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SPECIFICITY AND SOME OTHER PROPERTIES OF LIVER SERINE SULPHHYDRASE: EVIDENCE FOR ITS IDENTITY WITH CYSTATHIONINE β-SYNTHASE*

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SUMMARY

1. The catalytic properties of extensively purified preparations of chicken liver serine sulphhydrase (I; EC 4.2.1.22) and rat liver cystathionine β-synthase (II; EC 4.2.1.13) have been investigated in parallel. These two pyridoxal-*P*-dependent enzymes catalysed similar sets of reactions involving replacement of polar groups in L-serine, β-substituted analogues of serine, L-cysteine or its *S*-alkyl derivatives, on incubation with a variety of mercapto compounds, resulting in production of corresponding L-cysteine thioethers. Both enzymes failed to catalyse α,β-elimination reactions.

2. Measurement of the respective K_m values of the two enzymes showed that cystathionine β-synthase (II) exhibited higher affinities for substrate and cosubstrate in the reaction of cystathionine synthesis from L-serine and homocysteine, whereas serine sulphhydrase (I) had slightly higher affinities (and catalytic potency) in the displacement of H_2S from cysteine in the presence of β-mercaptoethanol.

3. Inhibitor sensitivities (I_{50} values) of the two enzymes were likewise closely similar: β-synthase II, like enzyme I, was strongly inhibited by hydroxylamine and aminooxyacetate (10^{-4} M); both were practically insensitive to DL-cycloserine and D- or DL-penicillamine—potent inhibitors for aminotransferases and α,β-eliminating enzymes (*e.g.* γ-cystathionase).

4. Fractionation of crude extracts from chicken and rat liver containing the enzymes under study, by electrofocusing, or subfractionation of purified preparations on Biogel P-200 columns revealed, in each case, the presence of only one protein peak with coincident activity maxima in reactions of (a) cystathionine synthesis from serine + homocysteine, and (b) H_2S release from L-cysteine in the presence of mercapto compounds. The isoelectric points, determined by ampholine electrophoresis, were slightly different for the chicken liver enzyme I (pH 6.0) and for enzyme II from rat liver (pH 5.5).

5. The body of evidence indicates the existence, in homoïothermic animals, of

* This publication is dedicated to Prof. Dr. Kurt Mothes (Halle/Saale, German Democratic Republic) on the occasion of his 70th birthday (November 3rd, 1970).

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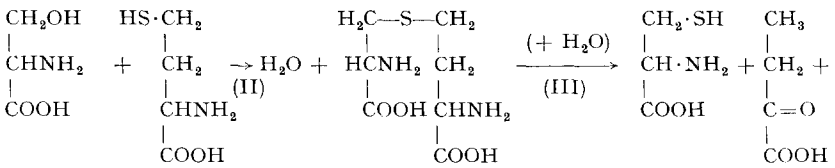
one single enzyme catalysing the formation of cystathionine (also of analogous β-thioethers and of free cysteine) either from L-serine or from L-cysteine by way of β-replacement reactions. The observed differences in some properties (K_m values, relative reaction velocities, isoelectric points, molecular weight *etc.*) of the enzymes obtained from different biological sources are attributable to genetically determined, species-specific variation of the enzyme. Certain similar β-replacing thiol-lyases from organisms of other classes (*e.g.* from microorganisms and higher plants, see refs. 28–30) probably belong to the same group, or family, of plurifunctional pyridoxal-*P*-dependent enzymes.

INTRODUCTION

L-Serine sulphhydrase (I; EC 4.2.1.22) was first detected, in 1957, in yeast cells by SCHLOSSMANN AND LYNEN[1]; it was later found in various animal and plant tissues[2,3] (*cf.* ref. 4). Serine sulphhydrase is known to catalyse the synthesis of L-cysteine from L-serine and H_2S and its reversal, *i.e.* the desulphhydration of cysteine, yielding H_2S and serine, according to Eqn. 1:

$$HOCH_2\cdot CHNH_2\cdot COOH + H_2S \underset{(I)}{\rightleftharpoons} HSCH_2\cdot CHNH_2\cdot COOH + H_2O \qquad (1)$$

A second, direct pathway was thus discovered for the biosynthesis of L-cysteine, in addition to the previously known two-step synthesis from serine and homocysteine *via* cystathionine[5], involving the sequential action of two pyridoxal-*P*-dependent enzymes, cystathionine β-synthase (II; EC 4.2.1.13) and γ-cystathionase (III; EC 4.2.1.15)[6], thus



