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# COMPARISON OF THE STRUCTURES OF L-GLUTAMATE DECARBOXYLASES FROM HUMAN AND RAT BRAINS

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SUMMARY

Human and rat L-glutamate decarboxylases have been purified to electrophoretic homogeneity. These two enzymes were compared using an immunochemical method, amino acid analysis and tryptic fingerprinting. Structural studies revealed several differences in the primary structure of the two enzymes, but the immunochemical method used did not distinguish between the antigenicity of the two proteins.

## INTRODUCTION

Several attempts have been made to purify mammalian L-glutamate decarboxylases (L-glutamate 1-carboxylyase; EC 4.1.1.15) by affinity chromatography [1,2]. Until now, these methods have not given reliable results. Recently, we purified L-glutamate decarboxylase of human brain using conventional techniques and an hydrophobic chromatographic step [3,4]. An 8,000-fold purified enzyme was obtained with a yield of about 3,8 %. The enzyme appeared to be pure by polyacrylamide gel electrophoresis under both denaturing and non-denaturing conditions, and only one N-terminal amino acid was found for the purified protein.

In the present study, the rat enzyme was purified to homogeneity using this same method, and the proteins from human and rat brains were compared using an immunochemical method, amino acid analysis and tryptic fingerprinting.

## MATERIALS AND METHODS

*Purification of L-glutamate decarboxylase of rat brain.* One hundred rats were decapitated and their brains were rapidly extracted. All procedures for homogenization, purification and measurement of enzyme activities were identical to those used for analysis of glutamate decarboxylase from human brain [3,4].

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*Radioimmunoassay for L-glutamate decarboxylase of rat brain.* Antiserum to rat glutamate decarboxylase was obtained in an adult rabbit 60 days after it received four subcutaneous injections of pure enzyme (each of 0.2 mg protein). Freund's complete adjuvant was used as the vehicle, and injections were made at 15-day intervals. The antiserum obtained gave one precipitin band against pure glutamate decarboxylase by immunoelectrophoresis and inhibited the pure enzyme by 70 %, as judged by immunoprecipitation.

[$^{125}$I]labelled rat enzyme was prepared by the method of Hunter [5] using chloramine-T. Specific radioactivities ranged from 8 to 10 µCi/mg.

Inhibition test : This test was performed with a dilution of antiserum (1/500) that gave 60-80 % binding of antigen. Unlabelled enzyme (human or rat) was incubated in a range of 0 to 40 · $10^{-9}$ g/400 µl in the presence of a constant level of labelled rat enzyme (about 10 · $10^{-9}$ g/400 µl). Phosphate buffer 20 mM, pH 7.4) was used as the incubation medium, and contained (as final concentrations) 1 % bovine serum albumin, 0.1 % sodium azide, 0.5 % Triton X-100, 1/500 rabbit immunized serum and 1/20 human normal serum. Incubations were carried out at 37°C; 6 h later, 0.2 ml of a 1/28 dilution of goat anti-rabbit gamma globulin was added to all samples and they were incubated for another 15 h at 4°C. The precipitates formed were isolated for gamma counting by filtration on GS Millipore filters (0.22 mµ).

*Tryptic fingerprinting.* Fifty µg of each enzyme were dissolved in 0.5 ml of 0.1 M ammonium bicarbonate solution and mixed with 20 µl of a 1 mg/ml solution of trypsin. The digestions were allowed to proceed for 24 h at 37°C. Then, the pH of the solutions was adjusted to 9.2 with NaOH (1.0 N), and the peptides were dansylated using 50 µCi (109 Ci/mole) of [$^{14}$C]dansyl chloride. The reactions were carried out at 37°C in the dark for 2 h in the presence of 0.1 % sodium dodecylsulphate. Separation of dansyl-OH and chromatography of dansyl-peptides on TLC plates were performed according to Zanetta *et al.* [6]. Radioactive spots were identified with a thin layer scanner II LB 2723 (Berthold).

*Cyanogen bromide cleavage.* CNBr cleavage of the two enzymes was carried out essentially by the method of Phillips and Azari [7]. Approximately 40 µg of each protein were dissolved in 250 µl of 70 % (v/v) formic acid. To this was added CNBr, producing a 500-fold molar excess of reagent over methionine content. The reaction was allowed to proceed for 15 h at 20°C. The reaction mixture was then lyophilized, and peptides were separated by polyacrylamide gel electrophoresis according to the method of Swank and Munkres [8].

## RESULTS AND DISCUSSION

Results of the enzyme purification procedure are shown in Table 1. Starting with 100 rat brains, we obtained about 200 µg of enzyme with a yield of 2.8 %.

