HOMOLANTHIONINE SYNTHESIS BY HUMAN LIVER CYSTATHIONASE

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SUMMARY: Cystathionase of human liver is shown to differ from the rat liver enzyme: it is a more acidic protein, chromatographing on DEAE-cellulose; it is not inhibited by rabbit antirat liver cystathionase serum; the optimum pH for reaction is higher than for the rat enzyme. However, as is the case with the rat, the homolanthionine synthase and homoserine dehydratase activities of human liver are not separated from the cystathionase activity by ammonium sulfate fractionation and starch block electrophoresis.

The formation of homolanthionine from homoserine and homocysteine is catalyzed by partially purified preparations of rat liver cystathionase (L-homoserine hydro-lyase (deaminating), EC 4.2.1.15) and not by rat liver cystathionine synthase (L-serine hydro-lyase (adding homocysteine), EC 4.2.1.21[1]) (1). Previous results with needle biopsy specimens of human liver did not permit assignment of homolanthionine synthesis to cystathionase (1), and it was pointed out that since the assays were of necessity carried out under conditions established as optimal for the enzymes from rat liver (1, 2), further studies of the human enzymes were needed. Despite extensive study of the transsulfuration enzymes of rat liver (cf. 3, 4), little has been reported concerning the human enzymes (5, 6, 7). We have now found that the enzymes of rat and human origin differ in certain physical, enzymatic and immunochemical properties, but that in man, as in the rat, the biosynthesis of homolanthionine appears to be mediated by cystathionase.

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[1] This nomenclature will be adopted in the forthcoming revision of the Enzyme Commission listing (E. C. Webb, personal communication). Non-standard abbreviations used: PLP, pyridoxal 5'-phosphate; DTT, dithiothreitol.

EXPERIMENTAL

Human liver, obtained at autopsy and kept frozen prior to use, or fresh liver from male Nelson-Wistar rats (Carworth) was homogenized with 8 volumes of cold 0.03 M potassium phosphate buffer, pH 6.9 (containing mM EDTA and, for human liver, 50 μM PLP and mM dithiothreitol (DTT)). The homogenate was centrifuged at 43,500 g for 1 hour, and the supernatant solution was fractionated with solid ammonium sulfate (8). The precipitated proteins were dissolved in a small volume of buffer and dialyzed overnight against the same buffer.

Columns of CM-cellulose and DEAE-cellulose (Whatman) were prepared by the manufacturer's directions. Electrophoresis was on starch block (9) and on disc gel (Canalco model 1200), using for the latter the methods of Davis (10) with human liver cystathionase and of Reisfeld et al. (11) (riboflavin being substituted for ammonium persulfate in the separating gel) with rat liver cystathionase; in most cases, the buffers contained 50 μM DTT and mM EDTA.

Enzymatic activities in rat liver were measured as previously described for cystathionase (2), except that 0.5 μmole of DTT was added to the assay, for homolanthionine synthase (1), and for homoserine dehydratase (12), except that DTT was used instead of 2,3-dimercaptopropanol and α-ketobutyrate was measured with 3-hydrazinoquinoline (13). Human liver was assayed similarly, but with the following modifications: cystathionase, optimum pH 9.2, incubation time 15 min; homolanthionine synthase, pH 8.8, 60 min; homoserine dehydratase, pH 8.0. Human liver cystathionine synthase was assayed by incubation for 45 min at 37.5° with 12 μmoles L-serine, 8 μmoles L-homocysteine (prepared from the thiolactone (2)), 0.015 μmole PLP, 1 μmole EDTA and 55.5 μmoles Tris-HCl buffer, pH 8.8, in a total volume of 0.4 ml. Reaction was stopped with 0.1 ml 25% CCl_3COOH; two 0.2-ml aliquots of protein-free solution were mixed with 0.2 ml of DTT solution (2 μmoles) and let stand for 30 min. One aliquot then was analyzed for cystathionine (14) and the other for cysteine (15). The amino acids react in both analyses, but their contributions to the absorbances are additive and the amounts of each can be calculated from the two simultane-

ous equations involving the absorbance values and the slopes of the four standard curves. Synthase activity is measured by the sum of cystathionine and cysteine produced; since human liver cystathionase is active at low PLP concentrations, in crude extracts part of the cystathionine formed by the synthase is metabolized to cysteine (cf. 16). In all cases, 1 unit (U) of enzyme is that amount catalyzing the formation of 1 μmole of product per min.

