Acta Chemica Scandinavica B 34 (1980) 363–367

Partial Purification of Cysteine Sulfinic Acid Decarboxylase from Calf Brain

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Cysteine sulfinic acid decarboxylase (EC 4.1.1.29) was purified from calf brain by pH precipitation, ammonium sulfate fractionation, gel filtration and DEAE-Sephadex A-50 chromatography. The enzyme preparation decarboxylated both cysteine sulfinic acid and cysteic acid at all steps of the purification and the ratio of the velocity of decarboxylation of cysteine sulfinic acid to that of cysteic acid was constant, about 2, throughout the purification procedure. The pH optimum was 7.2 both for cysteine sulfinic acid and cysteic acid. The molecular weight of the enzyme was estimated at 65 000 using gel filtration on a Sephadex G-200 column. Its K_m's were 0.9 mM for cysteine sulfinic acid and 1.6 mM for cysteic acid, with V_{max} values of 60.5 and 33.5 nmol/h(mg protein), respectively.

CSA decarboxylase* (EC 4.1.1.29) synthesizes taurine, a putative neurotransmitter substance, *via* hypotaurine by the decarboxylation of CSA. The same enzyme probably also decarboxylates cysteic acid, producing taurine directly. The pathway *via* hypotaurine is thought to represent the main biosynthetic route for taurine in animal tissue.[1-2] The distribution of CSA decarboxylase activity in synaptosomes is similar to that of taurine.[3] CSA decarboxylase activity in the brain has been found to be distributed between the soluble and particulate fractions, which have some different properties.[4-5] CSA decarboxylase has been partially purified and characterized from the liver of the rat,[6-8] ox and horse.[9] We describe here the partial purification of CSA decarboxylase from calf brain using as starting material those brain areas in which its activity is highest, namely the diencephalon, mesencephalon, cerebellar cortex and cerebellar white matter.[10] We also detail some properties of this brain CSA decarboxylase.

* CSA, cysteine sulfinic acid.

0302-4369/80/050363-05$02.50

RESULTS

Substrate specificity. The substrate specificity of the enzyme preparation was studied by determining the decarboxylase activity (Fig. 1) with two substrates, cysteine sulfinic acid and cysteic acid, during different stages of the purification (Table 1). Both substrates were decarboxylated at all stages, and the ratio of the velocity of decarboxylation of cysteine sulfinic acid to that of cysteic acid was constant at about 2 throughout the purification from the brain homogenate to the anionic exchange chromatography (Table 2, Fig. 2).

Molecular weight. The molecular weight of the brain CSA decarboxylase was estimated using a calibrated Sephadex G-200 column, the pooled

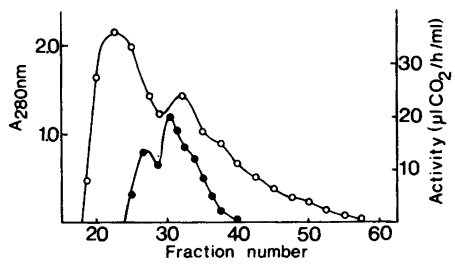

Fig. 1. Chromatogram of calf brain CSA decarboxylase on a Sephadex G-200 column (2.6 × 53 cm) in 0.05 M sodium phosphate buffer (0.1 mM PyrP, 1.0 mM AET, pH 7.2, flow rate 15 ml/h). Enzyme activity (●) and protein content (○) of the eluent fractions.

Table 1. Purification of the CSA decarboxylase from the calf brain.

Purification step	Volume/ ml	Total protein content/ mg	Total activity/ units	Specific activity/ units (mg protein)$^{-1}$	Purification coefficient	Recovery/ %
Homogenate	330	11200	207	0.018	1	100
Acid precipitation (supernatant)	200	1275	128	0.100	5.5	62
I $(NH_4)_2SO_4$ fractionation (30–65 % saturation)	6	475	116	0.244	13.3	56
Sephadex G-200 (pooled fraction)	26	105	75	0.714	38.8	36
II $(NH_4)_2SO_4$ fractionation (40–60 % saturation)	2	50	52	1.040	56.6	25
DEAE-Sephadex A-50	13	7	27	3.857	209.6	13

Sephadex G-200 enzyme then being re-chromatographed on an identical column. A plot of the elution volume of the five known proteins against the logarithm of their molecular weight is given in in Fig. 3. The molecular weight of the enzyme was 65 000. Since the standards used in the calibration were globular proteins, this molecular weight is based upon the assumption that CSA decarboxylase is itself a globular protein.

pH optimum. Optimal decarboxylation of cysteine sulfinic acid and cysteic acid by the purified enzyme was obtained at pH 7.2. The ammonium sulfate II enzyme was used for the pH estimation. The curves were parallel as shown in Fig. 4.