(2)

Serine sulphhydrase, extensively purified, and practically homogeneous under ultracentrifugation, was prepared from chicken liver by LAC[7] in this laboratory. It was shown[7,8] (*cf.* ref. 9) that the rate of cysteine desulphhydration by this enzyme (Reaction 1, from right to left) is markedly accelerated—like the rate of desulphhydration in β-replacement reactions of cysteine catalysed by the related enzyme of chicken yolk sac, cysteine lyase (IV)[10,11]—by the addition of certain mercapto compounds (thiols), *e.g.* β-mercaptoethanol, cysteamine and homocysteine. The respective cysteine thioethers, namely *S*-hydroxyethyl-L-cysteine, *S*-(2-aminoethyl)-L-cysteine ("thialysine") and cystathionine, were identified as the reaction products, in addition to H_2S. Serine sulphhydrase forms the same thioethers, at similar rates, from L-serine and the corresponding mercapto compounds. Enzyme I was much more active in the synthesis of cystathionine from L-serine and homocysteine than in the formation of cysteine from serine and H_2S (Reaction 1).

These findings suggested that the synthesis of cystathionine is, presumably, the main physiological function of serine sulphhydrase, and that this enzyme might be identical with cystathionine β-synthase (II).

The aim of the studies reported below was to further investigate the catalytic and other properties of chicken liver serine sulphhydrase, and to compare them with those of rat liver cystathionine β-synthase[12,13].

MATERIALS AND METHODS

DL-Homocysteine, L-cysteine, L-serine, DL-cystathionine, β-mercaptoethanol, methylmercaptan, thioglycollic acid, β-mercaptopropionic acid, DL-cycloserine and DL- and D-penicillamine were commercial preparations of the highest quality available. A sample of β-cyanoalanine was kindly provided by Prof. E. E. Snell. We are grateful to Dr. R. M. Khomutov for the gift of aminoacetic acid[14], DL-serine *O*-sulphate[15], β-chloro-DL-alanine[16] and DL-cycloserine, synthesized in his laboratory by the procedures indicated; and to Dr. P. Hermann (Halle/Saale) for samples of *S*-hydroxyethyl-L-cysteine, cysteamine, *N*-acetylcysteamine, *S*-(2-aminoethyl)-L-cysteine ("thialysine"), and N^{ω}-acetylthialysine.

Enzyme preparations

Purified L-serine sulphhydrase (I) was obtained from chicken liver according to the procedure of LAC[7], with minor modifications, namely, omission of fractionation of the enzyme on a DEAE-cellulose column and inclusion of a second run of gel filtration on a Sephadex G-200 column. High-purity preparations of the enzyme rapidly lost activity when stored at 4°, frozen (−20°) or lyophilized. They were kept in concentrated solution (after precipitation with $(NH_4)_2SO_4$) in 0.05 M Tris buffer (pH 8.4) in the presence of 10^{-4} M β-mercaptoethanol, and protected from contact with air.

Partially purified cystathionine β-synthase (II) was prepared from rat liver according to NAKAGAWA AND KIMURA[12]; as the last purification step, fractionation on a column of Biogel P-200 was used instead of separation on phosphocellulose and of the second fractionation on DEAE-cellulose. Enzyme preparations with specific activities of 8–12 units/mg of protein (cystathionine formation) were used for the experiments.

Assay of activity

The activity of serine sulphhydrase was measured as described previously[7,8] (*cf.* ref. 9): (A) By estimation of the initial rate of release of H_2S, determined spectrophotometrically (absorbance at 360 nm) in the form of a colloidal solution of lead sulphide. Experimental samples (total volume 2.0 ml) contained: 20–150 μg enzyme; 0.2 M Tris buffer (pH 8.4); 0.06 μmole pyridoxal-*P*; 15 μmoles L-cysteine; 45 μmoles β-mercaptoethanol (or other mercapto compound, where indicated), and 0.15 μmole lead acetate. Initial rates of desulphhydration were measured in the range of incubation periods (10–20 min) and enzyme amounts ensuring linear dependence of ΔH_2S on Δt and $\Delta[E]$. The reaction was stopped by cooling the samples in the ice-bath. The absorbance at 360 nm was compared with that of standard colloidal solutions of PbS.

