J. Biochem. Mol. Biol. Vol. 29, No. 4 pp. 335~342 July 31, 1996

Studies on the Purification and Partial Characterization of Cysteinesulfinic Acid Decarboxylase from Porcine Liver

Hongmie Lee Jin and Evan E. Jones*

Department of Animal Science and Biochemistry, North Carolina State University, Raleigh, North Carolina 27695-7621, USA

(Received March 5, 1996)

Abstract: Porcine liver cysteinesulfinic acid decarboxylase was purified approximately 460-fold by means of ammonium sulfate fractionation and sequential column chromatographic separation with Sephadex G-100, DEAE-cellulose and hydroxylapatite. The enzyme has a flat pH profile with maximum activity occurring between pH 6.0 and 7.6. Pyridoxal 5'-phosphate must be present in all buffers used for purification procedures in order to stabilize the enzyme. Addition of sulfhydryl reagents such as 2-mercaptoethanol are also necessary to maintain maximum enzyme activity throughout purification. The absorption spectrum shows that cysteinesulfinic acid decarboxylase is a pyridoxal 5'-phosphate-containing protein. The major absorption is at 280 nm with two smaller absorption regions, one at 425 nm which is ascribed to a Schiff's base between pyridoxal phosphate and protein, and another at 325 nm which is thought to be due to the interaction of 2-mercaptoethanol with the Schiff's base. A number of divalent cations tested did not affect enzyme activity with the exception of mercury, copper, and zinc which are inhibitory. The partially purified enzyme has an apparent K_m of 0.94 mM for cysteinesulfinate. Cysteic acid is a competitive inhibitor of the enzyme with a K_i of 1.32 mM. The molecular weight of the enzyme was estimated to be about 79,600 by using Sephadex G-200 column chromatography.

Key words: cysteinesulfinic acid, liver, porcine, taurine.

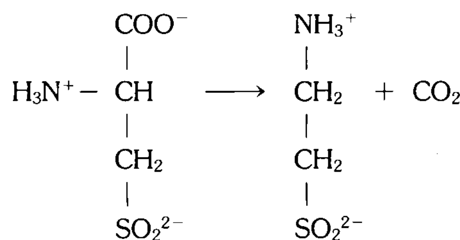
Taurine is a phylogenetically ancient compound, isolated more than 150 years ago from ox bile (Demarcay, 1838), and has a disjunctive distribution in the biosphere. It is present in high concentrations in algae and in the animal kingdom, including insects and arthropods, but it is generally absent or present only in trace amounts in the bacterial and plant kingdoms. In many animals, including mammals, it is one of the most common free-amino acids found in various tissues (Jacobsen and Smith, 1968).

Hayes *et al.* (1975) were among the first to demonstrate the effects of dietary taurine deficiency in cats. Taurine depleted cats show retinal degeneration and visual function changes such as reduced visual potential and poor visual acuity. It is now widely accepted that taurine is an essential nutrient for cats. Additional research by Hayes *et al.* (1980) showed a decrease in taurine concentration in most tissues of infant monkeys and a significant growth depression when those animals were fed a taurine-free, soy protein infant formula. In view of the effect of taurine deficiency on infant monkeys, there has been considerable interest in the role of taurine in human infant formulas.

*To whom correspondence should be addressed.
Tel: 1-919-515-4045, Fax: 1-919-515-7780.

The pig has been used as an animal model for human protein metabolism (Garlick *et al.*, 1976) and has recently been the central topic of discussion for research relating to human medicine (Tumbleson and Schook, 1995). Taurine supplementation of milk replacers for early weaned piglets led to a significant increase in plasma taurine, but no differences were observed for taurine concentrations in tissues such as eye, liver, and heart which suggests that the pig is capable of synthesizing taurine at an early stage of life (Stephen *et al.*, 1991).

The major route of taurine biosynthesis in mammalian liver involves the formation and decarboxylation of cysteinesulfinic acid by cysteine dioxygenase and cysteinesulfinic acid decarboxylase (CSAD, EC. 4.1.1.29), respectively. The reaction shown below is catalyzed by CSAD, and has been considered to have a rate-limiting role in the taurine biosynthetic pathway (Sturman, 1986; Stephen *et al.*, 1991).



It has been suggested that the species difference in hepatic CSAD activity accounts for the observed difference in taurine synthetic capacity among various species (De La Rosa and Stipanuk, 1985; Worden and Stipanuk, 1985; Chapman and Greenwood, 1988; Hayes, 1988). CSAD has been purified and studied extensively in rat liver (Lin *et al.*, 1971; Guion-Rain *et al.*, 1975; Weinstein and Griffith, 1987; Remy *et al.*, 1990) and bovine brain (Wu, 1982) but there has been no study reporting this enzyme in porcine liver. The purpose of the present study was to purify CSAD from porcine liver and to investigate some of the physical and chemical parameters of the enzyme and to compare some of these properties with the same enzyme isolated from other sources.

