BBA 63367

Reactions catalysed by serine sulfhydrase from chicken liver

In our laboratory, comparative investigations are in progress of the catalytic properties and active sites of several pyridoxal phosphate-dependent enzymes releasing H_2S from cysteine[1,2].

Serine sulfhydrase (EC 4.2.1.22) catalyses the synthesis of L-cysteine from L-serine and H_2S; as shown in Eqn. 1, the reaction is reversible[3–7].

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

Partial purification (50–100-fold) of serine sulfhydrase from various sources has been reported[3–7].

We have extensively purified (>500-fold) serine sulfhydrase from chicken liver. The purification procedure[8] includes ammonium sulfate fractionation (at 0.4 saturation), adsorption on calcium phosphate, separation on DEAE-cellulose and on Sephadex G-200 columns.

Activity assays were based on spectrophotometric determinations (a) of cysteine formed in Reaction 1 (ref. 4) and (b) of H_2S liberated in the reverse reaction (estimated at 360 mμ in the form of colloidal lead sulfide[1]).

TABLE I

EFFECT OF THIOLS ON THE DESULFHYDRATION OF L-CYSTEINE BY SERINE SULFHYDRASE

Assay mixtures (total vol., 2 ml) buffered with 0.2 M Tris (pH 8.3), each containing 0.2 μmole lead acetate, were incubated at 37°. Amounts of other added components: enzyme, 0.26 mg; pyridoxal phosphate, 0.2 μmole; L-cysteine, 13 μmoles; β-mercaptoethanol, 45 μmoles; DL-homocysteine, 13 μmoles; cysteamine, 20 μmoles.

System	*H_2S trapped as PbS (mμmoles)*	
	20-min incubation	*60-min incubation*
1. Enzyme + β-mercaptoethanol + pyridoxal phosphate	0	0
2. Enzyme + β-mercaptoethanol + pyridoxal phosphate + homocysteine	0	0
3. Enzyme + L-cysteine + pyridoxal phosphate	0	5 (17 at 120 min)
4. Enzyme + L-cysteine + pyridoxal phosphate + β-mercaptoethanol	126	115
5. Enzyme + L-cysteine + pyridoxal phosphate + homocysteine	126	124
6. Enzyme + L-cysteine + pyridoxal phosphate + cysteamine	126	120

As shown in Table I (System 3), small amounts of H_2S appear on incubation of L-cysteine with cysteine sulfhydrase for 1 h or longer. If in addition to cysteine the incubation mixtures contain an equimolar or larger amount of certain thiol compounds, *e.g.* β-mercaptoethanol, DL-homocysteine, or cysteamine, the rate of H_2S formation is greatly enhanced—after 20-min incubation, the limited amount of lead acetate added into the assay mixtures to trap H_2S is already converted practically completely to PbS (Systems 4, 5, 6); flocculation of colloidal PbS at higher concen-

trations precludes the use of larger quantities of lead acetate. No H_2S is released by the enzyme from homocysteine or other thiols in the absence of cysteine.

This thiol-induced increase in the rate of cysteine desulfhydration is closely similar to the effect of thiols in analogous experiments with cysteine lyase[2]. The products of reactions catalysed by serine sulfhydrase were identified with authentic reference standards (kind gift from Dr. P. Hermann) on filter paper (Whatman 1M) by high-voltage electrophoresis and chromatography; the spots were stained (on sheets run in parallel) with ninhydrin and with iodoplatinate reagent[9].

In experiments with cysteine and/or homocysteine the unreacted mercapto amino acids were precipitated with mercuric acetate from the trichloroacetic acid-fixed incubated mixtures prior to application on the Whatman sheets. Electrophoresis was carried out at pH 1.9 in the system formic acid–acetic acid–water (1:3:16, by vol.), usually at a potential gradient of 100 V/cm for 25 min. To improve spot separation, the band with ninhydrin-positive substances was excised transversally from dried electrophoretograms, sewn onto a fresh paper sheet and subjected to chromatography in the same direction, in the solvent system methyl ethyl ketone–propionic acid–water (15:5:6, by vol.)[4–6]. In the experiments with cysteamine (Fig. 1C) the order of electrophoresis and chromatography was inversed and the electrophoretic run was conducted for 30 min at 20–25 V/cm.

