PURIFICATION AND SPECIFICITY OF THE C-S-LYASE OF *ALBIZZIA LOPHANTA*

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(Received December 16th, 1959)

SUMMARY

Purification of the L-cysteine C-S-lyase of *Albizzia lophanta* seed endosperm has resulted in a 200-fold increase in specific activity as compared with the original extract. The enzyme is stable above pH 6.0 and below 60°, is activated by pyridoxal phosphate, and acts optimally on S-ethyl-L-cysteine at pH 8.0.

A survey of the specificity pattern of the enzyme with 40 potential substrates demonstrate that whereas the L-cysteine moiety, unaltered except for addition of oxygen to the sulfur atom and substitution of the hydrogen of the thiol group, is an absolute requirement for susceptibility to enzyme action, all or almost all S-substituted L-cysteine derivatives tested may serve as substrates, although there is considerable variation in the rate of hydrolysis to free thiol, pyruvate, and ammonia. In general it appears that those S-substituents whose structure suggest interference (either by hydrogen bonding or by steric hindrance) with the formation of a hydrogen bond (or chelate) between the hydroxyl at the 3-position of pyridoxal phosphate and the cysteine-derived group of the Schiff's base are poor substrates.

The utility of C-S lyases in the preparation, elucidation of structure and their possible role in the metabolism of sulfur compounds of biochemical interest are discussed.

INTRODUCTION

The occurrence of enzymes in higher plants capable of hydrolyzing S-substituted derivatives of L-cysteine has been demonstrated in bulbs of the genus *Allium*[1,2] and in the seed endosperm of the ornamental shrub *Albizzia lophanta*[3,4]. The former enzyme (alliinase) is associated in the fleshy tissue of the bulb with relatively large amounts of alliin (and other S-alkenyl and S-alkyl substituted cysteine sulfoxide derivatives) which interact when the tissue is damaged to yield pyruvate, ammonia and presumably unstable alkenyl or alkyl sulfenic acids which are further converted into the corresponding thiolsulfinates (allicin a.o.). The latter enzyme, upon the addition of water, interacts with djenkolic acid present in the endosperm to yield pyruvate, ammonia and highly odiferous substances, presumably methane dithiol and its decomposition products[3].

Although pyridoxal phosphate appears to be the coenzyme for both enzymes[3,5],

alliinase hydrolyses only S-substituted derivatives of L-cysteine sulfoxides whereas the C-S-lyase of *Albizzia* appears to exhibit a much broader specificity. Recently the present authors found that this enzyme can convert S-(2,4-dinitrophenyl)-L-cysteine to pyruvate, ammonia and 2,4-dinitrothiophenol[4]. The spectral difference between the substrate and the product in this reaction has served as the basis for the development of a continuous spectrophotometric assay of the enzyme. This assay has been utilized as a guide in its partial purification, procedures for which are presented in the present paper which also reports on some of the properties and on the specificity of action of this enzyme. The results suggest that the variation in reactivity of a large number of S-substituted L-cysteine derivatives can be interpreted in terms of the ability of the side groups of the S-substituents to interfere with formation of a stabilizing hydrogen bond (or chelate) between the amino group and the pyridine hydroxyl of the Schiff's base formed from the interaction of pyridoxal phosphate and the substrate.

MATERIAL AND METHODS

Enzyme source

Seeds of *Albizzia lophanta* were obtained locally from a commerical seed supply firm under the name of *Acacia lophanta*.

Enzyme activity

Details of the method for assaying enzyme activity are given in a recent publication[4]. The assay is based upon the change in absorbancy at 400 mμ due to the enzymic conversion of S-(2,4-dinitrophenyl)-L-cysteine to 2,4-dinitrothiophenol, (after correction for non-enzymic transarylation to N-(2,4-dinitrophenyl)-L-cysteine) at pH 6.5. One unit of activity is defined as that amount of enzyme in 3 ml of reaction mixture in a 1-cm cuvette which will cause the reaction to proceed at an initial rate of 0.054 O.D. units/min corresponding to conversion of 10^{-5} mmoles of substrate.

Enzyme activity was also estimated from the amount of pyruvate formed determined according to the method of MATSUO AND GREENBERG[6] with the following modifications. An aliquot of enzyme digest not greater than 0.1 ml was added directly to the 2,4-dinitrophenylhydrazine reagent and the final color was measured with an EEL colorimeter (green filter). One unit of enzyme will release 0.186 μmoles of pyruvate/min at 37° in 1 ml of enzyme reaction mixture containing an excess (0.05 *M*) of L-djenkolic acid, 0.02 μmoles pyridoxal phosphate and 0.04 μmoles of borate buffer at pH 8.0.