Optimal $(NH_4)_2SO_4$ concentration. The optimal $(NH_4)_2SO_4$ concentration for the precipitation of brain CSA decarboxylase was ascertained in preliminary experiments by fractionating the neutralized supernatant from pH precipitation stepwise between

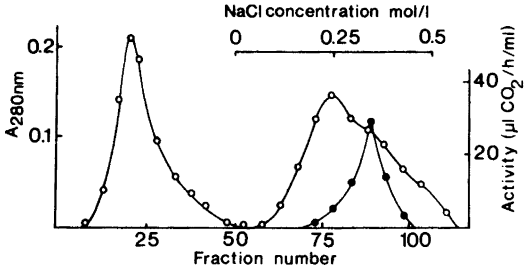

Fig. 2. Anion exchange chromatography of CSA decarboxylase on a DEAE-Sephadex A-50 column (1.6 × 32 cm) in 0.05 M sodium phosphate buffer (0.1 mM PyrP, 1.0 mM AET, pH 7.2, flow rate 25 ml/h) with a linear NaCl gradient from 0–0.5 M. Enzyme activity (●) and protein content (○) of the fractions collected.

Table 2. Ratio of the activity of CSA decarboxylase (CSAD) to that of cysteic acid decarboxylase (CAD) at different stages of the purification.

Purification procedure	CSAD-activity/ CAD-activity
Homogenate	1.7
pH precipitation	1.9
$(NH_4)_2SO_4$ fractionation	2.1
Sephadex G-200 (pooled fraction)	2.1
DEAE-Sephadex A-50 (most active fraction)	2.0

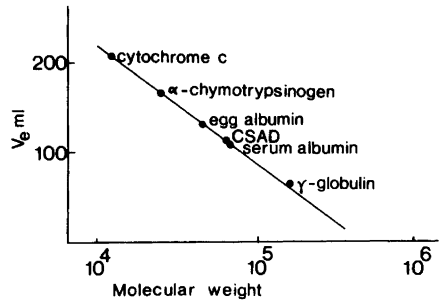

Fig. 3. Estimation of the molecular weight of brain CSA decarboxylase (CSAD) on a Sephadex G-200 column (2.6 × 53 cm). For details, see Materials and Methods.

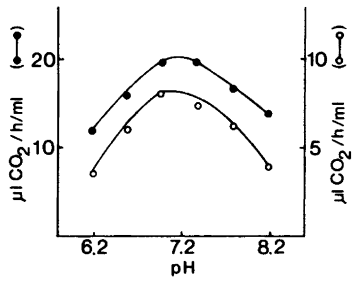

Fig. 4. pH-dependency of the partially purified decarboxylase. Enzyme sample from the second ammonium sulfate fraction in 0.2 M sodium phosphate buffer (pH 6.2–8.2) in the presence of protectors, 0.1 mM PyrP and 1.0 mM AET. Cysteine sulfinic acid (●) and cysteic acid (○) as substrates.

ammonium sulfate concentration of 25 % and 65 %. The optimum lay between 40 and 60 % $(NH_4)_2SO_4$ saturation. Cysteine sulfinic acid and cysteic acid decarboxylase activities were also parallel in the ammonium sulfate fractionation, as shown in Fig. 5.

Kinetic parameters. An enzyme sample for the determination of K_m was taken from the second ammonium sulfate fraction. The reciprocal plot of Lineweaver and Burk showed the K_m for cysteine sulfinic acid to be 0.9 mM and that for cysteic acid 1.6 mM. The V_{max} values were 60.5 and 33.5 nmol/h(mg protein), respectively (Fig. 6).