RESULTS

Differences between rat and human liver cystathionases. Precipitation with ammonium sulfate almost completely separates rat liver cystathionine synthase (30 to 45% saturation) from cystathionase (55 to 70% saturation) (1, 17). However, the human liver enzymes were precipitated over a wider range of saturations, shifted to higher values for the synthase and to lower values for cystathionase. Consequently, complete separation was not effected, although fractions were relatively enriched in one enzyme and depleted in the other (Table 1). The 30-45% fraction was used to determine the optimal assay conditions for cystathionine synthase, the 45-65% fraction was used for studies of cystathionase. In brief, the human enzymes have pH optima higher than those of the enzymes from rat liver. Substrate requirements are similar, except

Table 1. Enzymatic activities in fractions of human liver.

Human liver (21 g) was fractionated with ammonium sulfate (AMS) as described under "Experimental". Protein was determined by the biuret reaction (18).

Fraction	Protein	Cystathionase	Cystathionine synthase	Homolanthionine synthase	Homoserine dehydratase
	(mg)	(mU)	(mU)	(mU)	(mU)
Homogenate	3610	9260	10570	1628	5950
Soluble	2410	8210	8320	1633	5550
AMS 0-30%	134	66	492	16	106
AMS 30-45%	442	727	5720	119	458
AMS 45-65%	578	5720	1340	1060	3300
AMS >65%	905	0	trace	trace	trace
Total	2059	6513	7552	1195	3864
Recovery*	85%	70%	71%	73%	65%

*Calculated with respect to soluble fraction for protein, with respect to homogenate for enzyme activities.

that for serine by human cystathionine synthase. When the human enzymes are prepared in buffers containing PLP and DTT, a PLP requirement in the assay cannot be demonstrated. With crude extracts prepared without PLP and DTT, the activity of cystathionase in the absence of added PLP was 70% of the maximal value, found with 0.25 mM PLP. The results of these studies are incorporated into the assay procedures described; details will be reported subsequently.

Rat and human liver cystathionase differ markedly in isoelectric point. Rat liver cystathionase, a cation at neutral pH, is retained on columns of CM-cellulose, from which it can be eluted in a number of ways with a considerable increase in specific activity (1, 19, 20). The human liver enzyme is an anion under such conditions. It is not bound by CM-cellulose, but may be