Materials and Methods

Compounds and reagents

L-[1-^{14}C]-Cysteinesulfinic acid was purchased from Research Products Int. (Mount Prospect, USA). Pyridoxal 5′-phosphate, *L*-cysteinesulfinic acid, *L*-cysteic acid, 5,5′-dithio-bis(2-nitrobenzoic acid), and N-ethylmaleimide were from Sigma Chemical Company (St. Louis, USA). Sephadex G-100 and G-200 were obtained from Pharmacia Fine Chemicals (Piscataway, USA). DEAE-cellulose was purchased from Bio-Rad Laboratories (Richmond, USA). All other chemicals were reagent grade.

Assay of enzymatic activity

Standard assay conditions were those modified from the method described by Daniels and Stipanuk (1982). The incubation was carried out in a total volume of 1.5 ml in a 25-ml Erlenmeyer flask. The reaction mixture contained12 mM *L*-[1-^{14}C]-cysteinesulfinic acid (8 nCi), 0.5 mM DTT, 250 mM potassium phosphate buffer, pH 7.1 and enzyme protein up to 15 mg protein or not more than 80 U of enzyme activity. All components of the reaction mixture were added to the flask while being maintained at 4℃. A plastic center well was attached to a rubber septum and the flask was sealed. The center well contained 0.3 ml of a 1 : 2 mixture of 2-ethanolamine and ethylene glycol monomethyl ether and a piece of fluted-Whatman No. 1 filter paper. The reaction was initiated by transferring the flasks to a 37℃ shaking water bath and allowed to proceed for 30 min. Each reaction was stopped by injecting 1.0 ml of 10% trichloroacetic acid through the septum stopper into the reaction mixture. The flask was left in the shaking water bath for an additional 60 min to allow complete absorption of radio active carbon dioxide by the filter paper. The center well and its contents were removed and immediately placed in a scintillation vial along with 5 ml of Insta-gel counting solution. The radioactivity was determined with a Packard Tricarb Liquid Scintillation Spectrometer.

Enzyme unit

One unit of enzyme activity represents the amount of enzyme that produces 1 nmol of carbon dioxide per minute at 37℃.

Protein determination

The method of Lowry *et al.*, as modified by Zak and Cohen (1961) was used to determine the concentration of the protein in the homogenate supernatant solution and the dialyzed ammonium sulfate fraction using bovine serum albumin as a standard. The concentration of the more purified enzyme protein was determined by the equation of Layne (1957) using a Gilford Spectrophotometer.

Molecular weight determination

Gel filtration was used in molecular weight estimation according to the procedures described by Andrews (1964). A column (2.5×47 cm) of Sephadex G-200 was prepared by the reverse flow of 20 mM potassium phosphate buffer, pH 7.1, containing 0.1 M potassium chloride, 1.0 mM 2-ME, and 0.1 mM EDTA (elution buffer). Five molecular weight standards were passed through the column before and after the exclusion volume of CSAD was determined to establish a linear relationship between log molecular weight *vs.* elution volume. All standard proteins were dissolved and applied in 2 ml of elution buffer. The standard proteins used were horse heart cytochrome c (M_r 12,400; 0.5 mg), chymotrypsinogen (M_r 25,000; 0.5 mg), bovine serum albumin (M_r 67,000; 2 mg), bovine liver catalase (M_r 232,000; 3mg) and urease (M_r 490,000; 3 mg).

After concentration and dialysis against eluant buffer, 2 ml of the purified enzyme solution containing 3 mg of protein was applied to the bottom of the column followed by 2 ml of 10% sucrose solution. The flow rate was adjusted and maintained at 12 ml/h, and 2 ml fractions were collected. The appearance of standard proteins except cytochrome c in the effluent was determined by absorbance at 280 nm. Cytochrome c was detected by absorbance at 412 nm. CSAD was determined by enzymatic assay.

Results and Discussion

Purification of cysteinesulfinic acid decarboxylase crude extract

Fresh porcine liver was obtained from a local abbo-

Table 1. Purification of cysteinesulfinic acid decarboxylase from porcine liver

	Fraction	Total activity	Total protein (mg)	Specific acitivity (U/mg)	Yield (%)	Purifcation
I.	Crude homogenate	20,700	39,740	0.52	100	1
II.	10,800×g	19,400	19,200	1.01	94	2
III.	$(NH_4)_2SO_4$	13,600	3,930	3.46	66	7
IV.	Sephadex G-100	7,800	219	36.3	38	70
V.	DEAE-cellulose	2,300	14	167	11	321
VI.	Hydroxyapatite	13	0.06	238	0.7	460

toir and placed on ice at 4℃ for transport to the laboratory. The fresh liver was divided into 250 g portions and stored frozen at −20℃ until used for enzyme purification. Liver tissue could remain frozen for two months without affecting enzyme purification or loss of enzyme activity.