In experiments with L-serine as the primary substrate, the amount of thioethers formed as reaction products was the basis for activity assay. (B) Qualitative estimation of the thioethers was achieved by paper chromatography with the follow-

ing solvent mixtures: (1) isopropanol–formic acid–water (7:1:2, by vol.) for cystathionine and *S*-methylcysteine, and (2) *n*-butanol–methanol–acetic acid–water (4:4:1:2, by vol.) for *S*-hydroxyethylcysteine and N^{ω}-acetylthialysine. High-voltage electrophoresis on paper (15–30 min at pH 1.9 and at a potential gradient of 100 V/cm) was used for identification of thialysine, *S*-carboxymethylcysteine and *S*-(carboxyethyl-3)-L-cysteine (or 2-amino-4-thiapimelic acid).

The spots on chromato- and electrophoretograms were revealed by spraying with 0.5% acetone solution of ninhydrin or with iodoplatinate reagent[17], which forms bleached spots on a beige-coloured background with thiols, thioethers and disulphides on paper.

(C) Quantitative determinations of cystathionine and *S*-hydroxyethylcysteine were done by chromatography on paper followed by elution of the spots with a copper–ninhydrin reagent and spectrophotometry of the colour produced[18]. (D) Cystathionine was also determined spectrophotometrically with acid ninhydrin reagent, according to ref. 13*.

Method D is applicable for samples containing no cyst(e)ine. It was used for the assay of cystathionine β-synthase activity in samples of enzyme II incubated with L-serine and DL-homocysteine under conditions modified from those indicated in ref. 13. Samples (total volume 1 ml) contained: 20–60 μg enzyme (or more), 0.1 M Tris buffer (pH 8.4 or 8.6), 0.06 μmole pyridoxal-*P*, 10 μmoles L-serine, 15 μmoles DL-homocysteine. After 2–3 h incubation at 37°, trichloroacetic acid was added to 5% final concentration, to stop the reaction.

Protein concentrations were determined by measurement of absorbance at 280 nm or by the method of LOWRY *et al.*[19].

One unit is the amount of enzyme I or II that produces 1 μmole of product (H_2S or thioether) per 60 min under the conditions indicated. Specific activities are expressed as units per mg of protein. In the inhibition experiments, samples containing 20–60 μg of enzyme and 0.06 μmole of pyridoxal-*P* were preincubated for 20 min at 37° in the presence of inhibitor in the required concentration, and were then supplemented with the other components for assay of activity; activities of inhibited samples (ΔH_2S or Δ thioether in μmoles/mg) are expressed as percent of the activity of non-inhibited control samples. K_m and K_i values were determined graphically on double reciprocal plots (see DIXON AND WEBB[20]).

Electrofocusing in density gradient columns with ampholine buffers

This method[21,22] was used for determination of the isoelectric points of enzymes I (chicken liver) and II (rat liver), and for the separation of active proteins in solutions of partially purified or "crude" enzyme (the latter term designates the fraction precipitated by 40% saturation with $(NH_4)_2SO_4$ from the supernatant of liver homogenate).

Electrofocusing was performed in the 110-ml column with cooling jacket of the LKB apparatus[22], using ampholine mixtures producing pH gradients from 3–10 (or within narrower ranges), in a sucrose gradient of 40–0%. After electrophoresis for 36–40 h at 4° and a potential of 500 V from cathode to anode, the column was drained in 0.7–1.0-ml fractions, which were used for the measurement of pH and enzymic

* We are thankful to Prof. D. M. Greenberg for kindly making available to us a description of the procedure prior to its publication[13].

TABLE I

COSUBSTRATES FOR CHICKEN LIVER SERINE SULPHHYDRASE (I) AND RAT LIVER CYSTATHIONINE SYNTHASE (II)

Average reaction rates are expressed in percent of the rate (= 100%) in reference sample with DL-homocysteine and L-cysteine (Δ H_2S) or L-serine (Δ cystathionine). Relative rates of formation of thioethers other than cystathionine were (in some cases) estimated visually on paper chromatoelectrophoretograms. Specific activities of the enzyme preparations used were: enzyme I, 3–10 units/mg [Δ H_2S]; enzyme II, 5–8 units/mg [Δ H_2S] or 7–10 units/mg [Δ Cystathionine, Assay "D"].