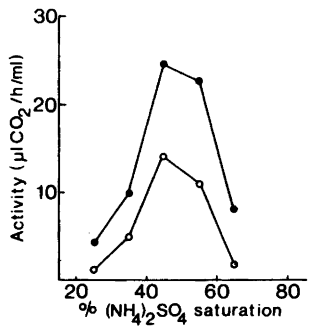

Fig. 5. Precipitation of the brain decarboxylase at various ammonium sulfate concentrations. Fractionation began from the neutralized supernatant after the pH precipitation. The pellets from the different $(NH_4)_2SO_4$ saturation were dissolved individually in 5 ml of 0.2 M sodium phosphate buffer (0.1 mM PyrP, 1.0 mM AET, pH 7.2). Enzyme activities were determined with cysteine sulfinic acid (●) and cysteic acid (○) as substrates.

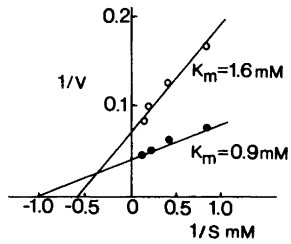

Fig. 6. Lineweaver-Burk plot of the decarboxylation of cysteine sulfinic acid (●) and cysteic acid (○), using partially purified enzyme from the second ammonium sulfate fraction. The substrate concentration varied from 1.1 to 9.4 mM in 0.2 M sodium phosphate buffer (0.1 mM PyrP, 1.0 mM AET, pH 7.2).

DISCUSSION

Purification by conventional methods produced a partially purified CSA decarboxylase preparation. The increase in the specific activity was 210-fold and the yield of the total activity about 13 % of that in the brain homogenate. Selection of the starting material from the most active brain areas can be regarded as a good initial concentration procedure. The purified decarboxylase is probably the soluble form of CSA decarboxylase, as only 60 % of the activity of the homogenate was observed in the supernatant fraction, although the particle bound form of the enzyme may also be present with the soluble form to some extent as a relatively dilute buffer was used in the homogenization of the brain tissue.[5] Heterogeneity of the preparation is shown especially in the Sephadex G-200 elution profile.

The fact that enzyme preparation decarboxylated both cysteine sulfinic acid and cysteic acid at all steps of the purification, and that the ratio of the decarboxylation velocities of these substrates was constant throughout the purification, suggests that both reactions are catalyzed by the same enzyme.[7,15] Cysteine sulfinic acid decarboxylase activity and cysteic acid decarboxylase activity were also seen to be parallel both when estimating the pH optimum and during ammonium sulfate fractionation. This uniformity supports the same hypothesis. It is interesting to observe that the ratio of the decarboxylation rates in favour of cysteine sulfinic acid is only 2 (present results and Refs. 1, 16, 17) whereas in liver it is much greater, 5–10 depending on species.[7,9,18] This reflects the molecular

difference between brain and liver enzymes and also indicates that the decarboxylation of cysteic acid may be of physiological significance in the biosynthesis of taurine in the brain.

The elution volume of the most active peak in the gel filtration on a Sephadex G-200 column corresponds to a molecular weight of 65 000. No molecular weight value has previously been reported for brain CSA decarboxylase. The pH optimum and kinetic parameters for the purified enzyme are in agreement with earlier results.[19]

EXPERIMENTAL

Animals. Fresh brains of male and female calves of 7–9 months of age were obtained from the local slaughterhouse. The material for the purification of CSA decarboxylase was taken from those brain areas in which the enzyme activity is highest.[10]

Substrates and co-factors. L-Cysteine sulfinic acid and 2-aminoethylisothiouronium bromide (AET) were obtained from Calbiochem (Los Angeles, CA, U.S.A.) and L-cysteic acid and pyridoxal-5′-phosphate (PyrP) from Fluka (Switzerland).

Enzyme assay. The decarboxylase activity was determined in a Warburg apparatus (Braun, Modell V 85). Krebs's manometric fluid containing 4.4 g of anhydrous NaBr, 30 mg of Triton X-100 and 30 mg of Evans Blue in 100 ml of H_2O was used for manometry.[11]

The main compartment of the Warburg flask contained 0.2 mM pyridoxal-5′-phosphate, 1.0 mM AET and 0.4–1.5 ml of suitably diluted enzyme solution in 0.2 M sodium phosphate buffer (7.2). The final volume in the flask was 3.2 ml. The sample was gassed with N_2 for 10 min before incubation at 37 °C. The reaction was started by tipping 0.2 ml of 0.15 M substrate solution from the side arm into the main compartment. The evolution of CO_2 was measured by taking readings every 10 min up to 1 h. A control flask without the enzyme preparation was also used.

