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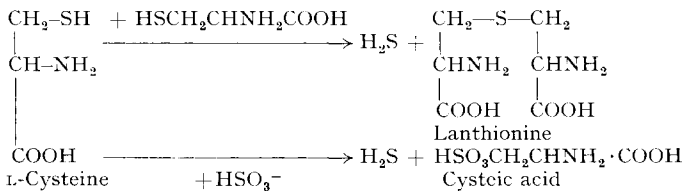
Reactions catalysed by cysteine lyase from the yolk sac of chicken embryo

Cysteine lyase (EC 4.4.1.1) is a pyridoxal phosphate-dependent enzyme discovered in the yolk sac of developing chicken embryos[1]; it has not yet been found in any other biological material.

In experiments with preparations of cysteine lyase purified 400-fold by our previously published procedure[2], the enzyme was shown to exhibit narrow substrate specificity towards L-cysteine. The purified cysteine lyase does not cleave cysteine to H_2S, pyruvate and ammonia by the α,β-elimination mechanism; it catalyses only reactions of replacement (substitution) at the β-C atom of L-cysteine, producing H_2S and lanthionine or (in the presence of added sulfite) H_2S and L-cysteic acid[3,4], according to Scheme 1:



(1)

The metabolic function of cysteine lyase in the developing embryo is apparently to provide for the biosynthesis of taurine *via* cysteic acid[1].

Since cysteine lyase can utilize either sulfite or a second cysteine molecule for replacement of the HS group in L-cysteine, it seemed plausible that the enzyme had no narrow specificity towards the cosubstrate interacting with cysteine. The following experiments were carried out to ascertain whether other thiol compounds could act as cosubstrates in reactions catalysed by cysteine lyase.

Test mixtures of 2 ml final volume were prepared, each containing 0.2 M Tris buffer (pH 8.5), 10 μg of enzyme, 10 μg of pyridoxal phosphate, 10^{-2} M L-cysteine, and 10^{-2} M lead acetate (as trapping reagent for H_2S). β-Mercaptoethanol or other thiol compounds (see Table I) were added in concentrations ranging from 1 to 20 mM.

TABLE I

THE AMOUNTS OF H_2S RELEASED BY HIGH-PURITY CYSTEINE LYASE FROM L-CYSTEINE IN THE PRESENCE OF VARIOUS THIOL COMPOUNDS

Thiol compound	*Concn. (mM)*	*Activity, H_2S (μmoles/mg)*
Control	—	1.08
β-Mercaptoethanol	1	2.00
	10	5.90
Thioglicolic acid	1	1.60
	10	2.30
β-Mercaptopropionic acid	1	1.50
	10	2.30
Dithiothreitol	1	2.01
	10	3.10

The samples were fixed by cooling after 10-min incubation at 39°, and the amount of colloidal PbS formed was measured immediately by spectrophotometry ($A_{360\ m\mu}$)[3,4]. The rates of cysteine desulfhydration are expressed in Table I as μmoles of H_2S released in 10 min by 1 mg of enzyme protein.

As seen from the data presented, the amounts of H_2S released by high-purity cysteine lyase from L-cysteine were markedly enhanced in the presence of various thiol compounds. No H_2S was formed from the thiols in the absence of cysteine or in complete control mixtures incubated with heat-denatured enzyme.

The other products formed from cysteine in the presence of β-mercaptoethanol or cysteamine were identified either by means of high-voltage electrophoresis (pH 1.9; 100 V/cm, 25 min) or by chromatography (solvent system: butanol–methanol–acetic acid–water, 40:40:10:20, by vol.) on "Whatman 1M" or "Leningrad M" filter paper.

In these experiments no lead acetate was added to the test mixtures, and non-reacted cysteine was precipitated with mercuric acetate prior to application of samples onto the filter paper.

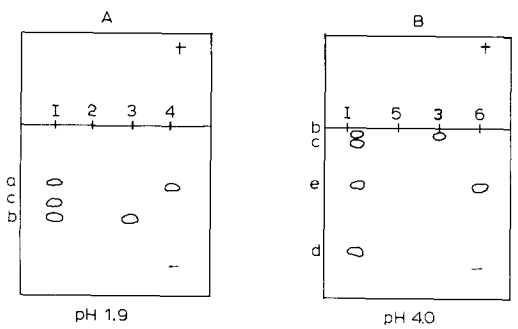


Fig. 1. Products formed by cysteine lyase from L-cysteine alone and in the presence of β-mercaptoethanol (A) or cysteamine (B). Scheme of electrophoretograms. Incubation period, 3 h. (Concentration of components and other experimental conditions as described in the text.) *Samples:* Control samples: heat-denatured enzyme with either cysteine and β-mercaptoethanol (2) or cysteine and cysteamine (5). Active enzyme with either cysteine (3), cysteine and β-mercaptoethanol (4) or cysteine and cysteamine (6). Standard solutions of reference compounds (1). *Spots*: a, *S*-hydroxyethylcysteine; b, lanthionine; c, cysteine; d, cysteamine, e, thialysine (All spots were positive to ninhydrin and to iodoplatinate reagent.)

The paper electrophoretograms and chromatograms were stained (a) with ninhydrin and (b) with iodoplatinate reagent[5].

Schematic drawings of the electrophoretograms are presented in Figs. 1A and 1B. It can be seen that the ninhydrin-positive products formed from L-cysteine are *S*-(2-hydroxyethyl)cysteine, in the presence of β-mercaptoethanol (A), and *S*-(2-amino-2-carboxyethyl)cysteine ("thialysine"), in the presence of cysteamine (B). Both compounds were identified by comparing the staining reactions and mobilities of their spots on chromatograms and electrophoretograms with those of synthetic *S*-hydroxyethylcysteine and thialysine, respectively, used as reference standards.

These experimental results confirm our conjecture as to the relative non-specificity of cysteine lyase with regard to the cosubstrate in the second reaction stage, when the enzyme-bound pyridoxylidene imine of L-cysteine[3] can react either with a second cysteine molecule or certain other thiol compounds, producing the

corresponding thioethers of cysteine, or with sulfite to yield cysteic acid, according to Scheme 2:

$$HSCH_2\text{–}CHNH_2\text{–}COOH + RH \rightarrow RCH_2\text{–}CHNH_2\text{–}COOH + H_2S \quad (2)$$

where R = $-SCH_2-CHNH_2-COOH$, $-SCH_2CH_2OH$, $-SCH_2CH_2NH_2$, or $-SO_3H$.

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

E. A. TOLOSA
N. K. CHEPURNOVA
R. M. KHOMUTOV
E. S. SEVERIN

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