Purification and Characterization of Glutamate Decarboxylase from Mouse Brain*

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SUMMARY

Glutamate decarboxylase catalyzes the formation of γ-aminobutyric acid, an important inhibitory transmitter in invertebrate and vertebrate nervous systems. Approximately 700-fold purification of the enzyme from mouse brain has been achieved by a combination of ammonium sulfate fractionation, gel filtration, calcium phosphate gel, and DEAE-Sephadex chromatography. The most highly purified preparation appeared to be monodisperse on high speed sedimentation equilibrium analysis and was found to have a partial specific volume, v, of 0.732 and a molecular weight of 85,000 ± 2,000. A single major protein band coincident with the enzyme activity was found with polyacrylamide gel. In addition, a faint band also was present.

In tests with 20 naturally occurring amino acids and γ-ketoglutarate the enzyme showed γ-decarboxylation only with glutamate to a great extent; but it also catalyzed γ-decarboxylation of aspartate to a slight extent. A sharp pH optimum at pH 7.0 has been observed. Kᵢ₅ values of 0.7 mM and 0.05 μM were found for glutamate and pyridoxal phosphate, respectively. The enzyme was dissociated into two physically indistinguishable subunits with a molecular weight of 44,000 ± 2,000 in 6 M guanidine HCl containing 0.1 M β-mercaptoethanol.

γ-Aminobutyric acid is an inhibitory transmitter in many invertebrate systems and also appears to be a major inhibitory transmitter in the vertebrate central nervous system. L-Glutamate decarboxylase (L-glutamate 1-carboxyl-lyase, EC 4.1.1.15) catalyzes the α-decarboxylation of L-glutamic acid to form γ-aminobutyric acid and carbon dioxide. L-Glutamate decarboxylase probably is the rate-limiting enzyme in determining steady state γ-aminobutyric acid levels in normal nerve tissues (1–3). However, relatively little is known with certainty about the detailed properties of this enzyme because previous attempts to purify this enzyme to homogeneity were unsuccessful due to its instability (4). L-Glutamate decarboxylase now has been obtained as a highly purified and stable enzyme from mouse brain. This communication presents the purification procedures and gives some properties of the enzyme.

EXPERIMENTAL PROCEDURES

Materials—Brains of adult Swiss albino mice were used as enzyme source. Some of the animals used were given to us locally by the Riker Laboratories, and the rest were purchased from Horton Laboratories. Ultrapure ammonium sulfate and guanidine HCl were obtained from Schwarz-Mann; Sephadex G-200 and DEAE-Sephadex were purchased from Pharmacia Fine Chemicals; calcium phosphate gel was obtained from Bio-Rad Laboratories; and 2-aminoethylisothiouronium bromide hydrobromide was obtained from Sigma Chemical Co. Pyridoxal phosphate was donated to us by Calbiochem.

Enzyme Assay—L-Glutamate decarboxylase was assayed according to a procedure and with apparatus described previously, measurement being made of the evolution of 14CO₂ from L-[1-14C]glutamic acid under anaerobic conditions (5). The 14CO₂ formed was absorbed in hyamine solution and counted in a liquid scintillation counter. In a typical assay, the incubation vessel contained 0.1 ml of 0.208 mM glutamic acid (0.74 μCi of L-[1-14C]-glutamate) in 0.1 mM potassium phosphate buffer containing 0.2 mM pyridoxal phosphate, pH 7.2. The reaction was started by injecting 1 ml of enzyme solution into 50 mM potassium phosphate buffer, pH 7.2, containing 0.2 mM pyridoxal phosphate and 1 mM 2-aminoethylisothiouronium bromide (standard buffer) into the incubation vessel. The incubation was performed in a Dubnoff metabolic incubator for 30 min at 37° at approximately 150 rpm and was terminated by injecting 0.1 ml of 8 N H₂SO₄ into the reaction mixture. The vessels were incubated for another 60 min to ensure a complete release of CO₂ and absorption in the hyamine base.

