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Abstract

Cysteine aminotransferase (L-cysteine: 2-oxoglutarate aminotransferase, EC 2.6.1.3) was purified over 400-fold from the high-speed supernatant fraction of rat liver. The purified enzyme was homogeneous as judged by gel filtration, isoelectric focusing and disc electrophoresis. The molecular weight of the enzyme was about 74,000 by gel filtration and the isoelectric point was 6.2 (4 degrees C). The enzyme catalyzed transamination between L-cysteine and 2-oxoglutarate and the reverse reaction. The optimum pH was 9.7. The Km value for L-cysteine was 22.2 mM, and that for 2-oxoglutaric acid was 0.06 mM. L-Aspartate was a potent inhibitor of the cysteine aminotransferase reaction. The enzyme was very active toward L-alanine 3-sulfinic acid at pH 8.0, and was also very active toward L-aspartic acid (Km = 1.6 mM). Ratios of activities for L-aspartic acid and L-cysteine were essentially constant during the purification of the enzyme. Evidence based on substrate specificity, enzyme inhibition, and physicochemical properties indicates that cytosolic cysteine aminotransferase is identical with cytosolic aspartate aminotransferase (L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1).

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PURIFICATION AND CHARACTERIZATION OF CYSTEINE AMINOTRANSFERASE FROM RAT LIVER CYTOSOL*

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Abstract. Cysteine aminotransferase (L-cysteine: 2-oxoglutarate aminotransferase, EC 2.6.1.3) was purified over 400-fold from the high-speed supernatant fraction of rat liver. The purified enzyme was homogeneous as judged by gel filtration, isoelectric focusing and disc electrophoresis. The molecular weight of the enzyme was about 74,000 by gel filtration and the isoelectric point was 6.2 (4℃). The enzyme catalyzed transamination between L-cysteine and 2-oxoglutarate and the reverse reaction. The optimum pH was 9.7. The Km value for L-cysteine was 22.2 mM, and that for 2-oxoglutaric acid was 0.06 mM. L-Aspartate was a potent inhibitor of the cysteine aminotransferase reaction. The enzyme was very active toward L-alanine 3-sulfinic acid at pH 8.0, and was also very active toward L-aspartic acid (Km = 1.6 mM). Ratios of activities for L-aspartic acid and L-cysteine were essentially constant during the purification of the enzyme. Evidence based on substrate specificity, enzyme inhibition, and physicochemical properties indicates that cytosolic cysteine aminotransferase is identical with cytosolic aspartate aminotransferase (L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1).

Key words : cysteine aminotransferase, enzyme purification, aspartate aminotransferase.

The discovery of S-(2-hydroxy-2-carboxyethylthio) cysteine (HCETC; β-mercaptolactate-cysteine disulfide) and S-(carboxymethylthio) cysteine (CMTC; mercaptoacetate-cysteine disulfide) (1, 2), and of β-mercaptolactate-cysteine disulfiduria patients (2-5) who excreted large amounts of HCETC attracted our attention to the transaminative metabolism of L-cysteine in mammals.

The transaminative pathway of cysteine metabolism functions in various rat tissues though it is not very large (6-10). The first step of the pathway is transamination of L-cysteine, which is catalyzed by at least four enzyme proteins in rat liver (11). One of these enzymes, mitochondrial cysteine aminotransferase (EC 2.6.1.3), was purified from rat liver and characterized (12). The present paper describes the purification and characterization of cysteine aminotransferase from the high-speed supernatant fraction of rat liver.

* A preliminary report of this paper was presented at The Second Symposium on Sulfur-containing Amino Acids (Sept. 1979, Tokyo). (Ref. 8)

