# PURIFICATION AND PARTIAL CHARACTERISATION OF 4-AMINOBUTYRATE 2-KETOGLUTARATE TRANSAMINASE FROM HUMAN BRAIN

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## 1. Introduction

A parallelism has been shown between the anticonvulsive effect and an increase in the cerebral GABA level after in vivo administration of some branched chain fatty acids such as *n*-dipropylacetate [1,2]. It has also been shown that these compounds improve conditioned behaviour [3]. It was later demonstrated that these compounds are competitive inhibitors of rat and mouse cerebal GABA-T**, thus probably explaining these effects [4]. This has stimulated interest in the search for non toxic compounds, slowly metabolised by the body, yet being powerful inhibitors of cerebral GABA-T. In order to test these compounds with human therapy in mind, it seemed pertinent to use GABA-T from human brain. We thus proceeded to purify this enzyme guided by the methods we employed for purification of GABA-T from rat brain [5]. This human GABA-T preparation will also enable a comparison to be made with brain GABA-T from other species, in particular that of the mouse [6] and that of the rat [5].

## 2. Materials and methods

The purification was made from a human brain removed 4 hr after death. After removal of the meninges, the cerebral trunk, the cerebellum and the corpus callosum, the remaining 980 g of cerebral material was homogenised in fractions in 6 l of 0.25 M sucrose containing 1 mM AET. All these operations and those which followed were performed at 4°C.

### 2.1. *Purification*

The homogenate was centrifuged at 1000 *g* for 15 min to precipitate the cellular debris and nuclei, then the supernatant was centrifuged at 6000 *g* for 30 min to precipitate the mitochondria. The pellet was washed by resuspension in 0.25 M sucrose, then recentrifuged. The mitochondrial pellet was lysed for 30 min in 2 l of water containing 1 mM AET and 0.1 mM EDTA. After centrifugation of the suspension at 50 000 *g* for 2.5 hr, the resultant pellet was frozen at −20°C, thawed and homogeneised in a Waring blendor with 2 l of water containing 1 mM AET, 0.1 mM EDTA and 10 mg/l PLP. The suspension was frozen, then thawed, and after centrifugation at 105 000 *g* for 90 min, the supernatant (1240 ml) was used as the source of GABA-T

The proteins were precipitated by ammonium sulphate addition at 4°C, the pH maintained at 7.2. The fraction precipitating between 45 and 67% ammonium sulphate saturation was redissolved in 75 ml of 2 mM phosphate buffer pH 7.2 containing 1 mM AET, 0.1 mM EDTA and 10 mg/l PLP. All further operations were conducted at this pH and with these concentrations of protectors. This solution was divided into 4 parts, each of which was successively chromatographed on a Sephadex G-200 column under the same conditions as those described for the rat enzyme [5].

All the enzyme eluted from the four G-200 runs was absorbed on a DEAE cellulose column and eluted with a linear gradient of phosphate buffer pH 7.2 between 2 and 50 mM. The enzyme eluted from this column

* This work is part of C.C.'s thesis.

** *Abbreviations:* GABA-T: 4-Aminibutyrate 2-Ketoglutarate Transaminase (EC 2.6.1.19), AET: 2-Aminoethylisothiouronium Bromide Hydrobromide, PLP: Pyridoxal Phosphate.

was absorbed on a DEAE Sephadex column and eluted with a linear gradient of phosphate pH 7.2 between 10 and 200 mM.

On these various supports, the human enzyme behaves in the same way as the rat enzyme [5].

2.2. *Determination of enzyme activity*

Two methods were used: (a) Salvador Alber's method with certain modifications [5] was used to identify the enzyme activity in the column eluates and to establish the pH optimum and temperature of maximum activity. (b) The method of Waksman and Roberts was used [7] to determine the $K_m$. Here, the [$^{14}C_1$]glutamate formed [$^{14}C_1$]α-keto-glutarate was separated via a Dowex column 50 X 8, $H^+$ form and the radioactivity measured by liquid scintillation counting.