Potential substrates

A collection of amino acids was obtained from (a) commercial sources, (b) through synthesis and through the generous gifts of the following individuals; (c) G. ALDERTON, (d) J. A. V. ARNSTEIN, (e) R. GMELIN, (f) D. M. GREENBERG, (g) J. B. NEILANDS, (h) H. TUPPY, (i) W. C. J. ROSS, (j) R. G. WESTALL and (k) R. M. ZACHARIUS. The letters in parentheses are used in a subsequent table to identify the source of each compound.

The following substrates were prepared in this laboratory. S-(2,4-dinitrophenyl)-L-cysteine and 2,4-dinitrothiophenol were synthesized according to procedures previously described[4,7]. The heretofore unknown S-benzyl-N-benzoyl-L-cysteine was

prepared by treating 3,1 g of S-benzyl-L-cysteine hydrochloride with benzoyl chloride in alkali at 0° and was isolated by acidification and filtration. The precipitate was washed with water and dried *in vacuo* to give 3.0 g of crude product which was purified by recrystallization, first from aqueous ethanol and then from a mixture of ethyl acetate and petroleum ether. An analytical specimen had m.p. 117° (uncorr.) and the following rotations $[\alpha]_D^{24}$ —39.9° (0.5 *N* NaOH), $[\alpha]_D^{24}$ —79.1° (EtOH). (Calc. for $C_{17}H_{17}O_3NS$: C 64.73; H 5.43; N 4.44. Found: C 64.99; H 5.43; N 4.03.)

The diastereoisomeric pair of S-benzyl-DL-thiothreonines, A and B, were prepared from the corresponding N-benzoyl-S-benzyl-DL-thiothreonines. 5 mg of each of these compounds was hydrolyzed in 5 ml of 6 *N* HCl in sealed glass tubes for 2 h at 110° and cooled. The hydrolysates were then passed through a small column containing Dowex-50 which was then washed with water and the amino acids were eluted with 2*N* ammonia. The eluates, after extraction with ether, were evaporated to dryness to yield 2 to 3 mg each of products, both of which exhibited an R_F of 0.73 on paper chromatograms (Whatmann No. 1 filter paper; solvent: *n*-butanol–acetic acid–water (120:30:50); spots detected with ninhydrin reagent). These thiothreonine derivatives were found to be completely refractory to the action of carboxypeptidase. S-ethyl-L-cysteine sulfone was prepared by treatment of 50 mg of S-ethyl-L-cysteine for 24 h at room temperature with 10 ml of 1 % hydrogen peroxide in 99 % formic acid containing 0.001 *M* molybdate. The resulting sulfone was precipitated and washed with ether and dried. A sample, repeatedly recrystallized from 80 % ethanol, had m.p. 180° (uncorr., decomp.). According to paper chromatography in *n*-butanol–acetic acid–water (120:30:50) a slight contamination with the corresponding sulfoxide proved very difficult to remove. The employed specimen, however, was estimated to be more than 98 % pure.

Cysteine derivatives of isobutyric acid were prepared by coupling free cysteine with β,β'-diiodoisobutyric acid (prepared according to the method of CORSE AND JANSEN[8]) in aqueous alkali. This procedure was not entirely satisfactory and better results attended attempts to condense free L-cysteine methyl ester, liberated *in situ* from its hydrochloride by CH_3ONa, and the methyl ester of β,β'-diiodoisobutyric acid in methanol solution containing an additional molecular equivalent of sodium methoxide. The latter ester was used as the oily reaction product formed upon treatment of the diiodoacid with excess of diazomethane in ethereal solution. The coupling proceeded at room temperature and the crude reaction product was saponified with aqueous NaOH for a few hours at room temperature, then neutralized by addition of HI. The anhydrous residue was then repeatedly extracted with absolute ethanol in order to remove NaI and other impurities. The resulting material did not possess any well-defined melting point. It could be purified by repeated precipitations from aqueous solutions at pH 7 by addition of ethanol. The resulting preparation was devoid of free cysteine (nitroprusside negative before and after cyanide) and served as a substrate for *Albizzia* C-S lyase, yielding free thiol, pyruvate and ammonia. However, paper chromatography in *n*-butanol–acetic acid–water (120:30:50) revealed the presence of two ninhydrin positive spots of which the fastest running was undoubtedly the desired condensation product, 3,3'-bis-(2-carboxy-2-aminoethylthio)-isobutyric acid. Various observations suggested that the tenaciously adhering contamination was the analogous 3-(2-carboxy-2-aminoethylthio)-3-hydroxyisobutyric acid formed by competitive hydrolysis of the diiodoacid.