MATERIALS AND METHODS

Materials. Male rats of Wistar strain weighing 200-250 g were used. L-Cysteine, L-alanine 3-sulfinic acid, monosodium 2-oxoglutarate, pyridoxal 5'-phosphate (PLP), dithiothreitol and ethylenediaminetetraacetic acid (EDTA) were obtained from Sigma Chemical Company, St. Louis, Mo., U.S.A. The following enzymes were purchased from Boehringer Manheim GmbH, West Germany: Glutamate dehydrogenase (EC 1.4.1.3) (in 50% glycerol) from beef liver, malate dehydrogenase (EC 1.1.1.37) (in 50% glycerol) from pig heart mitochondria and lactate dehydrogenase (EC 1.1.1.27) (in 50% glycerol) from hog muscle. Nicotinamide adenine dinucleotide, oxidized (NAD^+) and reduced (NADH), and adenosine 5'-diphosphate (ADP) were the products of Oriental Yeast Co. Ltd., Tokyo. DEAE-cellulose (DE 52) and CM-cellulose (CM 52) were obtained from Whatman Ltd., Kent, England. Sephadex G-150, Sephacryl S-200 (superfine) and Pharmalyte were from Pharmacia Fine Chemicals, Uppsala, Sweden.

Enzyme purification. Enzyme purification was carried out at 0-4 ℃ and centrifugation was performed at 10,000 × g for 30 min at 0-4 ℃ except otherwise indicated.

Step 1. Preparation of the supernatant fraction of rat liver. Livers from 10 rats were homogenized with 3 volumes of 0.14 M KCl containing 10 mM Tris-HCl, 1 mM EDTA and 0.1 mM dithiothreitol (pH 7.4) (buffer A) using a Potter-Elvehjem homogenizer equipped with a Teflon pestle. The homogenate was centrifuged at 54,000 × g for 60 min.

Step 2. Ammonium sulfate fraction. To the supernatant obtained above, 0.243 g/ml of solid ammonium sulfate was slowly added with stirring with a magnetic stirrer. After standing for 30 min, the precipitate formed was centrifuged off. Solid ammonium sulfate was added to the supernatant at a concentration of 0.245 g/ml and centrifugation was performed as above. The precipitate obtained was dissolved in 30 ml of 20 mM potassium phosphate buffer containing 0.5 mM EDTA and 0.1 mM dithiothreitol (pH 7.4) (buffer B), then dialyzed against 3 l of buffer B overnight.

Step 3. Heat treatment. After addition of neutralyzed 2-oxoglutarate solution at a final concentration of 10 mM, the enzyme solution was heated at 65 ℃ for 5 min, then centrifuged. To the resulting supernatant 0.565 g/ml of ammonium sulfate was added and centrifuged as above. The precipitate obtained was dissolved in 10 ml of buffer B and the solution was dialyzed against two changes of 3 l of 5 mM potassium phosphate buffer containing 0.1 mM EDTA and 0.1 mM dithiothreitol (pH 8.0) (buffer C) for 15 h.

Step 4. DEAE-cellulose column chromatography. The dialyzed solution was applied to a column (2 × 20 cm) of DE 52 which was equilibrated with buffer C. Elution was performed at a flow rate of 20 ml per h with a linear gradient of potassium prepared from 250 ml buffer C placed in a mixing chamber and 250 ml of buffer C containing 0.3 M potassium chloride placed in a reservoir. Five-ml fractions were collected. Fractions 40-50 were pooled, and the enzyme was precipitated by the addition of 0.565 g/ml of ammonium sulfate. The precipitate obtained by centrifugation was dissolved in 2 ml of 5 mM potassium phosphate buffer containing 0.5 mM EDTA and 0.05 mM dithiothreitol (pH 7.0) (buffer D), and dialyzed against 3 l of buffer D for 15 h.

Step 5. CM-cellulose column chromatography. The dialyzed enzyme solution was applied to a column (1.5 × 40 cm) of CM 52 equilibrated with buffer D. Elution was done with buffer D at a flow rate of 20 ml per h, and five-ml fractions were collected. Fractions 7 to 9 were pooled and the enzyme was precipitated with 0.565 g/ml of ammonium sulfate. The precipitate obtained by centrifugation was dissolved in 2 ml of buffer D, and dialyzed against 3 l of 10 mM potassium phosphate buffer containing 0.5 mM EDTA and 0.05 mM dithioth-

reitol (pH 7.4) (buffer E) for 18 h.

Step 6. Sephacryl S-200 column chromatography. The dialyzed enzyme solution was subjected to gel filtration using a column (2.6 × 85 cm) of Sephacryl S-200 equilibrated with buffer E, which was used as the elution buffer. Five-ml fractions were collected and fractions 55 to 59 were pooled.