Enzyme activity is expressed in μl CO_2/h or units of enzyme defined as the amount of enzyme required to produce 1 μl CO_2/min under assay conditions.

AET used in the assay system is a good stabilizer for CSA decarboxylase. This was ascertained in preliminary experiments.

Protein determinations. Protein concentration for the calculation of the specific activity of different stages of the purification were determined by the method of Lowry[12] with bovine serum albumin as the standard. The protein concentrations of the fractions in column chromatography were estimated by measuring absorbance at 280 nm.

Buffer. The buffer was the same at all steps of the purification: 0.05 M Na_2HPO_4/NaH_2PO_4 (pH 7.2) containing 1 mM 2-aminoethylisothiouronium bromide (AET) and 0.1 mM pyridoxal-5′-phosphate (PyrP).

Purification procedure. The dissected material (100 g) from the brain areas was homogenized in a Waring blender for 2 min in 3.5 vol. of 0.05 M sodium phosphate buffer, pH 7.2, containing 1.0 mM AET and 0.1 mM PyrP. The homogenate was centrifuged at 8000 *g* (Sorval RC-5) for 30 min. The pellet was then discarded and the supernatant acidified to pH 5.5 with 1 N HCl. The suspension was centrifuged at 12 000 *g* for 30 min. The precipitate was discarded and the supernatant neutralized to pH 7.2 with 0.5 M NH_4OH.

The next step was the first ammonium sulfate fractionation. Solid ammonium sulfate was slowly stirred into the neutralized supernatant. The pellet precipitated at $(NH_4)_2SO_4$ saturation of 30–65 % was centrifuged at 12 000 *g* for 20 min and dissolved in 6 ml of the above sodium phosphate buffer.

The active solution from the ammonium sulfate fractionation was applied to a Sephadex G-200 column (Pharmacia), 2.6 × 53 cm, and eluted with the sodium phosphate buffer. Fractions of 4 ml were collected, and those containing most of the enzyme activity were pooled for further purification (Fig. 1).

This was followed by a second ammonium sulfate fractionation. The Sephadex G-200 enzyme pool was precipitated by stirring solid $(NH_4)_2SO_4$ into the solution at 4 °C. The fraction precipitated at 40–60 % ammonium sulfate saturation was collected and the pellet dissolved in the sodium phosphate buffer and dialyzed for 20 h against 5 l of the same buffer.

The dialyzed active fraction was then applied to a DEAE-Sephadex A-50 column (Pharmacia), 1.6 × 35 cm, equilibrated with the above sodium phosphate buffer, containing protectors as in the former steps. The column was eluted first with 130 ml of the equilibrating buffer, and then with the same solution, but with a linear increase in NaCl concentration from zero up to 0.5 M in 190 ml of the elution buffer. The CSA decarboxylase eluted at an NaCl concentration of 0.4 M (Fig. 2). The results of the purification are summarized in Table 1.

Column chromatography. Sephadex G-200 and DEAE-Sephadex A-50 dextrans were pre-equilibrated with phosphate buffer according to the manufacturer's instructions (Pharmacia, Sweden). Pharmacia Columns K 26/70 and K 16/40 were used.

Molecular weight determination. The molecular weight of the calf brain CSA decarboxylase was estimated by gel filtration on a Sephadex G-200 column according to the technique of Andrews.[13] The standard proteins used were γ-globulin (MW

160 000), bovine serum albumin (MW 68 000), egg albumin (MW 45 000), α-chymotrypsinogen (MW 25 000) and cytochrome *c* (MW 12 400). Five mg of each protein were applied to the column (2.6 × 53 cm) in 2 ml of the elution buffer (0.05 M sodium phosphate, pH 7.2, 1 mM AET, 0.1 mM PyrP).

Polyacrylamide gel electrophoresis. Disc electrophoresis of the purified decarboxylase was performed with Shandon's apparatus according to Smith,[14] using 7.5 % (w/v) polyacrylamide gels in 0.3 M Tris-HCl buffer, pH 8.9. The gels were stained with 1 % (w/v) Amido Black 10 B in 0.9 M acetic acid.

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Received January 9, 1980.