RESULTS

Enzyme purification

200 g of *Albizzia lophanta* seeds were coarsely ground in a coffee mill and then shaken with trichloroethylene. Seed coat settled to the bottom of the container and endosperm, which floated to the top, was removed and dried overnight in the air to remove solvent. The endosperm was ground to give 90 to 95 g of fine meal which was then extracted with 360 ml of water for 1 h at room temperature under a hood. The suspension was squeezed through cheese cloth to yield 290 ml of Fraction A. The pH of fraction A was adjusted to 5.5 (from pH 7.3) with 6 ml of 1 *N* HCl, and the suspension was centrifuged at 2,100 × *g* for 30 min to yield 210 ml of supernate (Fraction B). Fraction B was neutralized with 1 *N* NaOH and heated to 65 ± 3° for 10 min, cooled and then centrifuged at 2,100 × *g* to yield 200 ml of supernate (Fraction C). To Fraction C was added 60 g of ammonium sulfate (0.42 saturated), the suspension was centrifuged at 2,100 × *g* for 30 min and the resulting precipitate was taken up in water to give 25 ml of Fraction D. To Fraction D was added saturated ammonium sulfate to 0.42 saturation at pH 6.54. The resulting precipitate was centrifuged and dissolved in water to give 15 ml of Fraction E which was then dialyzed for 48 h at 5° against distilled water. The precipitate, which started to form after 16 h of dialysis, was centrifuged off at 4,000 × *g* and lyophilized to yield 150 mg of Fraction F. Table I shows the total and specific activity of each fraction of the purification procedure. A 17 % yield of enzyme representing a 70-fold increase in specific activity was obtained.

TABLE I

PURIFICATION OF *Albizzia* L-CYSTEINE C-S-LYASE

Fraction	*Description*	*Volume ml*	*Total units*	*Specific activity units/mg protein*
A	Water extract	290	208,000	3
B	Supernate, pH 5.5	210	70,100	12
C	Supernate, heating	200	66,300	19
D	Ppt, 1st $(NH_4)_2SO_4$	25	47,200	79
E	Ppt, 2nd $(NH_4)_2SO_4$	15	43,900	145
F	Dialysis, lyophilize	—	35,000	210
G	Alumina treatment of Fraction D	20	5,000	680

Further purification could be achieved by adsorption on and elution from alumina. To 25 ml of an enzyme preparation corresponding to Fraction D (Table I) was added 1.6 ml of alumina for chromatography (Sigma). The precipitate from the ensuing centrifugation was washed with 10 ml of water and then eluted with three successive 10 ml portions of 5.0, 9.3 and 13.8 % solutions of ammonium sulfate solutions, respectively. The last two fractions contained about 10 % of the starting activity (Fraction G, Table I) and had specific activities of 643 and 700 units/mg of protein nitrogen, respectively. The turnover number of the most active fraction has been calculated as 13,000 molecules of pyruvate produced from L-djenkolic acid per minute per molecule of enzyme of molecular weight = 100,000 at 37° and pH 8,1 in the presence of $2 \cdot 10^{-5}$ *M* pyridoxal phosphate, a value which compares favorably with the

turnover number of 2,340 obtained by MATSUO AND GREENBERG[6] for the action of liver cystathionase acting on DL-cystathionine.

Properties of crude and purified preparations

Enzyme activity could be detected only in the endosperm. Extracts of the seed coat did not affect the activity of the endosperm extracts. These seed coat extracts solidified to a gel on standing overnight, thus indicating the presence of a strong pectin-pectinesterase system. The density of the seed coat was much greater than that of the endosperm, thus allowing their separation by flotation in inert solvents of relatively high density with little loss of activity. However, the trichloroethylene extractible material exhibited appreciable activity after removal of the solvent, amounting to about 1 % of the total activity present in a water extract of the endosperm.

Centrifugation of the crude extract, after squeezing through cheese cloth, resulted in a residue consisting of a layer of starch granules beneath a loosely packed yellow sediment and a milky yellow supernate. The material responsible for the opalescence could not be sedimented at 18,000 × *g*, readily passed through Celite pads and prevented heat and salt precipitation of the protein. After adjustment of the pH from 7.3 to 5.5, this colloidal material could be almost quantitatively removed by centrifugation at moderate speeds.