Step 7. Isoelectric focusing. Isoelectric focusing of the above enzyme solution was performed using a LKB-8100-1 electrofocusing column (110 ml), 0.5% Pharmalyte (pH range 3 to 10), and sucrose as the stabilizing medium. After electrofocusing at 500 volts for 65 h at 4℃, 2-ml fractions were collected. Fractions containing the enzyme were combined and dialyzed against two changes of 3 l of buffer E.

Enzyme assay. Cysteine aminotransferase activity was assayed by three methods (12). In method 1, L-glutamate formed in the forward reaction was determined with L-glutamate dehydrogenase. 3-Mercaptopyruvate formed in the same reaction was determined with lactate dehydrogenase (method 2). When many samples were assayed at the same time, method 3 was preferable, in which L-cysteine formed in the reverse reaction was determined by acidic ninhydrin reagent 2 (13). One unit of the enzyme was defined as the amount producing one μmol of L-glutamate or 3-mercaptopyruvate at 37℃ for 60 min under the present conditions. Aspartate aminotransferase (EC 2.6.1.1) activity was assayed according to Scandurra and Cannella (14). Alanine 3-sulfinate aminotransferase and cysteate aminotransferase activities were assayed by determining L-glutamate formed as in method 1 of the cysteine aminotransferase assay.

Protein determination. Protein concentration was determined by the method of Lowry et al. (15), biuret method (16), or ultra violet absorption (16) with bovine serum albumin as a standard.

Molecular weight estimation. The molecular weight of the enzyme was estimated according to Andrews (17) by gel filtration on a Sephadex G-150 column (2.64 × 90 cm) using 0.05 M Tris-HCl plus 0.1 M KCl (pH 8.0). Reference proteins (products of Boehringer Manheim GmbH) used were cytochrome C from horse heart, chymotripsinogen A from bovine pancreas, albumin from hen egg and from bovine serum, and aldorase from rabbit muscle. Samples of various steps of purification were subjected to the estimation of molecular weight.

Disc polyacrylamide gel electrophoresis. Electrophoresis was performed using pH 8.9-7.5% gel system (18) for 60 min. Protein was stained with Amido Black 10 B or coomasie brilliant blue.

RESULTS

Purification of cysteine aminotransferase. The results of the purification of cysteine aminotransferase from rat liver cytosol are summarized in Table 1. Overall yield was about 30 % and purification was about 400 fold.

Fig. 1A illustrates the effect of heat treatment on the activity of the enzyme. When the enzyme was heated at 70℃ for 5 min, the enzyme activity was reduced to about 50 % of the original, and was completely inactivated by heating at 80 ℃ for 5 min. The highest specific activity in the supernatant was obtained at 70℃. Fig. 1B shows the protective effect of 2-oxoglutarate on the heat inactivation of the enzyme. In the presence of 10 mM 2-oxoglutarate, about 75 %

TABLE 1. PURIFICATION OF CYTOSOLIC CYSTEINE AMINOTRANSFERASE FROM RAT LIVER

Step	Total protein (mg)	CAT Activity[a] Specific (units/mg)	CAT Activity[a] Total (units)	Recovery (%)	Purification (Fold)	GOT[b] activity (units/mg)	CAT/GOT
1. Supernatant	9240	0.282	2610	(100)	(1.0)	1.34	0.211
2. $(NH_4)_2SO_4$ Fractionation	5500	0.38	2090	80.1	1.4	2.29	0.167
3. Heat treatment	822	1.27	1040	39.8	4.5	10.6	0.120
4. DEAE-cellulose	52.3	18.7	975	37.4	66.3	138	0.136
5. CM-cellulose	31.7	25.8	817	31.3	91.5	195	0.133
6. Gel filtration	11.0	76.5	827	31.7	271	594	0.127
7. Isoelectric focusing	6.7	115	764	29.3	408	*c*	*c*

a: Cysteine aminotransferase activity was determined by method 1 as described under MATERIALS AND METHODS. *b*: Aspartate aminotransferase activity was assayed according to the method of Scandurra and Cannella (14). *c*: Not determined.