One enzyme unit is defined as 1 μmole of product formed per min at 37°; specific activity is in terms of units per mg of protein. The protein contents of various preparations were determined by a modified Lowry method (6, 7) and checked against a standard solution of crystalline bovine serum albumin.

Molecular Weight Determination and Partial Specific Volume—The apparent molecular weight and the partial specific volume...
of L-glutamate decarboxylase were determined simultaneously by measuring sedimentation equilibrium in two different solvents as described by Edelstein and Schachman (8).

The high speed sedimentation equilibrium method of Yphantis (9) was employed. Experiments were carried out at 4° with Yphantis' six-channel rectangular cell in a Spinco model E analytical ultracentrifuge equipped with Rayleigh interference optics. Runs were continued until no further change in the distribution of concentration in the cell occurred. Sapphire windows were used to minimize the optical distortion. Plates were analyzed in a Nikon microcomparator with 50-fold magnification. Samples in D₂O were prepared by dialyzing 1 ml of enzyme solution against 100 ml of D₂O buffer solution with three changes overnight. Samples also were prepared by dissolving the enzyme in 6 M guanidine HCl containing 0.1 M β-mercaptoethanol and dialyzing against the same solution. The densities of the solutions were determined with a pycnometer.

Determination of Molecular Weight by Gel Filtration—A Sephadex G-200 column (2.5 x 50 cm) was calibrated with three standard proteins; cytochrome c (horse heart, mol wt 12,400), ovalbumin (egg white, mol wt 45,000) and aldolase (rabbit muscle, mol wt 158,000). The flow rate was 22 ml per hour, and the fractions were monitored continuously at 280 nm by means of an ISCO ultraviolet analyzer. Elution volumes were calculated at the peaks of protein profiles.

Substrate Specificity—The enzyme solutions used in this and subsequent studies were those of the highest specific activity attained, 2 units per mg of protein. The assay procedure was the same as described above, except that glutamate was replaced with the compounds tested for substrate activity. Solutions of all 20 naturally occurring amino acids, as well as α-ketoglutarate, were made and tested in the same way as glutamate with the exception of L-tryptophan, L-cystine, L-phenylalanine, and di-serine, which were assayed at concentrations of 2.08 mM instead of 20.8 mM. All amino acids tested, other than glutamate, were uniformly labeled with 14C. In addition, L-[1-14C]aspartate and α-[1-14C]ketoglutarate were used.

Purification of L-[U-14C]Aspartic Acid—Separation of amino acids was carried out according to the procedures described by Moore and Stein (10, 11). L-[U-14C]Aspartic acid (15 μCi) with specific activity of 227 mCi per gm was applied to a Dowex 50-X8 column (0.9 x 60 cm), which had been previously calibrated with L-glutamate and L-aspartate. The column was eluted with 0.1 M sodium citrate buffer, pH 3.4, at a flow rate of 50 ml per hour. Four milliliters per fraction were collected, and 0.1 ml from each fraction was counted in a liquid scintillation counter. Solutions prepared from the purified L-[U-14C]Aspartic acid and L-aspartic acid were used for testing substrate specificity. The final concentrations in the assay medium were as follows: L-aspartic acid, 1.9 mM; L-[U-14C]aspartic acid, 0.07 μCi per ml; sodium citrate, 9 mM; potassium phosphate, 50 mM, pH 7.2; pyridoxal phosphate, 0.2 mM; 2-aminooxyisothiouroin bromide, 1 mM; and 10 μg of enzyme. The assay was carried out as before, except for the incubation time which was 2 hours instead of 30 min.

Amino Acid Analysis—In order to identify the reaction products, the incubation mixture was deproteinized with 2% sulfosalicylic acid and 40 μg each of L-glutamic acid, L-alanine, β-alanine, and γ-aminobutyric acid were added to the mixture. The solution was adjusted to pH 2 with NaOH and had a volume of 1.4. Then 0.5 ml of the above solution was applied through an automatic sample injector to Beckman model 119 automatic amino acid analyzer using Durrum type DC-1A resin. A stream divider was used to divert about one-seventh of the effluent from column into the analyzer for colorimetric analysis, while the remaining portion of the stream was collected at 1-min intervals with a fraction collector. The equipment had been previously standardized under the same conditions with a known standard amino acid mixture to synchronize within ±1 min over a period of 3 hours. Fractions from the collector then were analyzed for radioactivity in a Packard model 3320 Tri-Carb scintillation spectrometer.