A 250 g portion of liver was diced finely and the tissue was disrupted in an Omnimixer for one minute with a 3-fold volume (V/W) of 20 mM potassium phosphate buffer, pH 7.1, containing 1.0 mM 2-mercaptoethanol, 0.1 mM EDTA and 0.1 mM pyridoxal 5'-phosphate. All phosphate buffers (standard buffers) used throughout purification contained 1.0 mM 2-mercaptoethanol, 0.1 mM EDTA and 0.1 mM pyridoxal 5'-phosphate. The homogenate was filtered through four layers of cheesecloth and the filtrate was further homogenized in a Dounce tissue homogenizer with two strokes of both the loose and tight pestles. The homogenate (Fraction I, Table 1) was centrifuged at 10,800×g for 40 min in a Sorvall RC-5B refrigerated centrifuge. The supernatant solution (Fraction II, Table 1) was collected by decantation for further purification procedures. All purification procedures were performed at 4℃.

Ammonium sulfate fractionation

Fraction II was diluted with 20 mM standard buffer so as to provide a solution containing 15 mg protein per ml. Finely ground ammonium sulfate was slowly added, with gently stirring, to 40% saturation (saturation at 4℃ is 720 mg/ml). This ammonium sulfate solution was allowed to stir for 20 min and the protein precipitate was removed by centrifugation at 10,800×g for 20 min. The supernatant solution was decanted and the volume determined. Additional ammonium sulfate was slowly added to give a final concentration equal to 55% saturation. During the addition of ammonium sulfate, the pH was closely monitored and maintained at 7.1 by the addition of 1.0 N ammonium hydroxide. The 55% saturated solution was allowed to stir 20 min and then the precipitated protein was collected by centrifugation at 10,800×g for 20 min. The supernatant solution was decanted and the protein pellet was dissolved in a minimum amount of 20 mM standard buffer and dialyzed overnight against two changes of 20-fold excess standard buffer (Fraction III, Table 1).

Fractionation on Sephadex G-100

Fraction III was diluted to a protein concentration of 15 mg/ml and a 65-ml portion (4% of total bed volume) was applied to the bottom of a Sephadex G-100 column (5.2×80 cm) which had been pre-equilibrated with 20 mM standard buffer. The protein was eluted with 20 mM standard buffer at a flow rate of 60 ml/h and fractions of 8 ml were collected. Those protein fractions containing enzyme activity from four separate Sephadex G-100 columns were combined, concentrated with an XM-50 membrane and dialyzed against 2 changes of 20 mM standard buffer (Fraction IV, Table 1).

Chromatography on DEAE-cellulose

DEAE-cellulose was washed and fined according to the procedure described by Himmelhoch (1971) and equilibrated with 50 mM standard buffer before packing into a column (2.5×20 cm). Fraction IV was applied to the top of the DEAE-cellulose column, and that protein which was not absorbed was washed free of the column with 50 mM standard buffer. Enzyme protein was eluted from the column by using a linear gradient consisting of 100 ml each of 50 mM standard buffer and 50 mM standard buffer containing 300 mM potassium chloride. Fractions of 5 ml each were collected at a flow rate of 15 ml per h. Enzyme activity was eluted from the column at a potassium chloride concentration of approximately 100 mM. Those fractions which contained enzyme activity were combined and dialyzed against 10 mM potassium phosphate buffer, pH 7.3, containing 2-ME and pyridoxal phosphate (Hydroxylapatite application buffer).

Hydroxylapatite column chromatography

A column (1.0×14 cm) was packed with hydroxylapatite and washed with application buffer. Enzyme from

Fraction V was applied to the top of the hydroxyl apatite column and the column was washed with 40 ml of application buffer. Enzyme activity as well as other proteins were washed free of the column with a linear gradient made of 40 ml of 10 mM application buffer and 40 ml of 300 mM potassium phosphate buffer, pH 7.3, containing 1 mM 2-ME and 0.1 mM pyridoxal 5′-phosphate. Less than 5% of the enzyme was removed with the application buffer alone. The majority of the enzyme activity was removed by the early stage of linear gradient application. The enzyme activity was well resolved of the bulk of the protein eluting from the column with an increase of apporximately 1.5 fold in the specific activity (Fraction VI, Table 1). Even though this step in purification removed considerable protein from the enzyme activity, there was a loss of about 99% of the total enzyme units applied to this column. Attempts to restore enzyme activity by dialysis in buffers containing pyridoxal 5′-phosphate or reducing agents were not successful; therefore, those fractions obtained from DEAE-cellulose chromatography in Fraction V were dialyzed against 20 mM standard buffer and sterilized by filtering through a 0.22 micron nylon filter and stored at 4℃ for use in characterization studies.