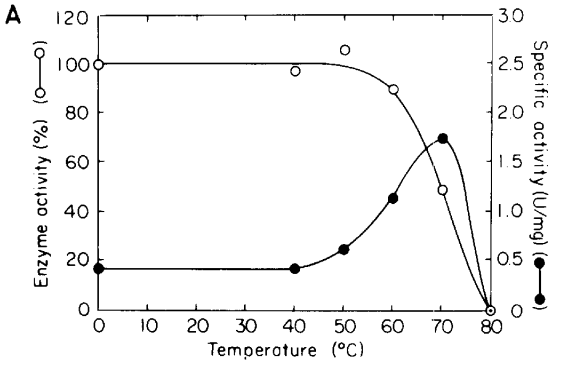

Fig. 1A. Effect of heat on cysteine aminotransferase activity. The enzyme solution of Step 2 of purification was heated at the temperature indicated for 5 min and centrifuged. Enzyme activity in the supernatant was determined. Protein was determined by the biuret method.

Fig. 1B. Effect of 2-oxoglutarate on heat treatment of cysteine aminotransferase. Heat treatment was performed as in 1A in the presence (●—●) or absence (○—○) of 10 mM 2-oxoglutarate.

of the original activity remained after heating at 70℃ for 5 min, and about 30 % even at 80℃. Therefore heat treatment was performed at 65℃ for 5 min in the presence of 10 mM 2-oxoglutarate in order to obtain good specific activity

and yield.

Fig. 2 is an elution profile of cysteine aminotransferase from the DEAE-cellulose column. The enzyme was eluted at a salt concentration of 70 mM. By this procedure about 15 fold purification was obtained with minimal loss of the enzyme.

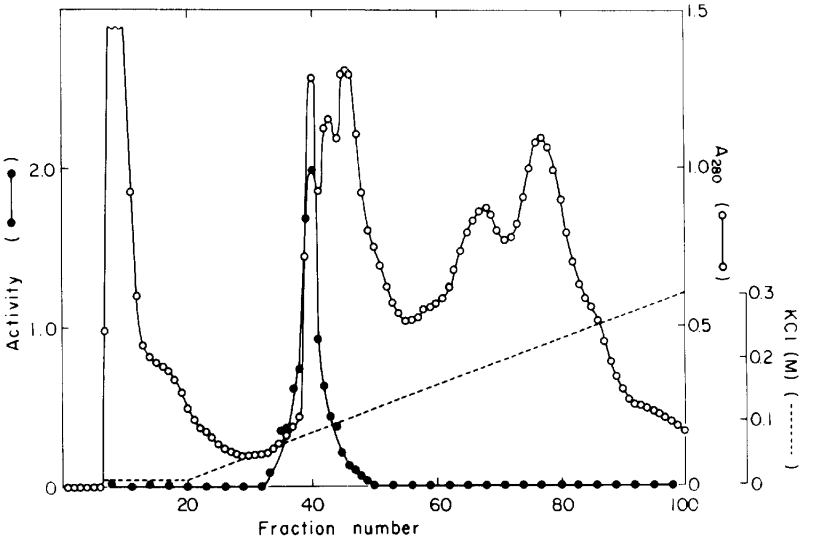

Fig. 2. Elution profile of cytosolic cysteine aminotransferase from DEAE-cellulose column (2 × 20 cm). Chromatography was performed as described under MATERIALS AND METHODS.

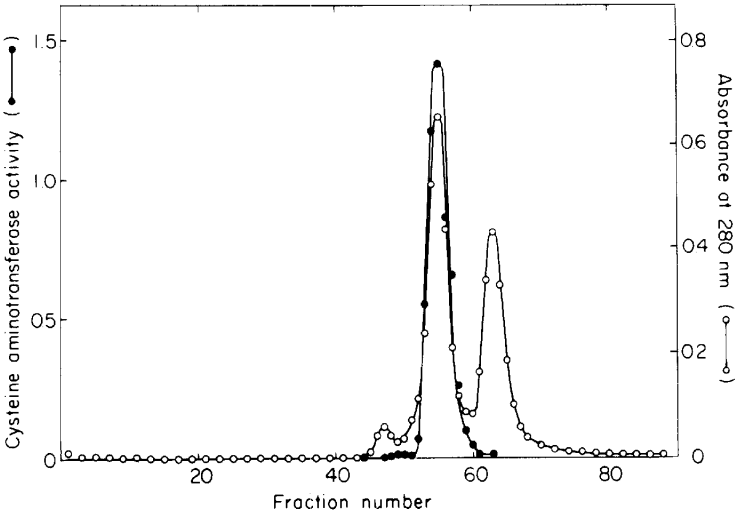

Fig. 3. Chromatography of cytosolic cysteine aminotransferase on Sephacryl S-200 column (2.6 × 85 cm). Experimental details are given in the text.