The enzyme in the extract was quite unstable below pH 5, *i.e.*, about 50 % of the activity was lost after 40 min at pH 4.0 and 5 %. The loss of activity upon heating the extract at 60°, 70° and at 80° amounted to 5 %, 12 % and 25 %, respectively.

The final preparation (corresponding to Fraction E, Table I)was quite stable. Thus a 0.3 % solution of the purified enzyme in 0.05 *M* phosphate at pH 6.5 retained 80 % of its original activity after 5 months at 5°. The above values on stability were found using the spectrophotometric assay method with S-(2,4-dinitrophenyl)-L-cysteine as substrate[4].

The effect of pH on the activity of the enzyme with S-ethyl-L-cysteine as substrate is shown in Fig. 1. The enzyme exhibited maximal activity between pH 7.8 and 8.2 and was completely inactive below pH 5.0.

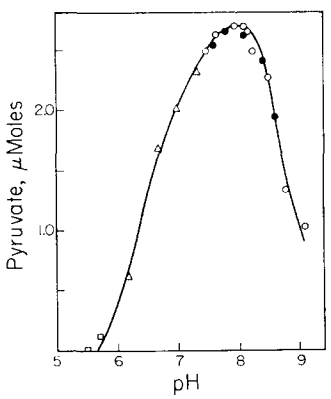

Fig. 1. Effect of pH on activity of *Albizzia* C-S lyase. 1 ml of reaction mixture contained 40 μmoles S-ethyl-L-cysteine, 40 μmoles buffer, 0.05 μmole pyridoxal phosphate and 4.5 units of enzyme. Reaction run for 10 min at 37°. The following buffers were used: ○, borate; ●, tris; △, phosphate; □, acetate.

Table II shows the effects of pyridoxal phosphate, mercaptoethanol and ethylenediamine tetra-acetate (EDTA) on the activity of the enzyme with S-ethyl-L-cysteine as substrate. Of these three substances only pyridoxal phosphate caused an increase in the rate of hydrolysis of the substrate. In the absence of pyridoxal phosphate neither mercaptoethanol nor EDTA influenced the activity. In the presence of pyridoxal phosphate there does appear to be a significant inhibition by these substances.

TABLE II

EFFECT OF MERCAPTOETHANOL AND EDTA ON *Albizzia* C-S-LYASE ACTIVITY

1 ml reaction mixture contained 40 μmoles of S-ethyl-L-cysteine, and 4.5 units of enzyme at pH 8.0 (borate buffer, 0.04 *M*) in the absence of EDTA and pH 7.8 in the presence of EDTA.

Substance added	*μmoles of pyruvate liberated in 10 min at 37°*	
	No pyridoxal phosphate	*Plus pyridoxal phosphate ($5 \cdot 10^{-5}$ M)*
None	2.10	2.82
Mercaptoethanol 0.007 *M*	2.02	2.40
EDTA 0.005 *M*	2.18	2.20
Mercaptoethanol + EDTA	1.60	1.95

TABLE III

EFFECT OF PYRIDOXAL PHOSPHATE ON C-S-LYASE ASSAY SYSTEM

Enzyme units	*Pyridoxal phosphate $M \times 10^{-5}$*	*Increase in absorbancy after**		
		5 min	*9 min*	*30 min*
1.00	0	0.280	0.435	1.03
1.00	1.8	0.315	0.483	—
1.00	18	0.340	0.560	—
0.82	18	0.282	0.430	1.02
1.00**	1.8	0.310	—	—
1.00***	1.8	0.309	—	—

* Reaction mixture which contains S-(2,4-dinitrophenyl)-L-cysteine as substrate, and procedures are those described for the enzyme assay[5].

** Pyridoxal phosphate incubated with substrate 10 min prior to addition of enzyme.

*** Pyridoxal phosphate incubated with enzyme 10 min prior to addition of substrate.

Table III shows the effect of pyridoxal phosphate on the enzyme activity with S-(2,4-dinitrophenyl)-L-cysteine as substrate. The increase percentage in initial velocity was about that observed with S-ethyl-L-cysteine as substrate. When the amount of enzyme in the presence of pyridoxal phosphate was decreased to give an initial velocity equal to that in its absence, no difference in the subsequent course of hydrolysis was observed. Prior incubation of pyridoxal phosphate with either substrate or enzyme did not alter its activating effect.