2.3. *Protein determination*

For the stages before column chromatography, Lowry's method was used, for later stages, owing to the low protein concentrations and high level of interfering substances (AET, PLP) the determinations were performed by densitometry of the protein bands stained with Coomassie Blue R 250 after electrophoresis on polyacrylamide gels. Protein standards were run under the same conditions using BSA. The results from this method were in agreement with those obtained by intergration of the α-amino acid peaks from the hydrolysed proteins on a Technicon amino acid analyser.

2.4. *Disc electrophoresis*

7.5% Polyacrylamide gels were used according to the technique of Joivin et al. [8]. The protein bands were stained with Coomassie Blue R 250.

2.5. *Determination of pH optimum*

Tris–HCl buffers, 0.3 M pH 7.4 to 9.4 containing GABA 50 mM, α-ketoglutarate 5 mM and the usual protectors (AET, EDTA, PLP) were used. The enzyme activity was measured during the first 30 min during which the reaction was linear with time.

2.6. *Temperature/activity relationship*

The above buffer was used though at the optimum pH of 8.6. The enzyme activity was measured during the first 30 min incubation at temperatures from 0–70°C.

2.7. *Determinations of apparent $K_m$'s*

The rate of formation of [$^{14}C_1$]glutamate was measured during the first 30 min incubation at 37°C in 0.3 M Tris–HCl buffer at pH 8.6.

## 3. Results

3.1. *Purification*

Under the above conditions, 80 μg of enzyme purified 1400-fold was obtained from one human brain. The yield of enzyme units was 0.14% (table 1).

3.2. *Electrophoresis*

A single band was obtained on 7.5% PAA gels, at pH 8.9 (fig. 1) 40 μg of protein was put on the gel. The densitometer trace from the stained gel suggests

Table 1
Purification of GABA-Transaminase from human brain

| | Total proteins (mg) | Yield % | Specific activity μmole/min/mg | Purification | Total activity μmole/min | Activity yield % |
|---|---|---|---|---|---|---|
| Sucrose homogenate | 87 100 | 100 | $27 \times 10^{-4}$ | 1 | 227 | 100 |
| Crude mitochondrial extract | 205 | 2.3 | $53 \times 10^{-3}$ | 19 | 10.9 | 4.8 |
| Ammonium sulphate | 37 | 0.42 | $86 \times 10^{-3}$ | 32 | 3.17 | 1.4 |
| Sephadex G-200 | 1.5 | $2 \times 10^{-3}$ | 0.65 | 230 | 0.975 | 0.42 |
| DEAE Cellulose | 0.378 | $4 \times 10^{-4}$ | 2.3 | 880 | 0.87 | 0.38 |
| DEAE Sephadex | 0.083 | $1 \times 10^{-4}$ | 4 | 1440 | 0.32 | 0.14 |

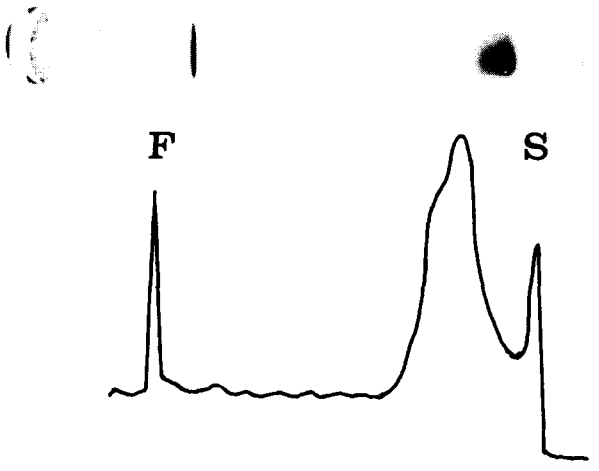


Fig. 1. Gel electrophoresis on 7.5% polyacrylamide of purified GABA transaminase. Top: Photography. Bottom: Densitometry. S: Start. F: Bromophenol Blue front.

