, in the absence of evidence for a system that catalyzes the synthesis of α-ketoglutaramate, seem to offer an alternative route to glutamine. The possibility that glutamine transaminase and the ω-amidase are physically linked within the cell (also considered by Hersh (1971)) requires investigation. The observation that rat liver glutamine synthetase is inhibited by L-alanine, glycine, and L-serine suggests that a regulatory mechanism exists in which products produced by glutamine transamination can inhibit the synthesis of glutamine (Tate and Meister, 1971). The broad α-keto acid specificity of glutamine transaminase suggests that transamination of glutamine may offer a physiological mechanism for the amination of many α-keto acids so as to provide the corresponding amino acids for protein synthesis; without such a mechanism the carbon chains of these structures would be lost by degradative reactions. The relatively high activity of methionine and its α-keto analog raises the question as to whether transamination represents quantitatively a more important pathway for the metabolism of this amino acid than has been generally supposed.

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Purification and Characterization of *S*-Adenosyl-L-methionine Decarboxylase from Rat Liver†

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ABSTRACT: *S*-Adenosyl-L-methionine decarboxylase has been purified more than 350-fold from rat liver by a new procedure. Certain characteristics of the decarboxylase are described. The enzyme is inhibited by known inhibitors of pyridoxal phosphate dependent enzymes, and at later stages of purification, by sulfhydryl group reagents. No metal cations were required by this enzyme and certain metals were inhibitory. The DEAE-cellulose fraction exhibited maximal activity only when pyridoxal phosphate was added to the assay. The molecular weight of the enzyme determined at various stages of purification in three different rat tissues, *i.e.*, liver, uterus, and ventral prostate, was approximately 50,000. Higher stages of purification failed to uncouple the decarboxylation of *S*-adenosyl-L-methionine from spermidine formation.

The polyamine, spermidine, is a naturally occurring polycation which occurs ubiquitously in animal and plant tissues and in microorganisms. Spermidine concentration and synthesis are increased in a number of systems that are characterized by rapid growth, such as neoplasms (Russell and Snyder, 1968), regenerating liver (Jänne *et al.*, 1964; Dykstra and Herbst, 1965; Russell and Snyder, 1968; Jänne and Raina, 1968; Russell and Lombardini, 1971), and embryonic tissues (Calderara *et al.*, 1965; Russell, 1970, 1971).

In bacteria, spermidine is synthesized by two enzymes: *S*-adenosyl-L-methionine is first decarboxylated by *S*-adenosyl-L-methionine decarboxylase and the decarboxylated *S*-adenosyl-L-methionine then serves as a propylamine donor for putrescine. A propylamine transferase then completes spermidine synthesis by transferring propylamine from decarboxylated *S*-adenosyl-L-methionine to putrescine (Tabor and Tabor, 1964). The bacterial decarboxylase requires magnesium, has a pH optimum of 7.4, and contains covalently bound pyruvate as the prosthetic group (Wickner *et al.*, 1970). The propylamine transferase, on the other hand, has no known cofactor requirements and exhibits a broad pH optimum that centers around 8.3 (Tabor, 1962).

In mammalian systems spermidine formation appears to be

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