Kₘ Values for Glutamate and Pyridoxal Phosphate—The assay conditions were the same as those described above, except for the concentration of glutamate which was varied. Six different concentrations of glutamate ranging from 0.058 to 19 mM were used. The concentration of pyridoxal phosphate was kept constant at 0.2 mM, a large excess. To check the linearity of the reaction in each experiment, the reaction was stopped at 10-, 20-, and 30-min intervals by injecting 0.1 ml of 8 M H₂SO₄ into the incubation vessel. Purified enzyme preparations (3 μg) were used.

When the effect of pyridoxal phosphate concentration on enzyme activity was studied, the concentration of glutamate was kept at 19 mM, 27 times the Kₘ value for glutamate. Eight different concentrations of pyridoxal phosphate ranging from 0.007 to 2 μM were used. Purified enzyme (0.48 μg) was used as described above.

Polyacrylamide Gel Electrophoresis and Enzyme Assay of Gel Slices—Polyacrylamide gel disc electrophoresis was performed by the method of Davis (12). Polyacrylamide 3.5% separating gel columns (2.5 x 8 cm) containing 0.14% N,N'-methylenebis-acrylamide, 0.0625% 3-dimethy lamino propionitrile, 0.75 M Tris, and 0.12 mM HCl, pH 8.9, were employed. Before application of the sample, 0.2 ml of 12.8 M β-mercaptoethanol was applied to the gel, and a current of 2 mA per gel was passed through for 30 min with standard anode and cathode buffer (0.025 M Tris and 0.192 M glycine, pH 8.4) containing 20 mg per liter of reduced glutathione. The samples consisted of 50 μg of the most purified enzyme preparation in 70 μl of 12% sucrose containing bromphenol blue to mark the front. Electrophoresis was carried out at 4° at 2.5 mA per column for 2½ hours with the standard anode and cathode buffer containing 20 mg per liter of reduced glutathione and 50 mg per liter of pyridoxal phosphate.

After electrophoresis, the gels were stained with 0.5% Amido black 10B in 7% acetic acid for 1 hour at room temperature, and then destained in 7% acetic acid electrophoretically in a Caudex quick gel destainer. Unstained gel was cut into 5-mm slices for assay of the enzyme activity. Each slice was chopped into small pieces and incubated for 2 hours in 1.1 ml of ice-cold solution containing 1 ml of 0.1 M potassium phosphate (pH 6.4), 0.2 mM pyridoxal phosphate, 1 mM 2-aminooxyisothiouroin bromide and 0.1 μl of dimethylsulfoxide. The medium was adjusted to pH 7.2 with 0.1 M K₂HPO₄ and stored at -20° overnight. Enzyme activity of the medium was measured at 37° as usual after gassing with water-saturated N₂ (99.98%) and adding 0.1 ml of the stock solution containing 0.1 M potassium phosphate buffer (pH 7.2), 0.208 μg glutamic acid (0.74 μCi of L-[1-14C]glutamic acid), and 0.2 mM pyridoxal phosphate.

RESULTS

Purification of L-Glutamate Decarboxylase

The successive steps in the purification of L-glutamate decarboxylase from 9000 mouse brains are summarized in Table I.
Purification of glutamate decarboxylase from mouse brain

Purification of glutamate decarboxylase was made from 9000 mouse brains.