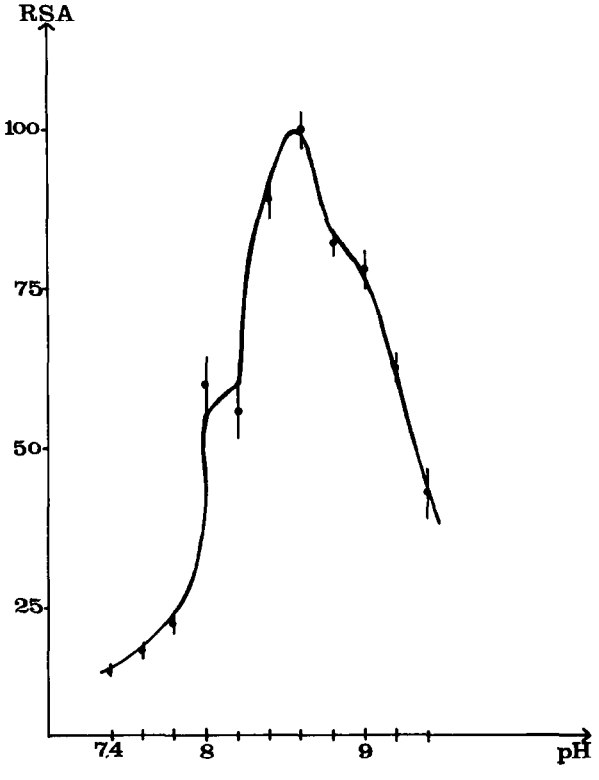


Fig. 2. Specific activity as a function of pH. Specific activity expressed as percent of maximum specific activity. RSA: Relative specific activity.

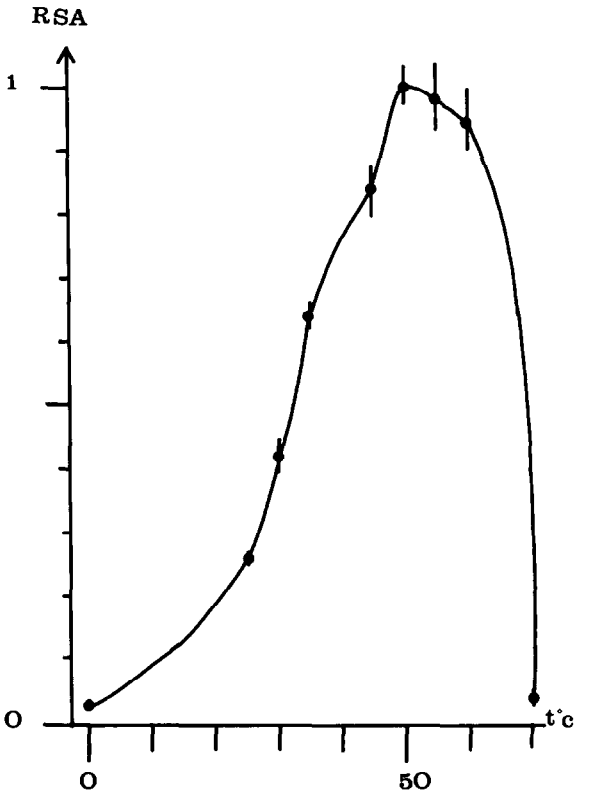


Fig. 3. Specific activity as a function of temperature. Specific activity expressed as percent of maximum specific activity. RSA: Relative specific activity.

a purity of 80% if one considers that the shoulder on the recording is due to a contamination.

### 3.3. *pH optimum*

The enzyme shows maximum activity at pH 8.6. However, the activity/pH curve shows a shoulder at pH 8. (fig.2).

### 3.4. *Temperature/activity relationship*

Under the conditions of the experiments, maximum enzyme activity is seen at 50°C. The reaction rate decreases slowly between 50° and 60°C, and very rapidly at higher temperatures. (fig. 3).

### 3.5. *Apparent $K_m$'s (fig. 4)*

(a) For GABA: with α-ketoglutarate at the concentration of 0.3 mM the apparent $K_m$ for GABA is $4 \cdot 10^{-4}$ M.