Properties of porcine cysteinesulfinic acid decarboxylase

Stability--Ammonium sulfate fractionated enzyme was stable for at least 6 weeks with little or no loss of activity when stored at −20℃. Enzyme which had been purified with Sephadex G-100 or DEAE-cellulose chromatography could not be stored at −20℃. The enzyme lost 30% of the activity after freezing for 10 days and was totally inactive after 6 weeks of storage in the frozen state. Greatest stability was achieved by filtering the DEAE-cellulose fraction with a 0.22 μm nylon filter and storing the filtrate at 4℃. In this condition, the enzyme was stable with less than 15% loss of activity over a 6 week period.

Effect of enzyme concentration and time

The enzyme preparation obtained from Fraction V, Table 1 was used to demonstrate that the enzyme activity was linear with respect to time. The reaction mixtures containing 8 mg of purified enzyme showed that the decarboxylation of cysteine sulfinate was linear for reaction times up to 45 min. The relationship between the amount of enzyme protein and enzyme activity showed that the reactivity was linear up to 16 mg of purified enzyme per reaction mixture and an incubation time of 30 min. Based upon these data, a standard reaction time of 30 min was established for subsequent characterization studies.

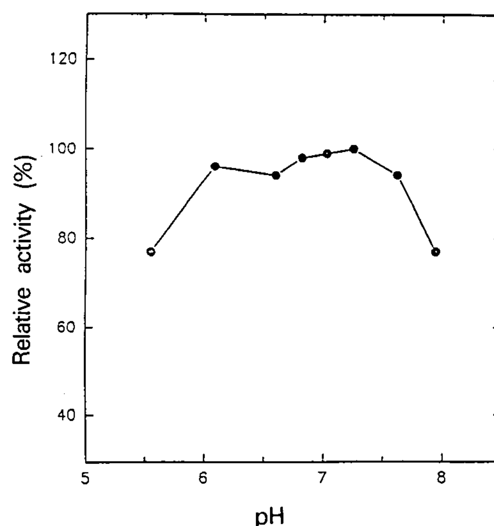


Fig. 1. The effect of pH on porcine liver cysteinesulfinic acid decarboxylase activity. Each reaction contained 40 μg of DEAE-cellulose column purified enzyme protein and the pH was determined at the end of the reaction period. All buffers used were 20 mM and contained potassium phosphate as the standard buffer reagent.

Effect of pH on reaction velocity

The porcine liver cysteine-sulfinic acid decarboxylase had a broad pH profile (Fig. 1). The highest level of activity was present in a reaction mixture with a pH of 7.2, but the activity between pH 6.0~7.2 and pH 7.2~7.6 was nearly 95% of the highest activity at pH 7.2. At the extremes of pH at 5.6 and 8.0, the relative activity remained at 75~80%. Thus, the porcine enzyme retains considerable activity over a range of 2.5 pH units.

Pyridoxal 5′-phosphate requirement

Pyridoxal 5′-phosphate was required to be added to all buffers used throughout the purification procedures in order to maintain stability of the enzyme. Removal of pyridoxal 5′-phosphate from the porcine liver CSAD solutions by dialysis decreased enzyme activity by 30~40% within 12 h. Moreover, if the purified enzyme was stored in pyridoxal 5′-phosphate-free buffer, pH 7.1, for 48 h, the specific activity decreased greater than three fold and activity could not be restored by addition of pyridoxal 5′-phosphate. These data suggest that pyridoxal 5′-phosphate is not covalently bound to the porcine decarboxylase. The tertiary structure of the enzyme appears to be maintained by the presence of pyridoxal 5′-phosphate and undergoes an irreversible conformational change upon removal of the cofactor. It is possible that a change of pH to a lower or higher extreme would affect the tertiary structure enough to