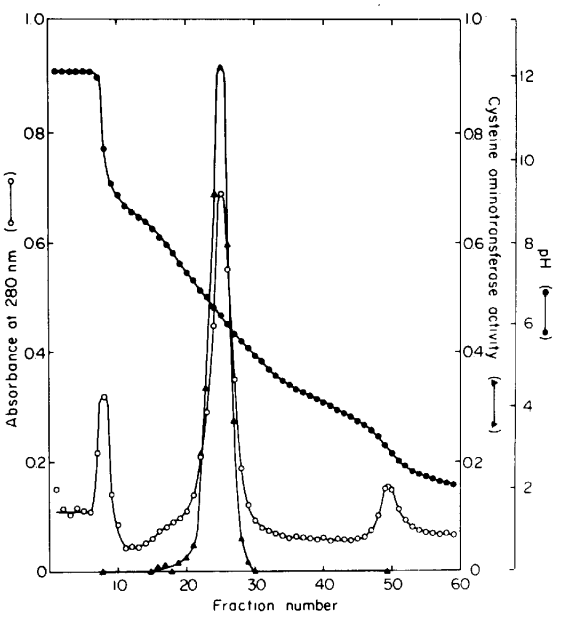

Fig. 4. Isoelectric focusing of purified cytosolic cysteine aminotransferase. Electrofocusing column containing Pharmalyte (0.5 %, pH range 3 to 10) and sucrose was used as described under MATERIALS AND METHODS.

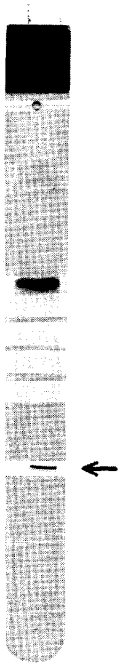

Fig. 5. Disc polyacrylamide gel electrophoresis of purified cytosolic cysteine aminotransferase from rat liver. Electrophoresis was performed using a pH 8.9-7.5 % gel system (18) for 60 min. Arrow indicates the position of bromphenol blue.

The enzyme was not retained on the CM-cellulose column under the present conditions and eluted in the void volume.

Chromatography of cysteine aminotransferase on a Sephacryl S-200 column

is shown in Fig. 3. The enzyme was eluted as a sharp symmetrical peak.

Fig. 4 illustrates a pattern of isoelectric focusing of the enzyme. A sharp symmetrical peak of cysteine aminotransferase activity and that of absorbance at 280 nm exhibited the maximum at pH 6.2 at 4℃.

The purified enzyme exhibited a single band on disc electrophoresis as shown in Fig. 5.

Properties of the enzyme. The molecular weight of the purified enzyme was estimated to be 74,000 ± 1,000 by gel filtration on Sephadex G-150 column. The isoelectric point was found to be pH 6.2 by isoelectric focusing at 4℃. The substrate specificity of the purified enzyme is shown in Table 2. The enzyme was most active toward L-alanine 3-sulfinic acid when assayed at pH 8.0 and was also very active toward L-aspartic acid. Activity to L-cysteine was about 5 % of that to L-aspartic acid. At pH 9.7, L-aspartic acid was the best substrate; L-alanine 3-sulfinic acid was also a good substrate. Activity toward L-cysteine was about 6 % of that to L-aspartic acid at pH 9.7. L-Cysteic acid was also a rather poor substrate for this enzyme. L-Alanine, L-methionine, L-phenylalanine and L-tyrosine were not substrates of this enzyme. The effect of pH on the enzyme activity is shown in Fig. 6. The optimum pH of cysteine aminotransferase activity was 9.7, and that of the activity for L-aspartic acid was between pH 8 and 10 showing a broad curve of pH-activity relationship with a maximum at pH 9.0. Optimum pH of the transamination of L-alanine 3-sulfinic acid and of L-cysteic acid was around 8.5. Km values, obtained by Lineweaver-Burk plot (Fig. 7), were 22.2 mM for L-cysteine, 1.6 mM for L-aspartic acid, and 0.06 mM for 2-oxoglutaric acid. Inhibition of cysteine aminotransferase reaction by various amino acids was examined. L-Aspartic acid was a potent inhibitor: The inhibition was 100 % and 69 % when the reaction was performed at pH 8.0 and 9.7, respectively. L-Alanine, L-tyrosine, L-phenylalanine and L-methionine exhibited little inhibition of the reaction both at pH