The enzyme was judged to be pure using the following criteria (same as for the human enzyme):

- Only one protein band existed after polyacrylamide gel electrophoresis

TABLE 1. Purification of rat brain glutamate decarboxylase.

| Fractions | Total protein mg | Specific activity units/mg prot. | Purification x | Total activity units | Activity yield % |
|---|---|---|---|---|---|
| Homogenate | 19,850 | 2.30 | | 45,600 | 100 |
| Supernatant | 2,405 | 4.93 | 2.14 | 11,860 | 26 |
| DEAE-Cellulose | 737 | 14.7 | 6.40 | 10,816 | 23.7 |
| Hydroxyapatite | 73 | 88.4 | 38.5 | 6,425 | 14 |
| Phenyl-Sepharose | 5.7 | 713.8 | 310 | 4,067 | 9 |
| QAE-Sephadex | 2.1 | 1,128.6 | 491 | 2,370 | 5.2 |
| Ultrogel | 0.200 | 6,370 | 2,770 | 1,274 | 2.8 |

1 unit = $10^{-9}$ moles/min

TABLE 2. Comparison of some properties of human and rat glutamate decarboxylases.

| | Human enzyme | Rat enzyme |
|---|---|---|
| Molecular weight : | | |
| native | 140,000 ±15,000 | 140,000 ±15,000 |
| subunits | 67,000 ± 5,000 | 67,000 ± 5,000 |
| Disulphide bridges | No | No |
| N-Terminal amino acid | Alanine | Alanine |
| pHi | 5.0 ±0.2 | 5.4 ±0.2 |
| pH optimum | 6.8 ±0.15 | 7.0 ±0.15 |
| Km, glutamate | 1.28 +0.07 mM | 1.10 ±0.07 mM |
| Km, pyridoxal phosphate | 0.13 ±0.09 $10^{-6}$ M | 0.50 ±0.09 $10^{-6}$ M |

TABLE 3. Amino acid composition of human and rat purified glutamate decarboxylases (GAD).

| Amino acids | Human GAD | | Rat GAD | |
|---|---|---|---|---|
| | Residues/ molecule | Percentage of total | Residues/ molecule | Percentage of total |
| Aspartic acid + asparagine | 118 | 10.5 | 103 | 9.5 |
| Threonine | 78 | 6.9 | 52 | 4.8 |
| Serine | 82 | 7.3 | 74 | 6.8 |
| Glutamic acid + glutamine | 138 | 12.3 | 91 | 8.4 |
| Proline | 34 | 3.0 | 56 | 5.2 |
| Glycine | 90 | 8.0 | 86 | 7.9 |
| Alanine | 93 | 8.3 | 55 | 5.1 |
| Half-Cystine | 19 | 1.7 | 23 | 2.1 |
| Valine | 68 | 6.0 | 73 | 6.7 |
| Methionine | 8 | 0.7 | 14 | 1.3 |
| Isoleucine | 68 | 6.0 | 66 | 6.1 |
| Leucine | 109 | 9.7 | 109 | 10.1 |
| Tyrosine | 29 | 2.6 | 25 | 2.3 |
| Phenylalanine | 52 | 4.6 | 39 | 3.6 |
| Lysine | 68 | 6.0 | 86 | 7.9 |
| Histidine | 12 | 1.0 | 54 | 5.0 |
| Arginine | 56 | 4.9 | 77 | 7.1 |
| Total | 1122 | 100 | 1083 | 100 |

The error in the amount of each amino acid was estimated at 3-5 residues per cent.

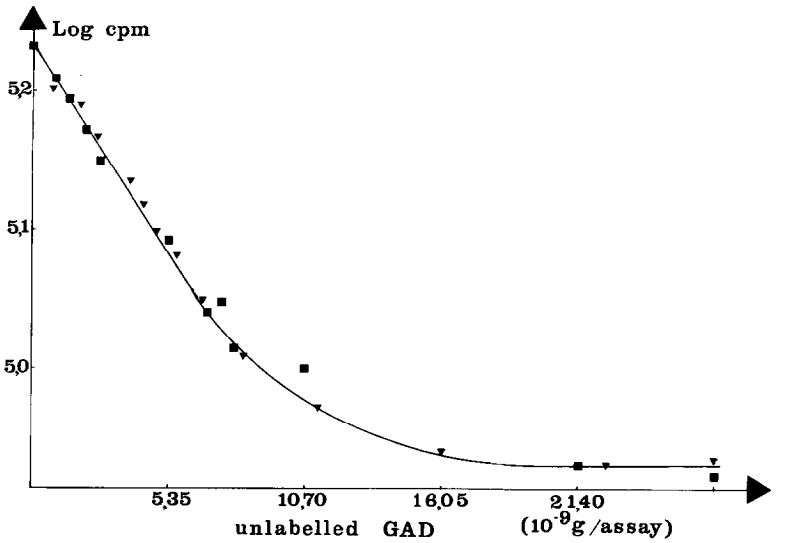



Fig. 1. Inhibition of the fixation of $^{125}$I rat enzyme by human (▽) and rat (□) L-glutamate decarboxylases. Antibodies were obtained from a rabbit injected with rat enzyme.