H_2S and serine were the only products formed on incubation of L-cysteine (also at higher concentrations) with high-purity serine sulfhydrase. With added mercapto-

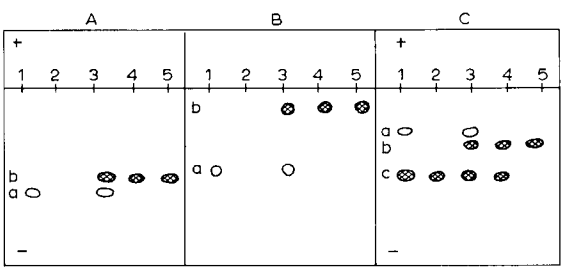

Fig. 1. Products of enzymatic reaction of L-serine and L-cysteine with thiols β-mercaptoethanol (A), DL-homocysteine (B) or cysteamine (C). Scheme of chromatoelectrophoretic separation. Incubation, 2 h at 37°; concentrations of components as in Table I; other experimental conditions, see text. *Samples:* Serine (1) or cysteine (2) with respective thiol, pyridoxal phosphate and heat-inactivated enzyme (controls); (3) like (1) and (4) like (2), but with active enzyme; standard solutions of *S*-hydroxyethyl-L-cysteine (5,A), cystathionine (5,B), "thialysine" (5,C). *Spots:* (A) a, serine; b, hydroxyethyl-L-cysteine. (B) a, serine; b, cystathionine. (C) a, serine; b, "thialysine"; c, cysteamine. Open spots, positive to ninhydrin; hatched spots, positive to ninhydrin and iodoplatinate.

ethanol the formation is observed of a thioether identified with synthetic *S*-hydroxyethylcysteine (Fig. 1A, Spot b). With added homocysteine there were formed considerable amounts of cystathionine (Fig. 1B, Spot b). The product formed in the presence of added cysteamine was identical in mobility and staining properties to *S*-(2-amino-2-carboxyethyl)cysteine ("thialysine") (Fig. 1C, Spot b).

The same thioethers were produced in approximately the same amounts by serine sulfhydrase from L-serine in the presence of the corresponding thiols (see Samples 3 in Figs. 1A, 1B and 1C).

In the reactions with L-serine no H_2S was released from the thiols. Dr. P. HERRMANN (Halle/Saale) has likewise demonstrated the formation, by semi-purified serine sulfhydrase from yeast[6], of thialysine from serine and cysteamine (personal communication).

It thus appears that serine sulfhydrase catalyses a number of β-substitution reactions between either L-serine or L-cysteine and certain thiol compounds, resulting in formation of the corresponding thioethers of cysteine, according to Eqns. 2 and 3:

$$HOCH_2 \cdot CHNH_2 \cdot COOH + RSH \rightarrow RSCH_2 \cdot CHNH_2 \cdot COOH + H_2O \quad (2)$$
$$HSCH_2 \cdot CHNH_2 \cdot COOH + RSH \rightarrow RSCH_2 \cdot CHNH_2 \cdot COOH + H_2S \quad (3)$$

In contrast to cysteine lyase, which catalyses a number of reactions of type 3 (but not of type 2), serine sulfhydrase is evidently incapable of utilizing, as cosubstrate, sulfite or a second molecule of cysteine[2].

The rate of cystathionine synthesis from L-serine (or L-cysteine) and homocysteine by serine sulfhydrase is several times higher than the rate of cysteine formation from L-serine and S^{2-}. This justifies the conjecture that the main metabolic function of "serine sulfhydrase" may consist in the conversion of homocysteine to cystathionine.

Work is in progress to investigate the possible identity of serine sulfhydrase with cystathionine β-synthase[10].

Laboratory of Enzyme Chemistry,
Institute of Molecular Biology
of the U.S.S.R. Academy of Sciences,
Moscow (U.S.S.R.)

A. E. BRAUNSTEIN
E. V. GORYACHENKOVA
NGUYEN DINH LAC

1 E. A. TOLOSA, E. V. GORYACHENKOVA, R. M. KHOMUTOV AND E. S. SEVERIN, *Mol. Biol. (Russ.)*, 2 (1968) 769.
2 E. A. TOLOSA, N. K. CHEPURNOVA, R. M. KHOMUTOV AND E. S. SEVERIN, *Biochim. Biophys. Acta*, 171 (1969) 369.
3 K. SCHLOSSMANN AND F. LYNEN, *Biochem. Z.*, 328 (1957) 591.
4 J. BRÜGGEMANN, K. SCHLOSSMANN, M. MERKENSCHLAGER AND M. WALDSCHMIDT, *Biochem. Z.*, 335 (1962) 392.
5 J. BRÜGGEMANN AND M. WALDSCHMIDT, *Biochem. Z.*, 335 (1962) 408.
6 K. SCHLOSSMANN, J. BRÜGGEMANN, AND F. LIJNEN, *Biochem. Z.*, 336 (1962) 258.
7 A. SENTENAC AND P. FROMAGEOT, *Biochim. Biophys. Acta*, 81 (1964) 289.
8 NGUYEN DINH LAC, *Biokhimiya*, 34 (1969), in the press.
9 G. TOENNIES AND J. KOLB, *J. Anal. Chem.*, 23 (1951) 823.
10 F. C. BROWN, J. MALLADY AND J. A. ROSZELL, *J. Biol. Chem.*, 241 (1966) 5220.

Received October 31st, 1968