Cosubstrate	*Reaction product*	*Serine sulphhydrase*		*Cystathionine β-synthase*	
		H_2S from L-*cysteine*	*Thioether from* L-*serine*	*H_2S from* L-*cysteine*	*Cystathionine from* L-*serine*
DL-Homocysteine	HOOC–CH(NH_2)–CH_2–S–CH_2–CH_2–CH(NH_2)–COOH	100	+++	100	100
β-Mercaptoethanol	HOOC–CH(NH_2)–CH_2–S–CH_2–CH_2–OH	85	+++	71	—
Cysteamine	HOOC–CH(NH_2)–CH_2–S–CH_2–CH_2–NH_2	27	++	42	—
N-Acetylcysteamine	HOOC–CH(NH_2)–CH_2–S–CH_2–CH_2–NH–CO–CH_3	21	++	—	—
Methyl mercaptan	HOOC–CH(NH_2)–CH_2–S–CH_3	9	+	24	—
Thioglycollic acid	HOOC–CH(NH_2)–CH_2–S–CH_2–COOH	6	+	11	—
β-Mercaptopropionic acid	HOOC–CH(NH_2)–CH_2–S–CH_2–CH_2–COOH	4	+	—	—
D-Penicillamine	— (none)	0	0	0	—

* The data presented in Tables I, II and III are relative reaction rates, expressed in percent of the rate (= 100, by convention) in the reference sample, and averaged from several (3–5) experiments with different batches of enzymes, having various specific activities; see MATERIALS AND METHODS). The entries on formation of cysteine thioethers other than cystathionine (from 0 to +++) are based on visual evaluation, on chromatograms, of relative amounts of the products formed.

activities. Protein contents and specific activities were determined on fractions of the eluate after removal of sucrose and ampholines by filtration through columns of Sephadex G-75.

RESULTS

Specificity towards cosubstrates

From the data presented in Table I it is evident that the initial rates of H_2S formation from L-cysteine, as catalysed by serine sulphhydrase (I), are markedly increased not only by DL-homocysteine, β-mercaptoethanol or cysteamine (*cf.* refs. 8 and 9), but also by other thiols, including *N*-acetylcysteamine, methyl mercaptan, thioglycollic acid and β-mercaptopropionic acid. In experiments in which the mercapto compounds listed above were used as cosubstrates for the reaction with serine as primary substrate, chromatography and electrophoresis on paper of the incubated samples allowed us to demonstrate the formation of the corresponding cysteine thioethers, namely: cystathionine, *S*-hydroxyethylcysteine, thialysine, N^{ω}-acetylthialysine, *S*-methylcysteine, *S*-carboxymethylcysteine or 2-amino-4-thiapimelic acid (Table I).

In the reaction of serine sulphhydrase with L-cysteine as primary substrate the most active cosubstrates for this enzyme were homocysteine and β-mercaptoethanol, and the least active were thioglycollic and β-mercaptopropionic acids. The serine sulphhydrase did not utilize D-penicillamine either as primary or as secondary substrate.

We next studied the substrate specificity of cystathionine β-synthase (II) for the sake of its comparison with serine sulphhydrase (I). When solutions of the rat liver enzyme (II) were fractionated on Biogel P-200, the major protein fractions were active in catalysing both cystathionine synthesis from L-serine and homocysteine (conventional cystathionine β-synthase activity) and the formation of H_2S from L-cysteine and mercaptoethanol (one of the typical β-replacement reactions of serine

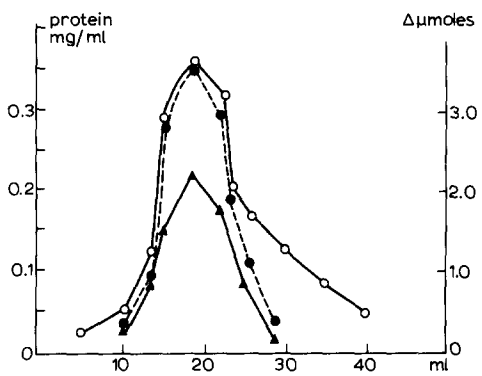


Fig. 1. Fractionation of semipurified cystathionine β-synthase from rat liver on Biogel P-200 column (2.3 cm × 40 cm), in potassium phosphate buffer (0.01 M, pH 7.8). The amount applied was 27 mg of protein with specific activity 4-5 units/mg protein (*Δ* cystathionine). ○—○, protein ($A_{280\ nm}$); ●---●, *Δ* cystathionine ($A_{455\ nm}$[13]); ▲—▲, *Δ* H_2S (as PbS, $A_{360\ nm}$)[7].

sulphhydrase) (Fig. 1). Coincident peaks of the two catalytic activities were observed in the fractions with highest protein content (Fig. 1, Fraction 18).