| Sample                  | Volume | Total activity | Total protein | Specific activity | Recovery of activity |
|-------------------------|--------|----------------|---------------|------------------|----------------------|
| Sucrose homogenate      | 30,000 | 900            | 531,000       | 3                | 100                  |
| Crude extract           | 4,800  | 274            | 13,500        | 20               | 29                   |
| First (NH₄)₂SO₄ (27-62%)| 300    | 223            | 6,000         | 37               | 23                   |
| First Sephadex G-200    | 1,080  | 116            | 1,500         | 77               | 12                   |
| Second (NH₄)₂SO₄ (30-68%)| 126    | 90             | 980           | 92               | 9                    |
| Calcium phosphate gel (pool) | 290   | 48             | 195           | 246              | 5                    |
| Third (NH₄)₂SO₄ (33-70%)| 23     | 39             | 120           | 325              | 4                    |
| DEAE-Sephadex (pool)    | 60     | 18             | 18            | 1,000            | 1.9                  |
| Fourth (NH₄)₂SO₄ (0-75%)| 3.5    | 17             | 17            | 1,000            | 1.8                  |
| Second Sephadex G-200   | 16.0   | 12             | 6             | 2,000            | 1.3                  |
| Fifth (NH₄)₂SO₄ (0-75%) | 2.0    | 12             | 6             | 2,000            | 1.3                  |

*One unit = 1 μmol of product formed per min at 37°C under standard conditions.

Eleven steps were employed in the purification and 1 to 2% of the total activity was recovered as a highly purified enzyme preparation representing 600- to 700-fold purification over the original homogenate.

**Step 1: Preparation of Starting Material**—The starting material was prepared according to the procedure described by Susz et al. (4) with some additions and modifications. In a typical preparation, 300 mice were killed by cervical dislocation. The whole brains minus the brain stem were removed rapidly, and 15% homogenate was made in ice-cold, N₂-saturated, 0.25 M sucrose in a motor-driven glass homogenizer with a Teflon pestle. The homogenate was centrifuged at 900 x g for 15 min. All operations and centrifugations were carried out at 0-4°C, and all buffer solutions contained 0.2 M pyridoxal phosphate and 1 mM 2-aminoethylisothiouronium bromide, unless otherwise mentioned. The supernatant liquid was centrifuged at 23,000 x g for 15 min. The supernatant liquid was discarded, and the pellet was again centrifuged at 73,000 x g for 20 min, and the supernatant liquid was poured off. The pellet was suspended in 144 ml of glass-distilled H₂O and stirred slowly at 4°C for 20 min; the suspension was centrifuged at 105,600 x g for 75 min. The supernatant fluid thus obtained was called the “crude extract.” No further enzyme activity was obtained with a second water extraction on the residue. Concentrated potassium phosphate buffer, 2-aminoethylisothiouronium bromide, and pyridoxal phosphate were added to the extract to give the following concentrations: potassium phosphate, 50 mM; 2-aminoethylisothiouronium bromide, 1 mM; and pyridoxal phosphate, 0.2 mM. The final pH was 7.2. The above preparation was stable for many months at -20°C and served as the starting material for further purification.

**Step 2: Ammonium Sulfate Fractionation**—Five batches of the extract prepared as above were combined, and solid ammonium sulfate (157 g per liter) was added gradually to the well stirred solution to give approximately 21% of saturation. The pH of the solution was maintained at 7.2 by gradual addition of 0.1 x NH₄OH during the addition of ammonium sulfate (about 0.5 ml of 0.1 x NH₄OH per g of ammonium sulfate was needed to maintain the pH at 7.2). After the addition of ammonium sulfate, the solution was stirred for another 15 to 20 min. The solution then was centrifuged at 13,200 x g for 30 min. The pellet was discarded and more ammonium sulfate (234 g per liter) was added to the supernatant fluid to give approximately 62% of saturation. The precipitate was dissolved in a minimal volume (40 to 50 ml) of the standard buffer. The solution was centrifuged at 105,600 x g for 30 min and applied to Sephadex G-200 directly. When storage was desirable, it was dialyzed against a large volume of the standard buffer to remove ammonium sulfate and stored at -20°C.