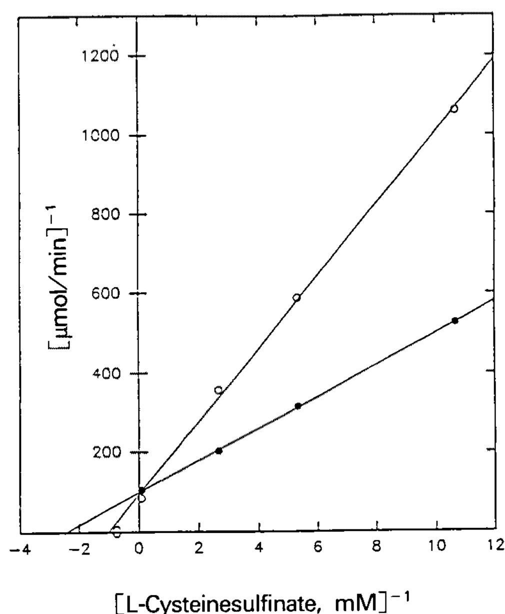


Fig. 2. Double reciprocal plot of cysteine sulfinate and cysteic acid concentration *vs.* activity of porcine liver cysteinesulfinic acid decarboxylase. Closed circles, L-cysteinesulfinic acid alone. Open circles, L-cysteinesulfinate plus 1.0 mM L-cysteic acid. Each reaction contained 50 μg of DEAE-cellulose purified enzyme protein. The reactions conditions were standard except for the variation of substrate concentration.

allow pyridoxal 5′-phosphate to recombine at the catalytically active center and remain bound as the enzyme was gradually returned to the pH of highest activity.

Reaction velocity and substrate concentration

Kinetic determinations were carried out with saturating levels of pyridoxal 5′-phosphate using four levels of substrate ranging in concentration from 0.1 to 10 times the estimated K_m values. Doublereciprocal plots of 1/v *versus* 1/S were prepared using linear regression analysis. An apparent K_m value of 0.94 mM was calculated for the substrate cysteine sulfinate (Fig. 2). Cysteic acid was shown to be a competitive inhibitor of cysteinesulfinic acid decarboxylase. Kinetic measurements in the presence of four levels of cysteic acid showed that this competitive inhibitor has a K_i of 1.32 mM (Fig. 2).

Sulfhydryl reagent requirement

Purified cysteinesulfinic acid decarboxylase appears to have a requirement for a reduced sulfhydryl reagent for maximal enzyme activity. As shown in Table 2, enzyme which had been dialyzed in buffers devoid of a sulfhydryl reducing agent lost enzyme activity very quickly. Greater than 60% of the activity was lost after 3 h of dialysis in 2-ME or DTT-free buffers. The activity could be restored, however, by the addition of DTT to the reaction mixture. The restoration of enzyme activity was linear with increasing concentrations of DTT up to 0.5 mM. Addition of 0.5 mM DTT to the reaction mixture resulted in instantaneous restoration of maximum activity of the porcine liver enzyme. These data are suggestive of the involvement of a cysteine residue at the catalytically active center which is involved with the mechanism of the pyridoxal 5′-phosphate-catalyzed decarboxylation reaction.

Table 2. Restoration of porcine cysteinesulfinic acid decarboxylase by dithiothreitol. Enzyme derived from the DEAE-cellulose fraction of Table 1 was dialyzed for 3 h against buffer which did not contain 2-ME or DTT. Enzyme activity was determined immediately following dialysis, and each reaction mixture contained 0.026 mg of enzyme protein. DTT was added to different reaction mixtures at the beginning of the reaction time and incubations was carried out for 30 min

Concentration of Dithiothreitol (M)	Decarboxylation of cysteinesulfinate (% of initial activity)
0	38
2×10^{-5}	59
10×10^{-5}	72
50×10^{-5}	100

Table 3. The effect of sulfhydryl reagents on porcine liver cysteinesulfinic acid decarboxylase activity. Each reaction mixture contained 0.026 mg of enzyme from the DEAE-cellulose fraction (Table 1) which had beeen dialyzed for 3 h against two changes of 100-fold excess buffer without 2-ME or DTT. The concentration of sulfhydryl reagents in the complete reaction mixture was 0.2 mM in 0.1 M Tris-HCl buffer pH 7.6

Reagent	Percent inhibition
N-ethylmaleimide	80
5,5′-Dithio-bis-(2-nitrobenzoate)	89
p-Hydroxymercuribenzoate	97
Iodoacetamide	78
Iodoacetic acid	17

Several thiol group reagents were also shown to inhibit porcine liver CSAD to varing degrees; as a mercaptide-forming mercurial, *p*-hydroxymercuribenzoate was most inhibitory of all the reagents tested (Table 3). Iodoacetic acid and iodoacetamide as alkylating agents and N-ethylmaleimide as a stable thioether forming agent showed lesser inhibition, namely, 17 &, 78%, and 80% respectively. These results are consistent with earlier observations that CSAD from other species requires a free sulfhydryl group for maximal activity (Davison, 1956; Jacobson *et al.*, 1964; Lin *et al.*, 1971).