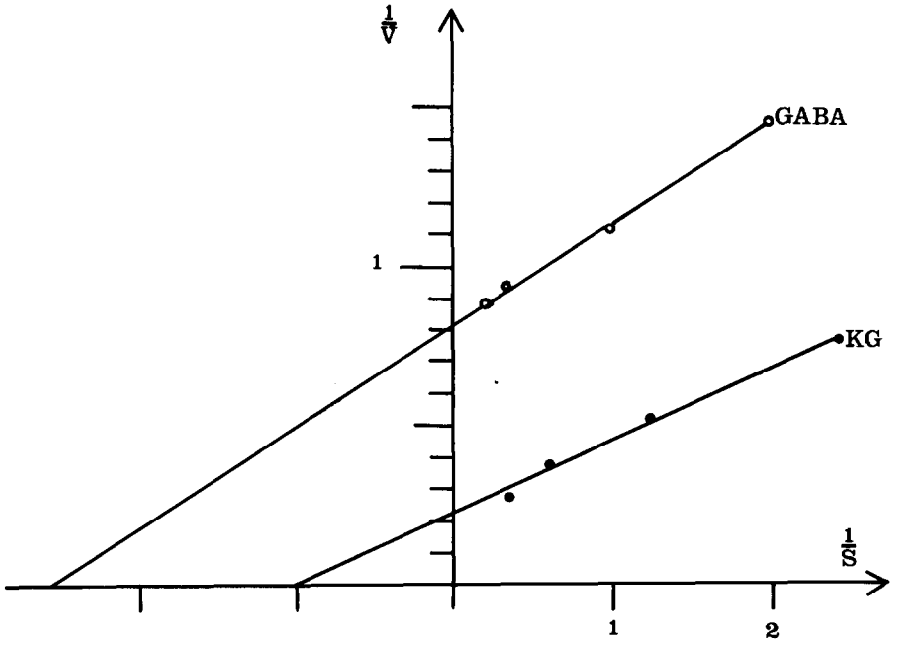


Fig. 4. Graphic determination of $K_m$ (apparent) of GABA and α-ketoglutarate. Lineweaver–Burk plot. Abcissa: reciprocal of concentration (mM) of GABA or α-ketoglutarate. Ordinate: reciprocal of reaction rate (μmole/hr).

(b) For α-ketoglutarate: with 10 mM GABA, the apparent $K_m$ for α-ketoglutarate is 1 mM.

## 4. Discussion

Because of the inevitable delays in obtaining a human brain, the initial specific activity of the homogenate is relatively low. It is possible that some of the enzyme is denatured yet is isolated together with the active enzyme during purification hence lowering the final specific activity. Nevertheless, the specific activity of the purified enzyme approaches that of the mouse brain enzyme prepared by Roberts et al. (specific activity of 300 nmole/hr/mg) but is much lower than that of the rat brain enzyme recently purified in our laboratory, (specific activity 600 nmoles/hr/mg) [5]. The purification of the human enzyme was estimated as 80% when taking the shoulder of the densitometer trace from PAA gels as a contaminant. However, a similar shoulder is seen on the pH/activity curve, suggesting the possibility of isoenzymes with pH optima 8 and 8.6. It is thus possible that much of the 20% impurity calculated from the densitometric trace consists of the isoenzyme with the pH optimum of 8. This isoenzyme could thus be similar to that obtained from mouse brain by Roberts et al. [6]. The enzyme remains active for about an hour at 60°C, hence it is more thermostable than the enzyme isolated from mouse and rat brain. The apparent $K_m$'s measured under the conditions described above are very similar to those measured under the same conditions for the rat enzyme ($K_m$ GABA: human $4 \cdot 10^{-4}$ M, rat $3 \cdot 10^{-4}$; $K_m$ α-ketoglutarate: human $1 \cdot 10^{-3}$ M, rat $1.2 \cdot 10^{-3}$ M).

Currently, the reality of the presence of isoenzyme is under investigation, with the view, if indeed there are isoenzymes, to isolate the pure species.

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