**Step 3: Chromatography on Sephadex G-200**—Sephadex G-200 gel was equilibrated with the standard buffer and packed into a column of 5.0 x 60 cm. About 40 to 50 ml (800 to 1000 mg of protein) of enzyme solution from Step 2 were applied to the column. The column was eluted with the standard buffer at a flow rate of 25 ml per hour. Fractions of about 18 ml were collected and stored at -20°C. Three batches of G-200 fractions with specific activity of between 0.05 to 0.17 unit per mg of protein were pooled, and ammonium sulfate fractionation was carried out as before. The precipitate between 30 to 68% saturation (176 g to 455 g of ammonium sulfate per liter) was collected and dissolved in a minimal volume of 1 mM potassium phosphate buffer, pH 7.2, and dialyzed against the same buffer overnight with two changes.

**Step 4: Chromatography on Calcium Phosphate Gel**—The amount of calcium phosphate gel (13) needed to adsorb all of the enzyme activity and the ionic strength needed to elute the enzyme were determined in small scale batchwise experiments. A slight excess of calcium phosphate gel was packed in a column (2.5 x 50 cm) with adapters to give a bed volume of 2.5 x 20 cm. The enzyme solution from the preceding steps was applied to a column which had been equilibrated with 1 mM potassium phosphate buffer, pH 7.2. After the application of the solution, an equal volume of the same buffer solution was introduced and the column was washed further with 100 to 150 ml of 25 mM potassium phosphate buffer, pH 7.2. A linear gradient made from 300 ml of 25 mM and 300 ml of 0.15 M potassium phosphate buffer, pH 7.2, then was employed. The enzyme activity started to appear after the beginning of the gradient, and the peak fraction appeared at 75 mm. The position of the peak fraction shifted toward higher ionic strength when the washing step was incomplete or when a larger amount of calcium phosphate gel was used. After the gradient, elution was continued with 0.15 M phosphate buffer.

Three batches of calcium phosphate gel fractions containing enzyme with a specific activity of 0.15 unit per mg of protein or higher were pooled and fractionated with ammonium sulfate. The precipitate coming out between 33 to 70% saturation (196 to 472 g of ammonium sulfate per liter) was collected, dissolved in a minimal volume of the standard buffer, and dialyzed against the buffer.

**Step 5: Chromatography on DEAE-Sephadex**—DEAE-Sephadex A-50 was equilibrated with the standard buffer and packed into a column of 2.5 x 50 cm. The enzyme solution from the preceding step was applied. An equal volume of the standard buffer was introduced after the sample and the column was equilibrated with the standard buffer and packed into a column of 5.0 x 60 cm. About 40 to 50 ml (800 to 1000 mg of protein) of enzyme solution from Step 2 was applied to the column. The column was equilibrated with the standard buffer at a flow rate of 25 ml per hour. Fractions of about 18 ml were collected and stored at -20°C. Three batches of G-200 fractions with specific activity of between 0.05 to 0.17 unit per mg of protein were pooled, and ammonium sulfate fractionation was carried out as before. The precipitate between 30 to 68% saturation (176 g to 455 g of ammonium sulfate per liter) was collected and dissolved in a minimal volume of 1 mM potassium phosphate buffer, pH 7.2, and dialyzed against the same buffer overnight with two changes.
washed with 100 ml of 0.1 M potassium phosphate buffer, pH 7.2. A linear gradient of potassium phosphate made from 160 ml of 0.1 M, pH 7.2, and 160 ml of 0.3 M, pH 6.4, was used for elution. The enzyme activity started to appear shortly after the gradient and peaked at 0.19 M phosphate buffer. Fractions with specific activities above 0.8, between 0.17 and 0.79, and below 0.17 unit per mg of protein were pooled separately into three solutions, concentrated by precipitation with (NH₄)₂SO₄ at 75% saturation (516 g per liter), and the precipitates were dissolved in 3.5 ml of the standard buffer.

Step 6: Chromatography on Second Sephadex G-200—Sephadex G-200 was prepared and packed as before except for the column size which was reduced to 2.5 × 50 cm. Three and one-half milliliters of the high specific activity fractions from the DEAE-Sephadex column at a concentration of 4.8 mg per ml were applied to the column. The column was eluted with the standard buffer at a flow rate of 22 ml per hour, and fractions were collected in 22-min intervals. Two peak fractions which had the same specific activity, 2 units per mg of protein, were concentrated with (NH₄)₂SO₄ as described above. The precipitates were dissolved and dialyzed against a large volume of the standard buffer.