TABLE 2. SUBSTRATE SPECIFICITY OF THE PURIFIED ENZYME[a]

Amino acids	Relative activity (%)	
	pH 8.0	pH 9.7
L-Alanine 3-sulfinic acid	190	92.3
L-Aspartic acid	100	100
L-Cysteic acid	14.9	9.6
L-Cysteine	5.3	6.3
L-Alanine	0.0	0.0

a: Assay method 1 was used. Five μmol L-amino acid and 5 μmol 2-oxoglutarate were incubated in 1.0 ml of incubation mixture containing 100 μmol potassium phosphate (pH 8.0) or 25 μmol sodium tetraborate (pH 9.7) and enzyme for 10 min. Activity for L-aspartic acid was taken as 100 %.

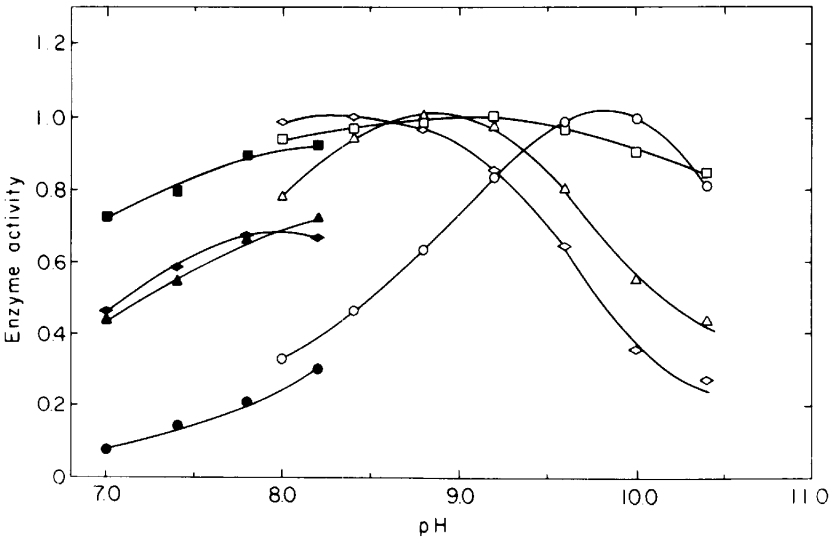

Fig. 6. Effect of pH on the activities for various substrates of purified cytosolic cysteine aminotransferase from rat liver. Activities for L-cysteine (●, ○), L-aspartate (■, □), L-alanine 3-sulfinate (◆, ◇) and L-cysteate (▲, △) were assayed as described under MATERIALS AND METHODS in 0.3 M potassium phosphate (closed symbols) or in 0.025 M sodium tetraborate buffer (open symbols).

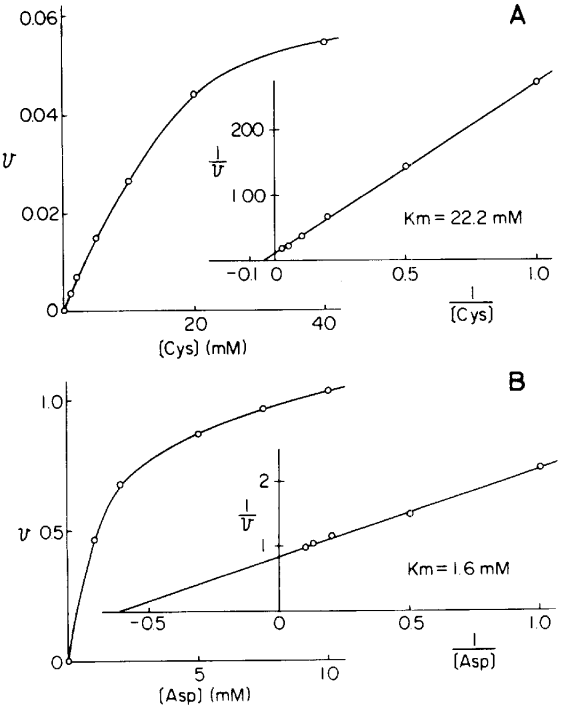

Fig. 7. Lineweaver Burk plot of cytosolic cysteine aminotransferase for L-cysteine (A) and L-aspartic acid (B).

