PURIFICATION AND PROPERTIES OF CYSTATHIONINE SYNTHASE FROM HUMAN LIVER

N. Tudball and Maureen A. Reed

Department of Biochemistry, University College, Cathays Park, Cardiff CF1 1XL, U.K.

Received September 3,1975

Summary

Cystathionine β-synthase has been isolated from human liver in two enzymically active forms. Both enzymes, α and β possess a molecular weight of 250,000 and are dependent upon pyridoxal phosphate as a cofactor.

Introduction

Cystathionine β-synthase [L-serine hydro-lyase (adding homocysteine), EC4.2.122] is a key enzyme in the transsulphuration pathway associated with normal methionine metabolism in mammals. Nagabhushanam and Greenberg (1965) reported the isolation of a homogeneous preparation of cystathionine synthase from rat-liver which also possessed L-serine debydratase [L-serine hydro-lyase (deaminating), EC4.2.1.13] activity. More recent work on purified rat-liver cystathionine synthase (Brown and Gordon, 1971; Nakagawa and Kimura, 1968; Kashiwamata and Greenberg, 1970) showed that the synthase and dehydratase were two distinct entities. However, Braunstein et al. (1971) have now presented evidence which indicates that cystathionine synthase and serine sulphydrase activities are properties of the same protein.

In humans, a congenital deficiency of cystathionine synthase is responsible for the condition known as homocystinuria (Mudd et al. 1964). Apart from one report (Porter, Grishaver and Jones, 1974) dealing with a partially purified preparation of the enzyme obtained from foetal tissue, information on the human enzyme is lacking. The present communication deals with the purification and properties of cystathionine synthase from adult human liver.

Methods

Routine estimation of protein was carried out by the method of Lowry

et al. (1951). For purified preparations of enzyme, the method of Warburg and Christian (1941) was also employed. Cystathionine β-synthase [L-serine hydro-lyase (adding homocysteine), EC4.2.1.22] was measured by the method of Mudd et al. (1965). The isoelectric focusing technique used was that of Vesterberg and Svensson (1966). Experiments were performed using a 110ml isoelectric focusing column (L.K.B.).

Results

Purification of the enzyme. Unless otherwise stated all extraction and purification steps were carried out at 4°C.

Stage 1. Post-mortem samples of liver (250g) which had previously been frozen and thawed, were minced and macerated in a Waring Blender with sufficient 0.1M-tris-hydrochloric acid buffer pH8.3 (containing EDTA and pyridoxal phosphate at final concns. of 10^{-3}M and 10^{-4}M respectively) to give a 25% (w/v) suspension. The resulting suspension was centrifuged at 18,000g for 30min and the supernatant collected. Any fat associated with the supernatant was removed by filtering through a fine (140μ) nylon mesh.

Stage 2. The supernatant from stage 1 was carefully adjusted to pH5.5 by the dropwise addition of 0.2M-acetic acid. Precipitated material was immediately removed by centrifuging at 18,000g for 10min. The clear supernatant was quickly adjusted to pH7.0 with 2M-NaOH.

Stage 3. The Stage 2 preparation was dialysed for 24h against two changes (total vol 20ℓ) of 0.08M-K_2HPO_4-KH_2PO_4-buffer pH6.5 and the nondiffusable material (725ml) added to a column (6cm x 30cm) of DEAE-cellulose equilibrated with the same buffer. The column was washed with starting buffer until the eluate was free from protein. Cystathionine synthase activity was eluted by washing with successive portions (650ml) of the starting buffer containing potassium chloride at final concentrations of 0.1M, 0.2M and 0.3M. The eluates were combined and dialysed for 24h against three changes (total vol 15ℓ) of starting buffer.

Stage 4. The dialysed eluates from Stage 3 were added to a column

(5cm x 26cm) of DEAE-cellulose equilibrated with 0.08M-K_2HPO_4-KH_2PO_4-buffer pH6.5 and the column washed with 750ml of the same buffer. Enzyme activity was eluted with a linear 0-0.5M-potassium chloride gradient, the eluate being collected in 10ml fractions. Two separate fractions designated α and β, both possessing cystathionine synthase activity were obtained. The active fractions (nos 23-26) and (nos 48-61) corresponding to α and β respectively were pooled separately and concentrated in a Diaflo ultrafiltration apparatus (Amicon Corp., Lexington, Mass., U.S.A) using an XM-50 membrane to yield solutions containing 4-5 mg protein/ml which were subsequently stored at -20°C.

Stage 5. Fractions α and β from Stage 4 were separately subjected to gel filtration on Sephadex G-200. The Stage 4 preparations were applied to a column (5cm x 76cm) of Sephadex G-200 equilibrated with 0.1M-Tris-hydrochloric acid buffer, pH7.5 containing pyridoxal phosphate at a final concentration of 10^{-4}M. The column was developed with the same buffer at a flow rate of 30ml/h and the eluate collected in 10ml fractions. Those fractions containing most of the enzyme activity were pooled and concentrated to yield solutions of approx. 1mg protein/ml. At this stage the respective preparations were stored at -20°C.

Stage 6. Each enzyme preparation from Stage 5 was concentrated further to yield solutions containing 2.5mg protein/ml and the resulting solution subjected to isoelectric focusing using Ampholine pH range 3-10. The separation was allowed to proceed for 48h, the power input being kept at 1.5W. At the end of this period fractions (1.5ml) were collected and each fraction assayed for enzyme activity, protein and pH. The fractions corresponding to enzymically active protein were pooled and the Ampholine and sucrose removed by gel filtration on a column (2.5cm x 40cm) of Sephadex G-50 (Vesterberg, 1969) equilibrated with 0.1M-Tris-hydrochloric acid buffer, pH7.5 containing pyridoxal phosphate at a final concentration of 10^{-4}M. The resulting enzyme preparations were concentrated as previously described to yield solutions containing approx. 1mg protein/ml and then stored at -20°C. The enzyme preparation derived from fraction α possessed

an isoelectric point of pH5.95. That derived from fraction β possessed an isoelectric point of pH5.75. These preparations have now been designated the α enzyme and β-enzyme respectively.