Polyacrylamide Gel Electrophoresis

A concentrated solution of the most purified preparation was examined on polyacrylamide gel electrophoresis for protein pattern and enzyme activity. There was a single major protein band which was coincident with the enzyme activity as shown in Fig. 1; a faint additional band also was present.

High Speed Sedimentation Equilibrium

The peak fraction appeared to be homogeneous as judged from a linear plot of the logarithm of concentration, C, against the square of the distance, γ, from the center of rotation, in high speed sedimentation equilibrium runs in both H₂O and D₂O solutions (Fig. 2). The partial specific volume, ⁿ, was calculated from the slopes in the figure. Our density measurements indicated there to be 93% D₂O, which gave  κ = 1.0144 by interpolation (κ = 1.0155 in a 100% D₂O solution). This, in turn, gave a ⁿ value of 0.732. Based on this value for ⁿ, the apparent molecular weights were calculated as 85,000 ± 2,000, 85,000 ± 1,000, and 84,000 ± 2,000 at protein concentrations of 0.1, 0.25, and 0.5 mg per ml, respectively. Thus, we obtained a weight average molecular weight of L-glutamate decarboxylase as 85,000 ± 2,000 at infinite dilution.

Estimation of Molecular Weight by Gel Filtration

The plot of log of molecular weight versus Vₑ (elution volume) (14, 15) resulted in a linear relationship. The molecular weight of L-glutamate decarboxylase was estimated to be 86,000 from the Vₑ and the calibration curve.

Substrate Specificity

All 20 naturally occurring amino acids, as well as α-ketoglutarate, have been tested by radioactive assay for substrate activity. Only L-[U-¹⁴C]aspartic acid showed about 3 to 5% of the activity found with L-glutamate as substrate. Direct evidence for α-decarboxylation of L-[U-¹⁴C]aspartic acid came...
FIG. 3 (left). Elution profile of the reaction mixture with L-aspartic acid as substrate on amino acid analyzer. The reaction mixture (0.5 ml) containing 0.95 pmole of L-aspartic acid, 0.035 µCi of L-[U-¹⁴C]aspartic acid, and reaction products were applied to amino acid analyzer. Standard amino acid mixture containing L-glutamic acid, L-alanine, β-alanine, and L-aminobutyric acid were also included as markers. Amino acids were identified as from identification of the reaction product as β-alanine (Fig. 3). Radioactivity appeared only in the position of L-aspartic acid and β-alanine, and none was found with L-glutamic acid and γ-aminobutyric acid. Furthermore, as calculated from the radioactivity, the molar ratio of CO₂ to β-alanine was about 1:1. Thus, the substrate activity observed with L-[U-¹⁴C]aspartic acid must be due to α-decarboxylation of L-aspartic acid and not to contamination by L-glutamic acid. In addition to the most purified enzyme preparation, preparations at various stages of purification also have been used to test the substrate activity of L-aspartic acid. Enzyme assays were carried out as before, except that L-glutamic acid was replaced by L-aspartic acid and m-[1-¹⁴C]aspartic acid, and the incubation time was 3 hours instead of 30 min. The ratios of ¹⁴CO₂ formed from DL-[1-¹⁴C]aspartic acid to that from L-[1-¹⁴C]glutamic acid were about 1:50, with enzyme preparations ranging from a specific activity of 0.037 to 2.0 units per mg of protein as shown in Table II.

Table II

| Enzyme preparations | Specific activity | ¹⁴CO₂ formed from DL-[1-¹⁴C]aspartic acid/ ¹⁴CO₂ formed from L-[1-¹⁴C]glutamic acid |
|----------------------|------------------|----------------------------------|
| (NH₄)₂SO₄ fraction between 27-62% of crude extract . . . . | 37 | 2.4 ± 0.5 |
| (NH₄)₂SO₄ fraction between 33-70% after calcium phosphate column . . . | 325 | 2.4 ± 0.2 |
| (NH₄)₂SO₄ fraction between 0-75% after second Sephadex G-200 column . . . | 2000 | 2.0 ± 0.2 |

pH Profile

The plot of enzyme activity in 50 mM potassium phosphate buffer versus pH is shown in Fig. 4. A relatively sharp pH profile was obtained with the optimum around 7.0.

