PURIFICATION AND SOME PROPERTIES OF CYSTEINE SULFINATE TRANSAMINASE

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1. Introduction

The catabolism of cysteine in rats may occur by two main pathways: the taurine pathway and the pyruvate pathway [1]. The latter involves two routes: a minor one which is the direct conversion of cysteine to pyruvate catalyzed by cysteine desulfhydrase and a major one which is the conversion of cysteine to pyruvate via cysteine sulfinic acid (CSA) catalyzed by, respectively, cysteine oxidase and cysteine sulfinate transaminase (CSA-T) [2]. Consequently, CSA is a key intermediate as it is the main precursor of taurine [3] and also one of the precursors of pyruvate.

We have investigated cysteine sulfinate transaminase, an enzyme which may play a role in the regulation of CSA level and consequently in the regulation of taurine biosynthesis. We report here a method for the purification of this transaminase, which has been sometimes considered to be either identical [4,5] to the well-known aspartate amino transferase (AAT), or different [6–8]. Some properties (K_m, V_{max}, mol. wt, substrate-specificity, thermic denaturation) of the purified enzyme have also been examined.

2. Materials and methods

Chemicals were purchased from Sigma Chemical Co., excepted for glutamate dehydrogenase, NAD and ADP, which were obtained from Boehringer. Sephacryl S-200, octylsepharose and DEAE–Sephadex were obtained from Pharmacia. Hydroxylapatite was prepared according to [9]. Enzyme activity was determined by measuring the fluorescence of NADH formed after two enzymatic steps as in [10]. Protein was determined by the Lowry method after precipitation of the protein with trichloroacetic acid to remove interfering substances [11]. Polyacrylamide gel electrophoresis was carried out as in [12].

3. Results

3.1. *Preparation of soluble rat brain fraction*

One hundred Wistar rats (200–250 g), bred in our laboratory, were killed for each purification, and the brains were quickly frozen in liquid nitrogen. Then, the brains were homogenized in Potter Elvejhem homogenizers in a solution containing Tris–HCl buffer (pH 8.6) 10 mM, pyridoxal phosphate 20 μM, 2-aminoethylisothiouronium bromide hydrobromide (AET) 100 μM, phenylmethylsulfonyl fluoride 1 mM (buffer I) in 480 ml final vol. The homogenate was centrifuged for 1 h at 100 000 × *g*, at 4°C. The supernatant was used for subsequent steps of purification.

3.2. *Ammonium sulfate precipitation*

Ammonium sulfate powder was slowly added over 2 h to 67% saturation to a continuously stirred solution. After 30 min centrifugation at 100 000 × *g*, the precipitate was discarded and ammonium sulfate was again slowly added to 85% saturation of the supernatant. The precipitate formed was removed by centrifugation at 100 000 × *g* 30 min. The precipitate was resuspended in 15 ml buffer I (fraction I). All steps were carried out at 0–4°C.

3.3. *DEAE–Sephadex chromatography*

Fraction I was applied to a DEAE–Sephadex A-50

column (2.6 X 50 cm) equilibrated with buffer I, and 5 ml fractions were collected using 20 ml/h flow rate. All of the fractions obtained from column chromatography were assayed for enzyme activity. Active fractions whose elution coincided with the beginning of large ultraviolet adsorbance peaks, were pooled and concentrated by filtration on a UM 10 membrane. At this stage, the enzyme was 20–30-fold purified in 10–15 ml final vol. (fraction II) containing ~25 mg protein.

3.4. *Hydroxylapatite chromatography*

Fraction II was applied to an hydroxylapatite column (1 X 25 cm) equilibrated with buffer I. The column was washed with 60 ml buffer I and then the proteins were eluted with a linear gradient of 500 ml (250 ml buffer I + 250 ml phosphate buffer 250 mM (pH 8.6); pyridoxal phosphate, 10 μM). CSA-T elution from the column began at 50 mM and was complete by 90 mM. The active fractions were pooled and reduced to ~10 ml (fraction III) by filtration on UM 10 membrane.