The results listed in Table I show that the β-synthase, like enzyme I, catalysed the same broad range of β-replacement reactions of L-cysteine and L-serine with a variety of mercapto compounds.

TABLE II

β-SUBSTITUTED SERINE ANALOGUES AS PRIMARY SUBSTRATES

The cosubstrate was DL-homocysteine. Activities were assayed by measurement (on chromatograms[18] and spectrophotometrically, by Procedure "D") of the amount of cystathionine produced. Relative activities of analogues are expressed as percent of that of L-serine. Specific activities of enzyme preparations used were: serine sulphhydrase, 0.75 unit/mg protein [Δ cystathionine assay "D"]; cystathionine synthase, 7 units/mg.

Substrate	*Enzyme*	
	Serine sulphhydrase (I)	*Cystathionine synthase (II)*
L-Serine	100	100
β-Chloro-DL-alanine	52	33
DL-Serine *O*-sulphate	20	20
β-Cyano-DL-alanine	23	6

Specificity towards primary substrates

Serine sulphhydrase has hitherto been reported to utilize only L-serine and L-cysteine as primary substrates[2,3,8] (*cf.* ref. 4; ref. 9). The previously observed inhibition of the enzyme in the presence of the structural analogues β-chloro-DL-alanine and DL-serine *O*-sulphate[7,8] (*cf.* ref. 9), was found by us to be reversible. Therefore we tested these compounds as possible primary substrates. As shown in Table II, serine sulphhydrase could, in the presence of homocysteine, utilize not only

TABLE III

CYSTEINE THIOETHERS AS PRIMARY SUBSTRATES

Experimental conditions and presentation of results as indicated in legend to Table II.

Substrate	*Enzyme*	
	Serine sulphhydrase (I)	*Cystathionine synthase (II)*
L-Serine	100	100
S-Carboxymethyl-L-cysteine	18	—*
S-Methyl-L-cysteine	17	20
S-Hydroxyethyl-L-cysteine	9	+
N^{ω}-Acetyl-L-thialysine	9	—*

* Not tested.

L-serine (or L-cysteine) but also the β-substituted serine analogous β-chloroalanine, serine *O*-sulphate, and β-cyanoalanine, for the synthesis of cystathionine. Moreover, it was found that cystathionine β-synthase (II) could also use the same compounds for the synthesis of cystathionine (Table II).

We also tested the capacity of serine sulphhydrase to act on the reaction products, *i.e.* on diverse cysteine thioethers. The enzyme was found to catalyse, in the presence of homocysteine, the formation of cystathionine from various cysteine thioethers (Table III) by replacement of the sulphur-containing β-substituent. The β-synthase from rat liver exhibited similar relative activities in cystathionine synthesis from primary substrates chosen at random among the thioethers listed (*e.g.* *S*-methyl-L-cysteine) (Table III). Reversibility of the reactions of thioether synthesis catalysed by the two enzymes was thus demonstrated.

Determination of K_m values

A study of the kinetics of reactions of thioether synthesis catalysed by enzymes I and II allowed us to determine their respective K_m values for substrates and co-

TABLE IV

K_m VALUES FOR SERINE SULPHHYDRASE AND CYSTATHIONINE β-SYNTHASE

The enzyme preparations used had the following specific activities: I, 6.0 units/mg [Δ H_2S]; II, 4–5 units/mg [Δ H_2S], or 7–8 units/mg [Δ cystathionine]. K_m values are expressed in mM.

Enzyme	L-*Cysteine (1)* + + β-*mercapto-ethanol (2)*		L-*Serine (3)* + + DL-*homo-cysteine (4)*	
	$K_{m\,(1)}$	$K_{m\,(2)}$	$K_{m\,(3)}$	$K_{m\,(4)}$
I. Serine sulphhydrase	22	13	34	83
II. Cystathionine β-synthase	36	24	08	18

substrates. K_m values were estimated in experiments involving the synthesis of *S*-hydroxyethylcysteine from L-cysteine and β-mercaptoethanol ("sulphhydrase assay") and the synthesis of cystathionine from L-serine and DL-homocysteine ("cystathionine β-synthase assay").