Effect of divalent cations

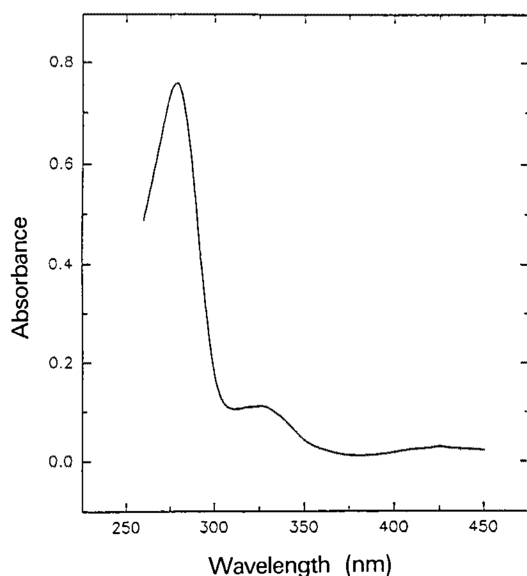


Fig. 3. The absorption spectrum of porcine liver cysteinesulfinic acid decarboxylase. Enzyme protein (0.8 mg/ml) obtained from DEAE-cellulose column chromatography was dissolved in standard buffer, pH 7.1, containing 1.0 mM 2-ME, 0.1 mM EDTA and 0.1 mM pyridoxal 5'-phosphate. The spectrum was determined witha Cary 15 Dual-Beam Spectrophotometer.

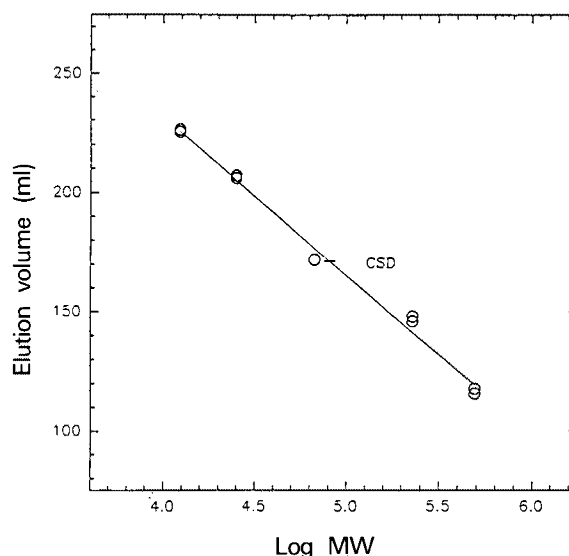


Fig. 4. Molecular weight determination of porcine liver cysteinesulfinic acid decarboxylase by size exclusion chromatography with Sephadex G-200. DEAE-cellulose purified CSAD was applied at the bottom of a 1.0×47 cm column in a 2 ml volume, containing 3 mg of total protein, followed by 2 ml of 10% sucrose solution in elution buffer. Proteins of known molecular weight (see methods) were applied prior to and after chromatography of CSAD in order to assure the same exclusion properties prior to, during, and after molecular weight determination.

All cations tested were chloride form except iron and zinc which were sulfate. Parallel incubations were made to show that sulfate and chloride anions exerted no effect on the enzyme activity. EDTA and was removed by dialysis so as not to interact with divalent cation additions. At a final concentration of 0.2 mM in the reaction mixture, divalent cations of mercury, copper, and zinc inhibited the porcine liver CSAD 100%, 60%, and 22%, respectively. Other divalent cations tested, calcium, cadmium, iron, magnesium, manganese, and nickel did not affect enzyme activity.

Absorption spectrum

The absorption spectrum of the porcine liver CSAD is shown in Fig. 3. The enzyme preparation obtained from DEAE-cellulose chromatography was concentrated by ultrafiltration in an Amicon Diaflo with an XM-50 membrane and dialyzed against 20 mM potassium phosphate buffer, pH 7.1, containing 1.0 mM 2-ME, 0.1 mM EDTA and 0.1 mM pyridoxal 5'-phosphate. The major absorption band was at 280 nm due to protein aromatic aminoacid residues. Two other regions of lesser intensity were present at 325 nm and 425 nm. The absorption band at 425 nm is ascribed to a phosphopyridoxal aldimine group and that at 325 is likely due to the association of 2-ME with the Schiff's base. Similar absorption profiles have been reported for another CSAD from rat liver (Guion-Rain *et al.*, 1975) and alanine aminotransferase (Saier, 1967).