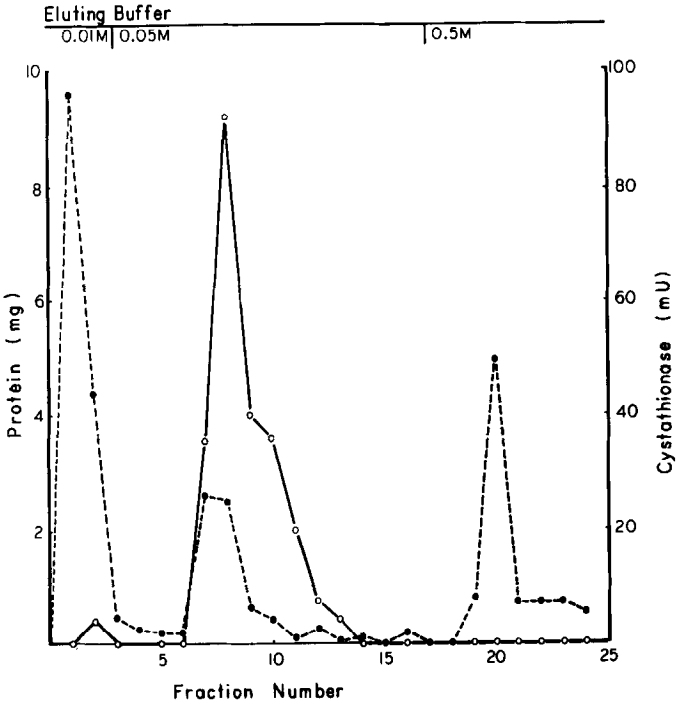

Fig. 1. Chromatography of human liver cystathionase on DEAE-cellulose. 1.0 ml of 45-65% ammonium sulfate fraction (cf. Table 1), containing 290 mU of cystathionase was applied to a 0.9 x 13.5 cm column equilibrated with 0.01 M potassium phosphate buffer, pH 6.9. Changes in the concentration of eluting buffer were made as indicated after fractions 3 and 16. All buffers contained 50 μM PLP, mM DTT and mM EDTA. The fraction size was 3.5 ml. Protein was measured by absorption in the ultraviolet (18). ●---●, protein; o—o, cystathionase activity.