The results are listed in Table IV, which shows that, in the latter reaction, the K_m values for serine and homocysteine were substantially lower (*i.e.* the affinities were higher) with cystathionine β-synthase, as compared with serine sulphhydrase. In the β-replacement reaction between L-cysteine and β-mercaptoethanol there was only a slight difference between the K_m values of the two enzymes for the substrate and cosubstrate: both K_m values were slightly lower for serine sulphhydrase.

Sensitivities to some inhibitors

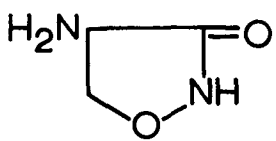
Among the substances formerly tested[7–9], hydroxylamine and aminooxyacetic acid were the strongest inhibitors of serine sulphhydrase; on the other hand, the enzyme displayed little or no sensitivity to pure DL-cycloserine, a potent inhibitor of some pyridoxal-*P*-dependent enzymes[23,24,26].

Together with these results[7–9], Table V lists some results obtained in parallel

TABLE V

INHIBITOR SENSITIVITIES OF CHICKEN LIVER SERINE SULPHHYDRASE AND RAT LIVER CYSTATHIONINE β-SYNTHASE

The preparation of β-synthase had a specific activity of 4–5 units/mg protein (Δ H_2S assay in reaction between L-cysteine and β-mercaptoethanol)

Inhibitor	*Inhibitor conc. (M)*	*Inhibition (%)*							
		Serine sulphhydrase				*Cystathionine synthase*			
		10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-2}	$5 \cdot 10^{-3}$	10^{-3}	10^{-4}
Hydroxylamine	H_2NOH	100	100	74	—	100	100	100	55
Aminooxyacetic acid	H_2NOCH_2COOH	100	100	87	22	100	100	100	100
DL-Cycloserine		0	0	0	0	5	—	0	—
D-Penicillamine	$(CH_3)_2 \cdot C(SH) \cdot CHNH_2COOH$	0	0	—	—	0	—	0	—
DL-Penicillamine		0	0	—	—	0	—	0	—

studies of the action of some selective inhibitors upon cystathionine β-synthase from rat liver. It can be seen that the two enzymes exhibited high and practically equal sensitivities towards hydroxylamine (10^{-3} M) and aminooxyacetic acid (10^{-4} M); at the concentrations indicated these agents completely inhibited both enzymes. The β-synthase, like serine sulphhydrase[8] (*cf.* ref. 9), is practically insensitive to DL-cycloserine and to the D- or DL-forms of penicillamine, which are strong specific inhibitors for some other pyridoxal-*P* enzymes[34], including the aminotransferases[33], β- or γ-eliminating lyases (*e.g.* γ-cystathionase), and α-decarboxylases[34].

Electrofocusing

In order to define the catalytic properties of serine sulphhydrase and cystathionine β-synthase under more exacting experimental conditions, an attempt was made to separate their known "typical" activities (see above) in purified preparations or "crude" enzyme fractions from liver extracts by the method of column electrophoresis in a pH gradient stabilized with ampholines ("electrofocusing"[21]). The results, presented in Figs. 2A and 2B, show the presence in extracts from chicken and rat liver of one single protein fraction, in either case, with coincident activities in assays