Molecular weight determination

Gel filtration of the purified porcine liver CSAD on a Sephadex G-100 column indicated the molecular weight of the enzyme to be about 79,600 (Fig. 4). This is within the range (60,000~83,000) reported for the rat liver enzyme as determined by other gel filtration methods (Lin *et al.*, 1971; Guion-Rain *et al.*, 1972; Weinstein and Griffith, 1987; Remy *et al.*, 1990). For the rat liver CSAD, the molecular weight determined by gel filtration was different from that determined by gel electrophoresis or analytical centrifugation (60,000~83,000 *vs.* 100,000; Guion-Rain *et al.*, 1975; Weinstein and Griffith, 1987). The rat liver enzyme has been reported by several researchers to be a dimer of M_r 53~55,000 subunits (Guion-Rain *et al.*, 1975; Weinstein and Griffith, 1987). No evidence of dimerization of the porcine liver enzyme has been observed in the buffer conditions used for molecular weight determination which included mercaptoethanol.

The purification procedure presented has provided a preparation of porine liver CSAD that has been purified about 460 fold. Attempts to use a heat treatment or purification by acid precipitation were not successful because of low increases in specificactivity and marked decreases in total yield of enzyme activity. Both heat and low pH most likely resulted in conformation changes and loss of pyridoxal 5'-phosphate from the enzyme and

neither could be restored sufficiently to warrant use of these procedures for enzyme purification.

It is also interesting to note that hydroxylapatite column chromatography purified bovine brain CSAD approximately 6 fold with a 22% yield when this purifica tion step was incorporated between Sephadex G-100 and DEAE-cellulose chromatography (Wu, 1982). In contrast, hydroxylapatite was not useful for the purificaiton of porcine CSAD. This step only provided about a 1.5 fold increase in specific activity but about 99% loss of total enzyme units; thus, enzyme obtained from the previous step of the purification procedure, DEAE-cellulose chromatography, was used for the characterization studies discussed herein.

The assay conditions used in this study were a modification of the method described by Daniels and Stipanuk (1982). The major modification was the change of the pyridoxal 5'-phosphate concentration in the assay buffer mixture from 0.75 mM to 0.1 mM. High levels of pyridoxal 5'-phosphate always resulted in nonenzymatic liberation of radioactive CO_2, from the substrate. Another modification was the decrease in the substrate concentration from 30 mM to 12 mM. By removing excess pyridoxal 5'-phosphate and decreasing the radioactive substrate concentration, the assay method resulted in an acceptable low background in the absence of enzyme and allowed the establishment of a linear relationship between enzyme concentration and product formed as well as the linearity with respect to time of reaction.

The porcine liver CSAD has a K_m for cysteinesulfinate of 0.94 mM. This is about 5 times higher than the K_m for rat liver as reported by Jacobson *et al.* (1964), Lin *et al.* (1972) and Guion-Rain *et al.* (1975), but it is about half the value of 2.0 mM reported for rat brain (Jacobson *et al.*, 1964).

There are some conflicting reports concerning the decarboxylation of cysteic acid by CSAD from rat liver as well as porcine liver. The double reciprocal plot of Fig. 2 shows that cysteic acid is a competitive inhibitor of the porcine liver CSAD with a K_i of 1.32 mM which is not too different from the K_m of cysteine-sulfinate. Although decarboxylation of cysteic acid was not measured in the present study, it would appear that both cysteic acid and cysteinesulfinate can serve as substrates for the porcine liver CSAD. Lin *et al.* (1971) reported that rat liver CSAD did not catalyze the decarboxylation of cysteic acid while several other investigators (Blaschko and Hope, 1954; Jacobsen *et al.*, 1964; Guion-Rain *et al.*, 1975) observed that the rat liver CSAD used cysteic acid as well as cysteinesulfinic acid as a substrate. The physiological role of cysteic acid is not clear. In view of the low concentration of cysteic acid in tissues or physiological fluids, this amino acid would not likely have a major impact upon the activity of CSAD with cysteinesulfinate as the substrate for synthesis of taurine.

One of the more interesting aspects of the enzyme mechanism of porcine liver CSAD involves the apparent requirement for the presence of reducing agents such as 2-ME or DTT. Attempts to remove the sulfhydryl reducing reagents by dialysis results in about 60% loss of enzyme activity. Restoration of activity can be achieved by addition of sulfhydryl reducing agents to the enzyme-buffer solution, and the restoration of enzyme activity appears to be reducing agent concentration dependent. The relationship between reducing agent concentration and enzyme activity suggests that there is a cysteine residue which participates in the decarboxylation reaction mechanism and that the cysteine is very succeptible to oxidation. The absorption spectrum which shows a band in the area of 325 nm is suggestive of the participation of a cysteine residue in the stabilization of the presumed Schiff base formed between pyridoxal 5'-phosphate and a probable lysine residue at the active center. If an oxygen-labile cysteine is involved in the reaction mechanism, it might be possible to show that dialysis of the enzyme in buffer which had been deoxygenated with nitrogen gas did not lose activity or lost activity more slowly upon dialysis in the absence of sulfhydryl reagents.