Specificity

The specificity pattern of the C-S-lyase is shown in Table IV. It appears that the L-cysteine moiety, unaltered except for substitution of the hydrogen of the thiol group and addition of oxygen to the sulfur atom, is an absolute requirement for susceptibility to enzyme action. Thus the following changes resulted in compounds which

TABLE IV

SPECIFICITY OF *Albizzia* C-S-LYASE

Reaction mixture at pH 8.1 (0.04 *N* borate) and 37° contained per ml: 10 μmoles of substrate, 0.02 μmoles of pyridoxal phosphate, 14 units (70 μg) enzyme (fraction E).

Substrate	*Pyruvate μmoles/10 min*	*Substrate*	*Pyruvate μmoles/10 min*
D-cy* (a)**	0.00	Cy-isobutyric acid (b)	2.74
L-cy (a)	1.10	S-(2,4-dinitrophenyl)-L-cy (b)***	0.19
S-methyl-L-cy (e)	4.76	Porphyrin *c* (synth.) (g)§	0.01
S-methyl-L-cy sulfoxide (e)	2.44	L-homocysteine (a)	0.00
S-ethyl-L-cy (e)	5.44	L-methionine (a)	0.00
S-ethyl-L-cy sulfone (b)	0.94	L-penicillamine (d)	0.00
S-propyl-L-cy (e)	6.00	S-benzylthiothreonines A and B (d)§§	0.00
S-benzyl-L-cy (e)	6.90	α-methyl-DL-cy (h)	0.00
Felinine (i)	0.60	N-benzoyl-S-benzyl-L-cy (b)	0.00
S-β-chloroethyl-L-cy (h)	3.92	S-benzyl-α-methyl-DL-cy (h)	0.00
S-β-bromoethyl-L-cy (b)	2.91	Glutathione (a)	0.00
S-β-carboxymethyl-L-cy (b)	0.40	S-methylglutathione (k)	0.00
S-β-carboxyethyl-L-cy (e)	0.26	Serine (a)	0.00
L-lanthionine sulfoxide (e)	1.35	Homoserine (f)	0.00
L-lanthionine (e)	0.75	Threonine (a)	0.00
β-methyl lanthionine (ex subtilin) (c)	0.26		
β,β-dimethyl lanthionine (d)	0.09		
DL-allocystathionine	0.25		
L-djenkolic acid (a)	6.25		
Methyl djenkolic acid (e)	3.20		
Isopropyldjenkolic acid (e)	0.81		
Isobutyldjenkolic acid (e)	0.19		

* Cy, abbreviation for cysteine.

** Letters in parentheses refer to source of compound as mentioned in MATERIALS AND METHODS.

*** Reaction mixture at 25° contained 0.2 μmole/ml of substrate at pH 6.5.

§ 1 ml digest at 37° and pH 8.1 contained 0.5 μmole substrate, 48 units of enzyme and 0.02 μmoles of pyridoxal phosphate.

§§ N-benzoyl-S-benzyl-L-thiothreonines and S-benzyl-L-thiothreonines used.

are not substrates: introduction of CH_2 into the chain (homocysteine); substitution of the α-hydrogen (α-methyl-DL-cysteine); substitution of one or more β-hydrogens (S-benzyl thiothreonine, penicillamine); substitution on the nitrogen (S-benzyl-N-benzoyl-L-cysteine, glutathione, S-methyl glutathione); replacement of the sulfur with oxygen (serine).

Alkylation of the sulfur atom increased the rate of hydrolysis (propyl > ethyl > methyl > hydrogen). Carboxylation of the terminal end of the S-alkyl group resulted in a drastic reduction of susceptibility to enzyme action, *i.e.*, compare S-ethyl-L-cysteine with β-carboxyethyl-L-cysteine and lanthionine. Halogenation resulted in only a moderate reduction in susceptibility. On the other hand, substitution of S-alkyl-α-hydrogen by an S-cysteine group resulted in an increase in the rate of hydrolysis as exemplified by L-djenkolic acid. It will be noted that another member of this category, "cysteine-isobutyric acid", was also cleaved rapidly.

In general it appears that substitution at the carbon atom, α to the sulfur in the S-substituent, decreased the susceptibility to hydrolysis. Thus felinine, S-1,1-dimethyl-3-hydroxypropyl-L-cysteine[9] was a much poorer substrate than S-propyl-L-cysteine; methyllanthionine and dimethyllanthionine (unsymm.) are poorer substrates than lanthionine; the substituted djenkolic acids are less susceptible to hydrolysis than

was djenkolic acid; and synthetic porphyrin *c* (see ref. 10), apparently identical with that from cytochrome (see ref. 11) was hydrolyzed at a negligible rate. Terminal arylation of the alkyl chain enhanced the hydrolysis rate (S-benzyl-L-cysteine) and direct arylation of the sulfur atom does not prevent hydrolysis (S-(2,4-dinitrophenyl)-L-cysteine).