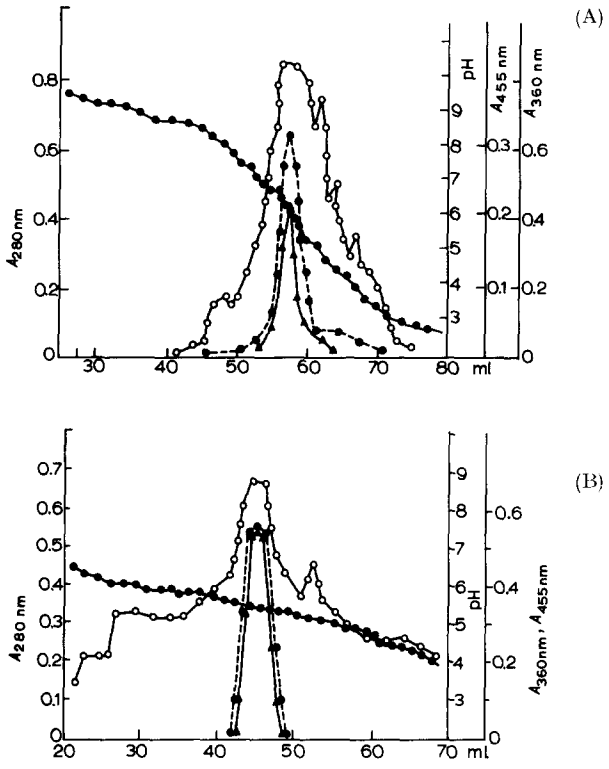


Fig. 2. Fractionation by ampholine electrophoresis of "crude" preparations of (A) serine sulphhydrase (from chicken liver), and (B) cystathionine synthase (from rat liver). For experimental conditions, see under MATERIALS AND METHODS. Amount applied on column: 80–120 mg protein. ●—●, pH values; ○—○, protein, $A_{280\,nm}$; ●---●, Δ cystathionine ($A_{455\,nm}$)[13]; ▲—▲, Δ H_2S[7].

for the two enzymes, *i.e.* in the reactions (a) between cysteine and β-mercaptoethanol (ΔH_2S), and (b) between serine and homocysteine (Δ cystathionine).

Similar results were obtained in electrofocusing runs done on high purity preparations of the two enzymes. In these experiments, significantly different isoelectric points were found for the chicken enzyme I (at pH 6.0) and for the rat liver enzyme II (at pH 5.5).

DISCUSSION

In our studies concerning the specificities of serine sulphhydrase from chicken liver for β-substituted α-amino acids (primary substrates) and for cosubstrates (or secondary substrates), reported in ref. 8 (*cf.* ref. 9) and in the present paper, it was established that the enzyme catalyses reactions of replacement of polar groups in position β of L-serine and some of its β-substituted analogues, of L-cysteine and its thioethers, upon incubation with a variety of mercapto compounds (thiols) or with hydrogen sulphide; some, at least, of these β-replacement reactions (Eqns. 1 and 3–5), were shown to be reversible.

$$HOCH_2\cdot CHNH_2\cdot COOH + H_2S \rightarrow HSCH_2\cdot CHNH_2\cdot COOH + H_2O \quad (1)$$

$$HOCH_2\cdot CHNH_2\cdot COOH + RSH \rightarrow RSCH_2\cdot CHNH_2\cdot COOH + H_2O \quad (3)$$

$$HSCH_2\cdot CHNH_2\cdot COOH + RSH \rightarrow RSCH_2\cdot CHNH_2\cdot COOH + H_2S \quad (4)$$

$$XCH_2\cdot CHNH_2\cdot COOH + RSH \rightleftharpoons RSCH_2\cdot CHNH_2\cdot COOH + XH \quad (5)$$

(R = –Alkyl; X = –S-Alkyl, –Cl, $-OSO_3H$, –CN)

The only reaction formerly shown to be catalysed by cystathionine β-synthase was the formation of cystathionine from L-serine and homocysteine[5,12,13]. The evidence presented above shows that extensively purified and electrophoretically homogeneous preparations of β-synthase from rat liver catalysed, like high purity serine sulphhydrase, a variety of β-replacement reactions conforming to Eqns. 1 and 3–5; substrate and cosubstrate specificities and relative reaction rates were essentially similar for the two enzyme preparations.

Kinetic experiments have shown that the chicken liver enzyme I had slightly higher affinities for cysteine and β-mercaptoethanol (in *S*-hydroxyethyl cysteine formation), as compared with enzyme II from rat liver. Conversely, the affinities for serine and homocysteine in the reaction of cystathionine synthesis were substantially higher with rat liver enzyme II. In spite of these differences in K_m values (and in relative reaction velocities), it appears that the main physiological function of "serine sulphhydrase", like that of rat liver β-synthase, consists in the synthesis of cystathionine from serine and homocysteine. The rat and chicken liver enzymes exhibited identical inhibitor sensitivities. The strongest inhibition was observed with hydroxylamine and aminooxyacetate (which is both a carbonyl reagent and a substrate analogue).

The enzymes under study were insensitive to DL-cycloserine (up to 10^{-2} M). The following steps appear to be involved in the irreversible inhibition by cycloserine of aminotransferases (and probably in that of other types of pyridoxal-*P*-dependent enzymes[23,24]. The inhibitor (a *quasi*-amino acid) forms an aldimine intermediate with

enzyme-bound pyridoxal-*P*; prototropic rearrangement of this aldimine to the corresponding ketimine of pyridoxamine-*P* is followed by decyclization of the inhibitor's isoxazolidone ring and blocking, by the resulting reactive acyl residue, of an essential group of the catalytic site.