Table 1 shows the results of a typical purification.

Properties of human cystathionine synthase

The molecular weights of enzymes α and β were determined by gel filtration on a column of Sephadex G-200 (1.5xm x 90cm) equilibrated with

Table 1

Purification of Cystathionine β-Synthase from Human Liver

	Total volume (ml)	Total protein (mg)	Total activity (units)*	Specific activity (units/mg protein)	Recovery
Supernatant	725	15,950	561.8	0.035	100
pH5.5 treatment	680	10,120	460.6	0.046	82
Stepwise elution on DEAE-cellulose	1900	360	387.6	1.078	69
Gradient elution on DEAE-cellulose					
Peak α (Nos. 23-26)	20	87	191.1	2.197	34
Peak β (Nos. 48-61)	20	115	163.2	1.419	29
Gel-filtration on Sephadex G-200					
Peak α	12	12.2	151.4	12.39	26.9
Peak β	13	12.0	141.1	11.76	25.1
Isoelectric focusing					
Peak α	3	2.4	64.6	26.78	11.5
Peak β	3	2.2	57.3	25.90	10.2

* 1 unit of activity is defined as that amount producing 1μmole of cystathionine/2h.

0.1M Tris-HCl buffer, pH8.3. Calibration of the column was carried out using preparations of cytochrome C, chymotrypsinogen, ovalbumin, albumin, aldolase, fibrinogen and ferritin. Both enzymes possessed molecular weights of approximately 250,000, a value which has been recorded for the rat-liver enzyme (Nakagawa and Kimura, 1968).

The K_m value with respect to L-serine was 0.91×10^{-3}M for both the α and β enzymes, whereas the K_m value with respect to homocysteine was 3.98×10^{-3}M for enzyme α and 3.41×10^{-3}M for enzyme β. The human enzymes thus possess greater affinities for both substrates than does the corresponding rat system (Nakagawa and Kimura, 1968; Kashiwamata and Greenberg, 1969; Brown and Gordon, 1970). The pH activity curve for the enzyme β peaked sharply at pH8.25 whereas the corresponding curve for enzyme β exhibited a broad region of maximum activity over the range pH7.9-8.3.

Antibody prepared against enzyme β was also cross-reactive with enzyme α, however its effectiveness in this respect (as judged by its relative inhibition of enzyme activity) was less than 50% that obtained with enzyme β.

Both enzymes possessed absorption maxima at 410nm in 0.08M phosphate buffer pH7.5. After reduction with $NaBH_4$, according to the method of Churchich (1965) a new maximum in each case was found at 325nm. When the emission spectra of the reduced enzymes were examined following excitation at 325nm, then new maxima at 392nm were observed. These spectral characteristics were indicative of pyridoxal phosphate being bound as a protonated Schiff base which agrees with the conclusions of Kashiwamata and Greenberg (1970) for the rat-liver enzyme.

Further information concerning the involvement of pyridoxal phosphate was afforded by resolution experiments. Preparations of both enzymes were resolved by dialysis against L-cysteine as described by Brown and Gordon (1971). As a result of this treatment enzyme α lost 51% whereas enzyme β lost 57% of their respective initial activities. Preincubation of the resulting activities in the presence of pyridoxal phosphate (final conc. 10^{-4}M) for 30min, raised the activities of enzyme α and β to 89% and 92% of their respective initial activities.

The above properties of human liver cystathionine β-synthase are in accord with the known properties of the rat-liver system. Nonetheless, the human liver seems to possess two distinct synthase activities. Further investigations are in progress which are aimed at defining the nature of these activities more precisely.

Acknowledgements

This work was supported by a grant from the Mental Health Research Fund.

References

Braunstein, A.E., Goryachenkova, E.V., Tolosa, E.V., Willhardt, I.H. and Yefremova, L.L., (1971) Biochim. Biophys. Acta, 242, 247-260.

Brown, F.C. and Gordon, P.H. (1971) Can. J. Biochem. 49, 484-491.

Churchich, J.E. (1965) Biochemistry, 4, 1405-1410.

Kashiwamata, S. and Greenberg, D.M. (1970) Biochim. Biophys. Acta, 212, 488-500.

Lowry, O.H., Rosebrough, N.J. Farr, A.L. and Randall, R.J. (1951) J. biol. Chem. 193, 265-275.

Mudd, S.H., Finkelstein, J.D., Irreverre, F. and Laster, L. (1964) Science, 143, 1443-1445.

Mudd, S.H., Finkelstein, J.D., Irreverre, F. and Laster, L. (1965) J. biol. Chem. 240, 4382-4392.

Nagabhushanam, A. and Greenberg, D.M. (1965) J. biol. Chem. 240, 3002-3008.

Nakagawa, H. and Kimura, H. (1968) Biochim. Biophys. Res. Commun. 32, 208-214.

Porter, P.N., Grishaver, M.S. and Jones, O.W. (1974) Biochim. Biophys. Acta, 364, 128-139.

Vesterberg, O. and Svensson, H. (1966) Acta, chem. scand. 20, 820-834.

Warburg, O. and Christian, W. (1941) Biochem. Z. 310, 384-421.