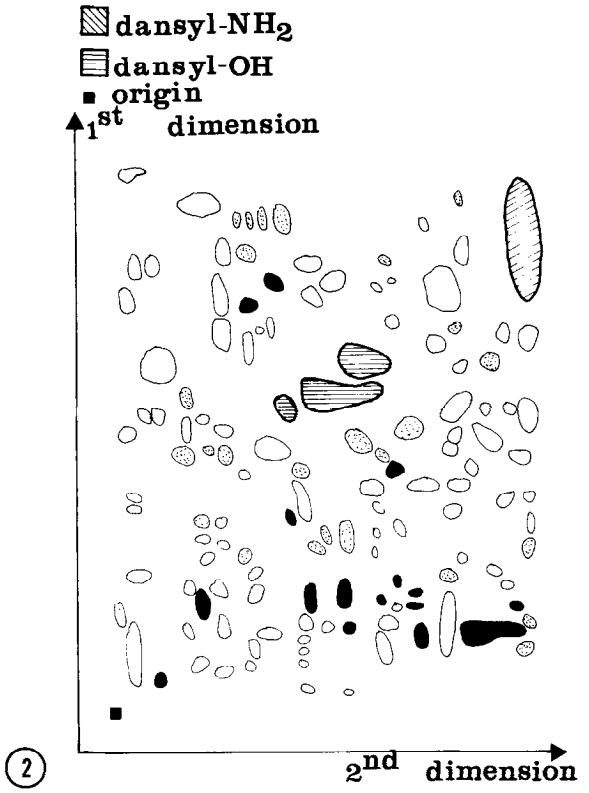
under both denaturing and non-denaturing conditions. (Fig. 3).

- After electrofocussing on a thin layer plate, according to the technique of Radola [9], the active fraction, layered on a polyacrylamide-SDS gel, gave only one band.

- Determination of the N-terminal amino acid, by the technique of Casola [10], revealed only one radioactive α-dansyl amino acid : alanine. The same experiments used for characterizing the human enzyme [3,4] were conducted with the rat enzyme. Comparative results are shown in Table 2.

Inhibition by the two enzymes of the fixation of $^{125}$I rat enzyme on antibodies is shown in Fig. 1. No detectable differences existed between the two enzymes; i.e., the antibody against rat enzyme exhibited approximately the same affinity for human and rat enzymes.

Tryptic fingerprints (Fig. 2) showed 16 specific radioactive spots for the human enzyme, 26 specific radioactive spots for the rat enzyme, and 92 spots that were common to both enzymes.



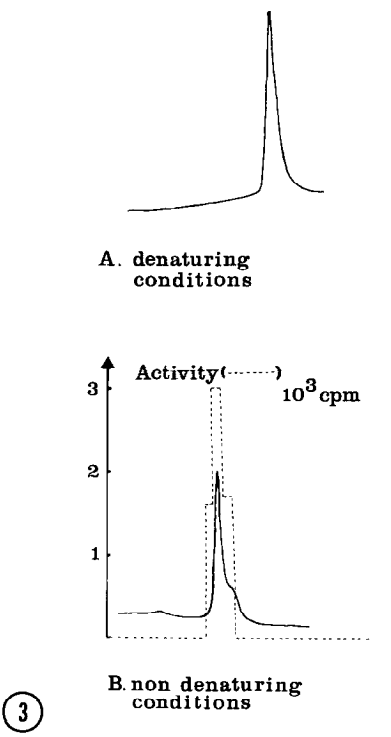




Fig. 2. Tryptic fingerprints of human and rat L-glutamate decarboxylases. ▨, specific to rat enzyme; ■, specific to human enzyme; □, common spots.

Fig. 3. Densitometry of GAD submitted to electrophoresis on polyacrylamide gels. (A), denaturing conditions. (B) non denaturing conditions.

After cyanogen bromide cleavage, polyacrylamide gels revealed 6 protein bands, 4 of these having about the same molecular weight for each enzyme.

The amino acid composition of the rat enzyme was determined in exactly the same manner as that for the human enzyme [4]. The comparative composition is shown in Table 3. Although the numbers of total amino acids are similar, there exist some differences; e.g., in the numbers of threonine, proline, alanine, phenylalanine, lysine, arginine and histidine residues.

Saito *et al.* [11] reported that L-glutamate decarboxylases of mouse and human brains were rather indistinguishable by double diffusion and microcom-

plement fixation tests. However, we think that our preparations of rat and human L-glutamate decarboxylases are more homogeneous than previously reported for the mouse enzyme [12]. In fact, in the latter study, the authors reported a separation of 4 bands using SDS-polyacrylamide gel electrophoresis [13]. Based on the comparison of rat and human brain L-glutamate decarboxylases reported here, we cannot distinguish between the two enzymes by the radioimmunological method employed. Thus, the human enzyme can be measured by radioimmunoassay using antibody against rat enzyme. Nevertheless, structural studies have revealed several differences in the primary structure of the two enzymes. These differences are interesting from a phylogenetic point of view and demonstrate clearly the limit of the immunological method.

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