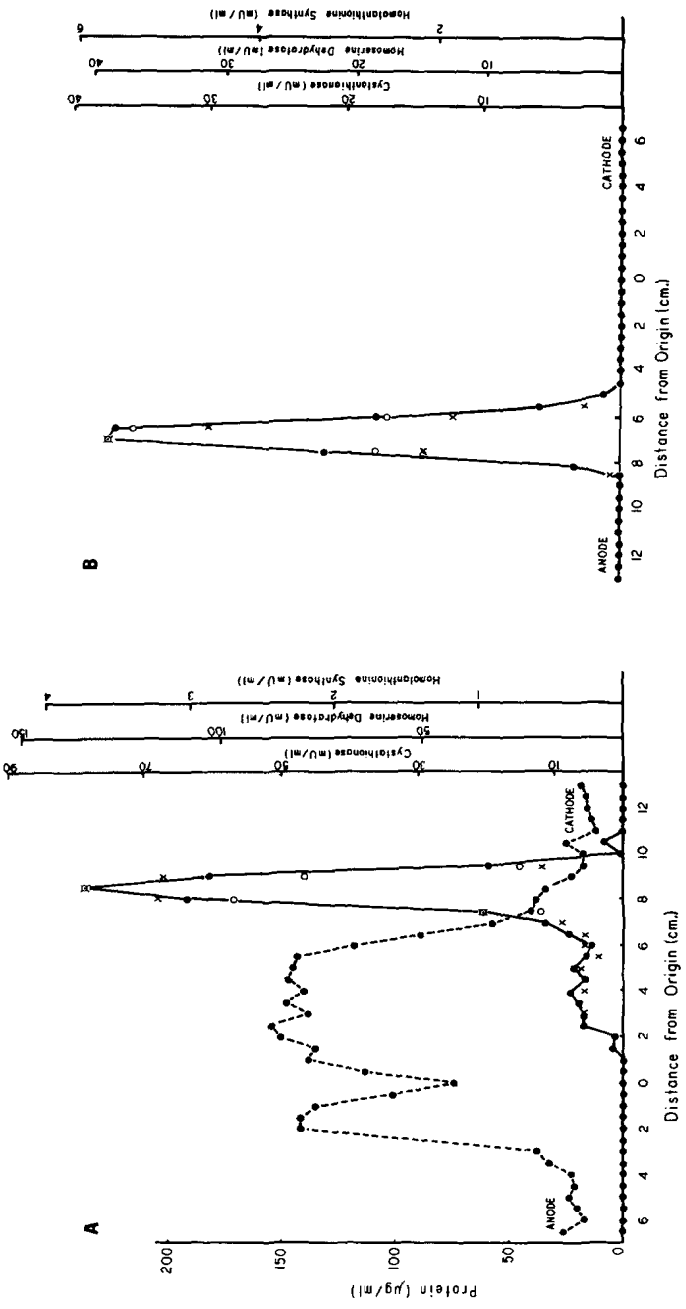

Fig. 2. Electrophoresis of cystathionase on starch block. A. Rat liver cystathionase. The buffer was 0.1 M sodium acetate, pH 5.6, containing 50 μM PLP and mM EDTA. The sample consisted of 1.5 ml of 55-70% ammonium sulfate fraction (cf. Ref. 1), containing 1725 mU of cystathionase. B. Human liver cystathionase. The buffer was 0.05 M sodium barbital, pH 8.6, containing 50 μM PLP, mM DTT and mM EDTA. The sample consisted of 2.0 ml of 45-65% ammonium sulfate fraction (cf. Table 1), containing 450 mU of cystathionase. In both cases, electrophoresis proceeded at 4° for 19 hours at 150 volts. The blocks were sliced into 0.5-cm segments and each segment was extracted with 2 or 3 ml of 0.05 M potassium phosphate buffer, pH 6.9, containing PLP, EDTA and DTT as noted above. Protein was measured by the method of Lowry et al. (23). ●, cystathionase activity; o, homoserine dehydratase activity; X, homolanthionine synthase activity; ----, protein.

chromatographed on DEAE-cellulose, as shown in Fig. 1. A 3- to 4-fold purification was obtained, with a recovery of 80% of the enzyme applied.

Further evidence of the dissimilarity of the two cystathionases was obtained immunochemically. Rabbit antibody to rat liver cystathionase was prepared (21, 22), using as antigen a preparation purified by electrophoresis on starch block (as in Fig. 2A) and then on polyacrylamide disc gel. The material administered to the rabbits showed a single protein band on the disc gel. The rabbit antiserum inhibited both the cystathionase and homoserine dehydratase activities of rat liver cystathionase (59% inhibition of 67 mU of cystathionase by 30 µl of serum), but had no effect on either activity of human liver cystathionase.

Homolanthionine synthesis. Although the human and rat liver cystathionase molecules must differ considerably in the details of composition and structure, there is a similarity of enzymatic specificity. Evidence has been presented that rat liver cystathionase has homoserine dehydratase (12, 20, 24, 25) and homolanthionine synthase (1) activities. This is demonstrated by the electrophoretic separation shown in Fig. 2A. With extract of human liver, fractionation with ammonium sulfate (Table 1) resulted in a parallel distribution of cystathionase, homoserine dehydratase and homolanthionine synthase (Mudd et al. (7) have reported in a footnote that homolanthionine synthesis was not associated with cystathionine synthase). Further purification of the cystathionase fraction was obtained by electrophoresis on starch block (Fig. 2B); the extent of purification is indicated by the decreased amount and number of proteins visible on disc gel electrophoresis of the cystathionase fraction (Fig. 3). In the active fractions from the starch block, cystathionase, homoserine dehydratase and homolanthionine synthase were not separated. Procedures for further purification of human liver cystathionase and for the preparation of antibody are under study in order to establish definitively that the three activities reside on the same molecule.

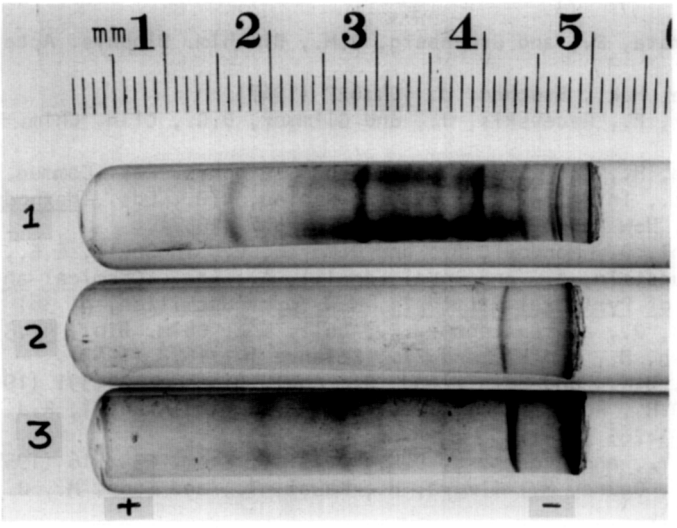

Fig. 3. Disc gel electrophoresis of human liver cystathionase preparations. Tris-glycine buffer, pH 8.3, was used. The gels were stained with Coomassie Blue. 1. Ammonium sulfate fraction (45-65%; cf. Table 1), containing 0.22 mU of cystathionase. 2. Concentrated peak fractions from starch block electrophoresis (Fig. 2B), containing 4 mU of cystathionase. 3. As in 2, 10 mU of cystathionase.

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