8.0 and 9.7. Aminotransferase activities towards L-cysteine and L-aspartic acid were essentially constant during the purification of this enzyme.

DISCUSSION

Degradation of cysteine may proceed through two different routes. One route involves the oxidation of the sulfur atom before its cleavage from the carbon skeleton of cysteine, which is catalyzed by cysteine dioxygenase (EC 1.13.11.20). This pathway is considered to be the major route of cysteine catabolism (19, 20) and its final products are inorganic sulfate and taurine. The other route is the removal of the sulfur of cysteine without oxidation. The latter process can occur in two ways: one is catalyzed by cystathionase (cystathionine γ-lyase, EC 4.4.1.1) and the other involves transamination of cysteine, followed by transsulfuration of the resulting 3-mercaptopyruvate. This transaminative pathway of cysteine metabolism has been found to occur in various rat tissues (6-10). The physiological role of this pathway has not yet been established. However its significance appears to be the splitting and transferring of the sulfur atom to sufur acceptors without oxidation. Sörbo proposed that the natural sulfur acceptor was sulfite and that the product was thiosulfate (21).

Cysteine aminotransferase was purified from rat liver mitochondria and characterized (12). It was suggested that the enzyme was identical with mitochondrial aspartate aminotransferase (12). In the present study, cytosolic cysteine aminotransferase was purified from rat liver to a homogeneous state as judged by gel filtration, isoelectric focusing and disc electrophoresis. The purified enzyme catalyzed the transamination reaction of L-alanine 3-sulfinic acid, L-cysteic acid and L-cysteine. The enzyme was also very active toward L-aspartic acid at a rate comparable to that toward L-alanine 3-sulfinic acid. The cysteine aminotransferase reaction was completely inhibited by L-aspartic acid at pH 8.0. The ratio of activities for L-cysteine and L-aspartic acid was constant during the purification of the enzyme. Huynh *et al.* reported the purification and physicochemical properties of cytosolic aspartate aminotransferase from rat liver (22). The properties of these two enzymes are very similar. Km values of the present enzyme for L-aspartic acid (1.6 mM) and 2-oxoglutaric acid (0.06 mM) are almost same as those of aspartate aminotransferase (1.81 and 0.06 mM, respectively). Isoelectric points of these enzymes are very close, although determined by different methods. The optimum pH for aspartic acid and the molecular weight of these two enzymes are also close. These data suggest that cytosolic cysteine aminotransferase may be identical with cytosolic aspartate aminotransferase.

Ip *et al.* reported purification and partial characterization of cysteine aminotransferase from rat liver (23). The molecular weight and isoelectric point are similar to the above mentioned two enzymes. However, data such as substrate

specificity and Km values were not reported. Therefore further study of the identity of these enzymes is necessary.