The inhibition of porcine liver CSAD by *p*-hydroxymercuribenzoate, 5,5'-dithio-bis-(2-nitrobenzoate), and N-ethylmaleimidealso suggests the involvement of cysteine at the active center of the enzyme; however, these reagents could cause conformational changes or steric hinderance for substrate binding. The two reagents, iodoacetic acid and iodoacetamide also inhibit the enzyme and the inhibition by these compounds is not likely due to steric hinderance but to alkylation of the sulfhydryl group of cysteine. The data presented in Table 3 show quite a difference in inhibition between iodoacetic acid (17%) and iodoacetamide (78%). This difference may be an indication of the ionic environment in the active center of the enzyme. If there is a negatively charged amino acid which can attract the protonated alpha amino group of cysteinesulfinate, then the amide nitrogen of iodoacetamide might also move into the same area easily whereas the fully ionized carboxyl group of iodoacetic acid would be repelled; thus, alkylation of the cysteine sulfhydryl by iodoacetic acid would be much more difficult than with iodoacetamide.

The apparent involvement of the sulfhydryl group, a sulfhydryl reducing reagent, and the pyridoxal 5'-phosphate requirement make this an intriguing enzyme mechanism. It is obvious that additional studies will be

required to elucidate the reaction mechanism and those amino acid residues that participate in the decarboxylation of cysteinesulfinic acid.

References

Andrews, P. (1964) *Biochem. J.* **91**, 222.

Blaschko, H. and Hope, O. B. (1954) *J. Physiol.* (London) **126**, 52P.

Chapman, G. E. and Greenwood, C. E. (1988) *Nutr. Res.* **8**, 955.

Daniels, K. M. and Stipanuk, M. H. (1982) *J. Nutr.* **112**, 2130.

De La Rosa, J. and Stipanuk, M. H. (1985) *Comp. Biochem. Physiol.* **81B**, 565.

Demarcay, H. (1838) *Ann. Pharm.* **27**, 270.

Davison, A. H. (1956) *Biochem. Biophys. Acta.* **19**, 66.

Garlick, P. J., Burk, T. L. and Swick, R. W. (1976) *Am. J. Physiol.* **230**, 1108.

Guion-Rain, M. C., Portemer, C. and Chatagner, F. (1975) *Biochem. Biophys. Acta.* **384**, 265.

Hayes, K. C., Carey, R. E. and Schmidt, S. Y. (1975) *Science* **188**, 949.

Hayes, K. C., Stephen, Z. F. and Sturman, J. A. (1980) *J. Nutr.* **110**, 2058.

Hayes, K. C. (1988) in Modern Nutriton in Health and Disease (Shils, M. E. and Young, V. R. eds.) 7th ed. pp. 464-470, Lea and Febiger, Philadelphia.

Himmelhoch, S. R. (1971) *Methods Enzymol.* **22**, 273.

Jacobsen, J. G., Thomas, L. L. and Smith, L. H. Jr. (1964) *Biochem. Biophys. Acta* **85**, 103.

Jacobsen, J. G. and Smith, L. H. (1968) *Physiol. Rev.* **48**, 424.

Layne, E. (1957) *Methods Enzymol.* **3**, 447.

Lin, Y-C., Demeio, R. H. and Metrione, R. M. (1971) *Biochim. Biophys. Acta.* **250**, 558.

Remy, A., Henry, S. and Tappaz, M. (1990) *J. Neurochem.* **54**, 870.

Saier, M. H. and Jenkins, W. T. (1967) *J. Biol. Chem.* **242**, 92.

Stephen, L .L., Chavez, E. R. and Sarwar, G. (1991) *Nutr. Res.* **11**, 197.

Sturman, J. A. (1986) *Nutr. Res.* (Special Issue) **1**, 11.

Tumbleson, M. and Schook, L. (1995) International Symposium: Swine in Biomedical Research, University of Maryland, USA.

Weinstein, C. L. and Griffith, O. W. (1987) *J. Biol. Chem.* **262**, 7254.

Worden, J. A. and Stipanuk, M. H. (1985) *Comp. Biochem. Physiol.* **82B**, 233.

Wu, J-Y. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4270.

Zak, B. and Cohen, J. (1961) *Clin. Chim. Acta.* **6**, 665.