3.5. *Octylsepharose chromatography*

NaCl powder was added to fraction III to 2 M final conc. Then, fraction III was passed through an octyl-sepharose column (0.8 X 18 cm) and the column was washed with buffer I which also contained NaCl (2 M). The pooled active fractions were concentrated to 5 ml (fraction IV). At this stage, 1–3% of the initial activity was recovered and 200–250-fold purification was obtained.

3.6. *Gel filtration on Sephacryl S-200 superfine column*

Fraction IV was loaded onto a Sephacryl S-200 superfine column (2.8 X 80 cm) equilibrated with buffer I and 3.5 ml fractions were collected. The active fractions were concentrated to 0.3–0.5 mg enzyme/ml, and 10% glycerol was added. The enzyme (fraction V) could then be stored at –30°C without loss of activity for several months. The above procedure was performed at 0–4°C. The results of a typical purification from 100 rat brains are shown in table 1.

4. Discussion

4.1. *Purity and properties of the enzyme*

A single protein band was found after polyacrylamide gradient gel electrophoresis of the purified enzyme (20 μg) either in the presence (polyacrylamide

Table 1

Procedure	Total activity (mmol/h)	Total protein (mg)	Spec. act. (mmol/h/mg) protein)	Yield (%)	Purification -fold
Homogenate	928	19 432	47.8	–	–
Supernatant	351	3769	93.3	37.8	2
Ammonium sulfate precipitatior 67–85% (fraction I)	284	477	597	30.6	12
DEAE–Sephadex (fraction II)	69	28	2466	7.4	51
Hydroxylapatite (fraction III)	42	5.1	8265	4.5	172
Octylsepharose (fraction IV)	26	2.1	12 471	2.8	260
Sephacryl S-200 (purified enzyme) fraction V)	16	1.2	13 632	1.7	285

Typical purification of rat brain soluble CSA-T. The enzyme was isolated from 100 brains. Activity was assayed as in section 2

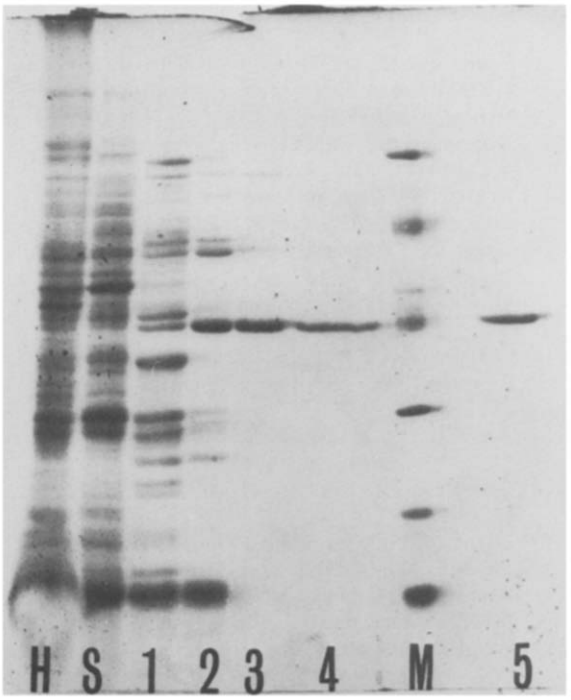

Fig.1. Polyacrylamide gel electrophoresis (gradient 10–20%): H, homogenate; S, supernatant; 1, fraction I; 2, fraction II; 3, fraction III; 4, fraction IV; M, enzymes of known molecular weight; 5, fractions V (purified enzyme). For definition of fraction and for molecular weight of the enzymes used as references, see text.

gradient, 10–20%) (fig.1) or in the absence of (polyacrylamide gradient 4–6%) sodium docecylsulfate (SDS). By means of SDS–polyacrylamide gel electrophoresis, a 41 000 ± 3000 mol. wt was determined using as references phosphorylase *b* (94 000), bovine serum albumin (67 000), ovalbumin (43 000), carbonic anhydrase (30 000), soy bean trypsin inhibitor (20 100) and α-lactalbumine (14 400) (see fig.1).