Dissociation of L-Glutamate Decarboxylase by Guanidine HCl and β-Mercaptoethanol

The enzyme preparation, which appeared to be monodisperse in the high speed sedimentation equilibrium runs, was treated with 6 M guanidine HCl and 0.1 M β-mercaptoethanol. The resultant solution was analyzed by the high speed sedimentation equilibrium method as described before, except in that the speed was 42,040 rpm at 20°C. The plot of logarithm of concentration against the square of the distance from the center of rotation is shown in Fig. 2. The linear plot of log µ versus γ² suggests that the enzyme solution treated with 6 M guanidine HCl and 0.1 M β-mercaptoethanol is homogeneous in molecular size. It also suggests the homogeneity of the native enzyme preparation. By assuming the same partial specific volume,
\(\delta\), as that of the native enzyme, 0.732, the slope of the plot gave a molecular weight of 44,000 ± 2,000, which is about half of the size of the native enzyme. Therefore, it seems that L-glutamate decarboxylase is dissociated into two ultracentrifugally indistinguishable subunits by 6 M guanidine HCl and 0.1 M \(\beta\)-mercaptoethanol.

**DISCUSSION**

The methods described in this paper for the purification of L-glutamate decarboxylase from mouse brain have proven to be highly reproducible in our hands. The procedure employed for the preparation of the initial extract liberated only approximately 30% of the total enzyme activity in mouse brain. However, although it was possible to recover almost all of the enzyme activity by employing more vigorous conditions of homogenization, the activity in starting material prepared in this way proved to be present in a highly aggregated form, appearing in the void volume of Bio-Gel A-0.5m which has an exclusion limit of 500,000. The material was refractory to disaggregation, and the enzyme activity in the solution was not amenable to ammonium sulfate fractionation.

Within the limits of the method, data obtained from high speed sedimentation equilibrium runs on the most highly purified material in aqueous buffer, D_2O buffer, or in concentrated guanidine HCl containing \(\beta\)-mercaptoethanol showed the material to be monodisperse. Evidences of \(\alpha\)-decarboxylation of L-aspartic acid by this enzyme came from several observations. First of all, with L-aspartic acid containing purified L-[\(\text{U}^{14}\text{C}\)]-aspartic acid as substrate, the reaction products were identified as \(\beta\)-alanine and CO_2 with a molar ratio of 1:1. Second, the ratio of \(\text{H}^4\text{CO}_2\) formed from [1-\(\text{U}^{14}\text{C}\)]aspartic acid and [1-\(\text{U}^{14}\text{C}\)]glutamic acid was about the same by enzyme preparations with purities differing more than 50-fold. Third, L-aspartic acid was found to be a relatively potent competitive inhibitor of the enzyme with the \(K_i\) being 3.1 \(\text{nm}\) whereas the \(K_m\) for L-glutamate is 0.7 \(\text{nm}\) (17). The latter observation makes it unlikely that the activity observed could be attributable to a trace contamination of L-glutamic acid. Hence, it seems appropriate to conclude that the \(\alpha\)-decarboxylation of L-aspartic acid observed was probably catalyzed by glutamate decarboxylase. It is interesting that bacterial glutamate decarboxylase can also decarboxylate \(\alpha\)-methyl-DL-glutamic acid and L-glutamine in addition to L-glutamate (18). Some other amino acid decarboxylases have also been reported to decarboxylate more than one substrate, e.g. arginine decarboxylase can decarboxylate both L-arginine and L-canavanine (19); lysine decarboxylase decarboxylates L-lysine, 5-hydroxylysine, and S-(2-amino-ethyl)-L-cysteine (20).

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