In accordance with this mode of action it was found, in this laboratory and elsewhere, that DL-cycloserine (and its homologous analogues) strongly inhibit those B_6-dependent enzymes whose reaction mechanism comprises aldimin $\rightleftharpoons$ ketimine rearrangement of Schiff-base intermediates[25,26], such as aminotransferases[22,23], aspartate β-decarboxylase and γ-cystathionase[26]. On the other hand, pyridoxal-*P* enzymes whose presumable catalytic mechanism does not involve ketimine intermediates[25,26], *e.g.* glutamate α-decarboxylase[32] and the enzymes considered in this paper, proved practically insensitive to cycloserine[8] (*cf.* ref. 9). 1,2-Aminothiols (*e.g.* penicillamine, cysteamine, cysteine, *etc.*), by combining with the CO group of pyridoxal-*P* to thiazolidine derivatives, strongly inhibit the action of certain B_6-dependent enzymes[33–36], including the plurifunctional β,γ-(and α,β-)eliminating enzyme, γ-cystathionase (ref. 35 and E. V. GORYACHENKOVA, unpublished); but the β-replacing cysteine lyase from chicken yolk sac[10] and the two enzymes under study in the present paper proved resistant to inhibition by D- or DL-penicillamine (a substrate analogue). It has been suggested[8,10] (*cf.* ref. 9) that this striking difference might be due to binding of primary substrates (and substrate-like β-thio amino acids) in a conformation with β-substituent *trans* to α-hydrogen (*cis* to the α-NH_2 group) in the active site of the penicillamine-sensitive γ- or β-eliminating γ-cystathionase, and in the opposite conformation (*cis* to α-H-atom) in enzymes with strict β-replacement specificity, like cysteine lyase[10], serine sulphhydrase and cystathionine β-synthase. The former, but not the latter, conformation is favourable for elimination reactions and allows thiazolidine formation.

The results of experiments on gel filtration and electrofocusing of purified and crude enzyme preparations from chicken and rat liver revealed the presence, in either case, of only one protein fraction having coincident peak activities in the assays for "serine sulphydrase" and "cystathionine synthase".

As stated in RESULTS, enzymes I and II significantly differed in isoelectric points, K_m values and relative reaction velocities. No precise determinations have been reported of their molecular weights, but approximate estimates differ: approx. 125 000 for "serine sulphhydrase[7] and approx. 250 000 for rat liver cystathionine β-synthase[12,13].

The sum of data available indicates the presence, in homoïothermic animals, of one single plurifunctional pyridoxal-*P*-dependent lyase, catalysing a series of β-replacement reactions between L-serine or L-cysteine (also β-substituted serine analogues and cysteine thioethers) as primary substrates, and a variety of mercapto compounds (thiols) or H_2S as cosubstrates; these include the originally reported characteristic reaction of "serine sulphhydrase"[1–4] and that of "cystathionine β-synthase"[5,12,13]. The analogous "serine sulphhydrase" of yeast cells[1,27] has similar substrate and reaction specificities, but differs from the liver enzyme in relative reaction velocities with individual cosubstrates; it is probably identical with the "*S*-methylcysteine synthase" of WOLFF *et al.*[28].

This plurifunctional enzyme might appropriately be classified and designated as follows[31]:

(1) Code No.	*(2) Reaction*	*(3) Recommended name*
4.2.1.22	Serine (cysteine) + RSH(H_2S) = cysteine thioether + + H_2O(H_2S)	Cystathionine β-synthase

(4) Other names	*(5) Basis for classification (Systematic name)*
Cysteine synthase (methylcysteine synthase); serine sulphhydrase	Serine hydro-lyase (adding thiols or H_2S)

(6) Comments
A pyridoxal phosphate protein. Also catalyses β-replacement reactions between some β-substituted serine analogues (β-chloroalanine, *etc.*) or cysteine thioethers, and mercapto compounds.

The above-mentioned differences in some catalytic (K_m values, relative reaction velocities) and physical (*e.g.* molecular weights, isoelectric points) parameters are attributable to genetically determined, species-specific variations in the structure and properties of this enzyme. Certain pyridoxal-*P*-requiring enzymes from higher plants[29] or microorganisms[27,28,30], reported to catalyse more or less specifically the synthesis of cysteine and/or cysteine thioethers from serine (respectively, *O*-acylated serines) and H_2S or thiols, are probably either variants of this enzymic entity or closely related members of the same group, or family, of C_3-specific β-replacing lyases.

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