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REFERENCES

1. Ubuka, T., Kobayashi, K., Yao, K., Kodama, H., Fujii, K., Hirayama, K., Kuwaki, T. and Mizuhara, S.: S-(2-Hydroxy-2-carboxyethylthio) cysteine and S-(carboxymethylthio) cysteine in human urine. *Biochim. Biophys. Acta* **158,** 493-495, 1968.
2. Crawhall, J.C.: β-Mercaptolactate-cysteine disulfiduria. In *The Metabolic Basis of Inherited Disease,* 4th Ed. ed. J.B. Stanbury, J.B. Wyngaarden and D.S. Frederickson, McGraw-Hill Book Company, New York, pp. 504-513, 1978.
3. Crawhall, J.C., Parker, R., Sneddon, W., Young, E.P., Ampola, M.G., Efron, M.L. and Bixby, E.M.: β-Mercaptolactate-cysteine disulfide: Analog of cystine in the urine of a mentally retarded patient. *Science* **160,** 419-420, 1968.
4. Niederwieser, A., Gilberti, P. and Baerlocher, K.: β-Mercaptolactate-cysteine disulfiduria in two normal sisters. Isolation and characterization of β-mercaptolactate-cysteine disulfide. *Clin. Chim. Acta* **43,** 405-416, 1973.
5. Law, E.A. and Fowler, B.: β-Mercaptolactate-cysteine disulfiduria in a mentally retarded Scotish male. *J. Ment. Defic. Res.* **20,** 99-104, 1976.
6. Ubuka, T., Yuasa, S., Ishimoto, Y. and Shimomura, M.: Desulfuration of L-cysteine through transamination and transsulfuration in rat liver. *Physiol. Chem. Phys.* **9,** 241-246, 1977.
7. Ishimoto, Y.: Transaminative pathway of cysteine metabolism in rat tissues. *Physiol. Chem. Phys.* **11,** 189-191, 1979.
8. Ubuka, T., Ishimoto, Y. and Akagi, R.: On the transaminative pathway of cysteine. *Ganryuaminosan* (Sulfur-containing Amino Acids) **2,** 283-296, 1979 (in Japanese).
9. Ubuka, T., Ishimoto, Y. and Akagi, R.: Transaminative metabolism of L-cysteine in rat tissues. *J. Inher. Metab. Dis.* **4,** 65-66, 1981.
10. Ubuka, T., Yuasa, S., Akagi, R., Masuoka, N., Kinuta, M. and Mizuhara, S.: Transminative metabolism of L-cysteine in rats. *Abstracts of XII International Congress of Nutrition* p. 94, 1981.
11. Ubuka, T., Umemura, S., Ishimoto, Y. and Shimomura, M.: Transamination of L-cysteine in rat liver mitochondria. *Physiol. Chem. Phys.* **9,** 91-96, 1977.
12. Ubuka, T., Umemura, S., Yuasa, S., Kinuta, M. and Watanabe, K.: Purification and characterization of mitochondrial cysteine aminotransferase from rat liver. *Physiol. Chem. Phys.* **10,** 483-500, 1978.
13. Gaitonde, M.K.: A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acids. *Biochem. J.* **104,** 627-633, 1967.
14. Scandurra, R. and Cannella, C.: Mitochondrial aspartate aminotransferase from beef kidney. *Eur. J. Biochem.* **26,** 196-206, 1972.
15. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265-275, 1951.
16. Layne, E.: Spectrophotometric and turbidimetric methods for measuring proteins. In *Methods in Enzymology,* Vol III, ed. S.P. Colowick and N.O. Kaplan, Academic Press, New York, pp. 447-454, 1957.
17. Andrews, P.: Estimation of the molecular weights of proteins by Sephadex gel filtration.

Biochem. J. **91,** 222-233, 1964.

18. Maurer, H.R.: *Electrophoresis and Related Techniques of Polyacrylamide Gel Electrophoresis.* 2nd Ed. Walter de Gruyter, Berlin, p. 43, 1971.
19. Jocelyn, P.C.: *Biochemistry of the SH Group.* Academic Press, London, pp. 170-175, 1972.
20. Yamaguchi, K., Sakakibara, S., Asamizu, J. and Ueda, I.: Introduction and activation of cysteine oxidase of rat liver. II. The measurement of cysteine metabolism *in vivo* and the activation of *in vivo* activity of cysteine oxidase. *Biochim. Biophys. Acta* **297,** 48-59, 1973.
21. Sorbo, B.: Enzymic transfer of sulfur from mercaptopyruvate to sulfite or sulfinates. *Biochim. Biophys. Acta* **24,** 324-329, 1957.
22. Huynh, Q.K., Sakakibara, R., Watanabe, T. and Wada, H.: Glutamic oxaloacetic transaminase isozymes from rat liver. Purification and physiological characterization. *J. Biochem.* **88,** 231-239, 1980.
23. Ip, M.P.C., Thibert, R.J. and Schmidt, Jr., D.E.: Purification and partial characterization of cysteine-glutamate transaminase from rat liver. *Can. J. Biochem.* **55,** 958-964, 1977.