The highly purified enzyme (mixed v/v with Freund adjuvant) 1 mg, was injected subcutaneously into a rabbit. Three weeks after the first injection, 1 mg protein was again injected. Two weeks after the second injection, a third injection was made intravenously. After 1 week, the rabbit was bled and its blood was collected. Antiserum was prepared and then stored at −20°C until assayed. The supernatant, fraction I and purified enzyme were tested against the antiserum according to [13]. Only 1 precipitation line appeared

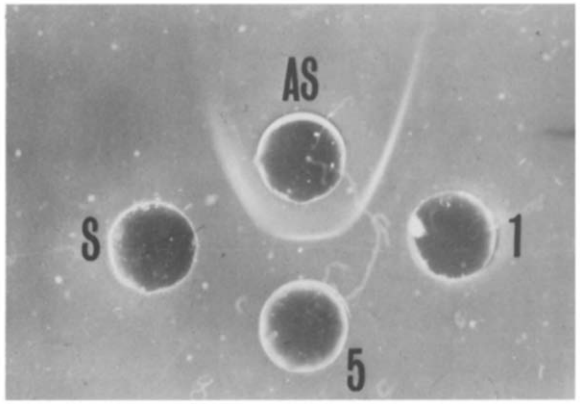

Fig.2. Ouchterlony test. Antiserum against supernatant, fraction I and purified enzyme; AS, antiserum; S, supernatant; 1, fraction I; 5, purified enzyme, fraction V.

(see fig.2). Immunoelectrophoresis confirmed these results.

4.2. *Properties*

The optimal pH for the activity of this transaminase is about 8.6; no activity was detectable up to pH 7.4. A thermic denaturation study indicated that enzymatic activity is completely destroyed at 60°C. Apparent Michaelis constants of K_m 9.4 mM and V_{max} 45 mmol/h/mg protein was determined for CSA at a fixed concentration of α-oxoglutarate (10 mM) and at pH 8.6 (fig.3). Substrate specificity was tested. In addition to

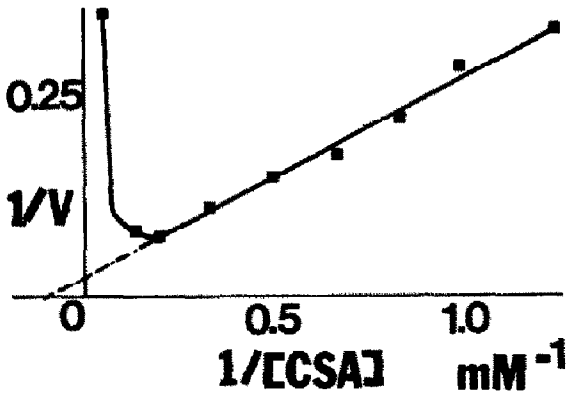

Fig.3. Lineweaver-Burk plots of initial velocities against CSA concentrations at a fixed concentration of α-oxoglutaric acid (10 mM) and at pH 8.6.

Table 2

Substrate	% spec. act.
CSA	100
Aspartic acid	32
Cysteic acid	30
Homocysteine	7.7

Substrate specificity of the cysteine sulfinate transaminase. No activity was found with cysteine, taurine, hypotaurine, glycine, α-aminobutyric acid, α-aminoisobutyrate, β-alanine GABA, serine, ciliatine and glycocyamine

CSA, aspartate and cysteic acid were transaminated by the purified enzyme as shown in table 2.

4.3. *Conclusion*

These results, as suggested [6–8,10] have revealed that this transaminase, when purified to homogeneity, had different properties from those of aspartate aminotransferase purified from rat brain [14]. pH optimum for rat brain AAT was 7.4 whereas it was 8.6 for CSA-T. At pH 7.4 no CSA-T activity was detectable. Thermic stability was also quite different for both enzymes. When rat liver transaminase (pH optimum 8.5) was purified by following the transamination of pyridoxamine with oxaloacetate [15]; it was found that this transaminase differed from rat liver AAT. It was concluded that the purified pyridoxamine oxaloacetic transaminase may be an apoenzyme of an unidentified transaminase which could be identical to CSA-T.

Other properties of CSA-T are now under investigation in order to find further differences between rat brain CSA-T and AAT and a possible identity with the purified pyridoxamine oxaloacetic transaminase.

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