DISCUSSION

While the presence of the L-cysteine moiety in the substrate is an absolute requirement for action of *Albizzia* C-S-lyase, it appears that in general all or most S-substituted L-cysteines may serve as substrates although there is a considerable variation in the rate of hydrolysis, depending upon the structure of the S-substituent. Although many factors probably enter into the determination of susceptibility to hydrolysis as a function of structure of the S-substituent, inspection of molecular models of the Schiff's base formed from interaction of the L-cysteine derivatives with pyridoxal phosphate suggest a rational basis for this variation, *viz.*, the substitution of carboxyl groups in the terminal end of the S-alkyl-substituents and the introduction of branching groups in the carbon atom α to the sulfur, interfere with hydrogen bond (or chelate) formation between the hydroxyl at the 3-position of the pyridoxal and the imino nitrogen of the Schiff's base derived from the α-amino group of the amino acid. According to SNELL[12] this stabilizes the aldoxime structure, and otherwise facilitates the subsequent removal of electrons to the pyridine nitrogen, thereby labilizing the α,β-bond in the amino acid. The terminal carboxyl is present in a spatial relation to the imino nitrogen in such a manner that it is capable of forming a hydrogen bond with the latter, thus competing with the pyridine-substituted hydroxyl, whereas the position of the α-substituents suggests steric hindrance to the formation of such a bond. A model of lanthionine sulfoxide, which is a much better substrate than lanthionine, reveals that the bond angle of the sulfur is altered to a degree such that the above-mentioned hydrogen bond formation is much less likely than in lanthionine. A similar relation holds for the carboxyl groups of djenkolic acid.

In connection with the reactivity of the L-cysteine sulfoxides, of which there exist two stereoisomers, due to the asymmetry of the SO group, STOLL AND SEEBECK[1] found that alliine ((+)S-allyl-L-cysteine sulfoxide) was hydrolyzed more rapidly than (−)-S-allyl-L-cysteine sulfoxide by garlic alliinase. This finding is an exception to the general rule that either only one of two stereoisomeric forms of a substance serves as substrate for an enzyme or that the two isomers serve equally well as substrates if the optically active center is not involved in the enzyme action. Molecular models of the two isomers of the Schiff's base of these diastereoisomers show that the S-oxygen of one form interferes to a greater extent with the above-mentioned chelate or hydrogen bond formation than does the alternate form.

The extremely high rate of hydrolysis of S-benzyl-L-cysteine suggests that the benzene ring enhances the electron-attracting function of the heterocyclic nitrogen, thus facilitating electron displacement from the α-carbon atom.

The specificity pattern exhibited by liver cystathionase[13] differs strikingly from that of *Albizzia* C-S-lyase in that (a) the former forms cysteine and α-ketobutyric acid from L-cystathionine (b) homoserine and homocysteine are substrates for cystathionase but not for C-S-lyase; (c) the S-alkyl-L-cysteines are much poorer substrates for cystathionase than are L-djenkolic acid and cystathionine. These differences may

reflect differences in the mode of elimination. SNELL[12] considers cystathionase action as an example of γ-elimination whereas the *Albizzia* C-S-lyase action can best be explained as proceeding via an α,β-elimination mechanism. The enzyme also differs from cystathionase in its pH optimum and in the effect of mercaptoethanol on the activity[14].

The properties of *Albizzia* C-S-lyase indicate that it may find some use in the preparation and elucidation of the structure of sulfur compounds of biochemical interest. Thus the refactoriness of synthetic porphyrin *c* indicates substitution at the carbon atom of the porphyrin moiety α to the sulfur atom and thus constitutes corroborative evidence for the identity of this compound with that obtained from cytochrome *c*. The thiol resulting from the action of this enzyme on felinine (1,1-dimethyl-3-hydroxypropanethiol) may be of interest in mevalonic acid metabolism. Action of this enzyme on the β-methyl lanthionines from subtilin[15] and on that from yeast[16] should yield thiothreonines A and B[17], the absolute configurations of which have recently been established by ARNSTEIN[18]. Thus the absolute configuration of the substituted β-carbon of these two lanthionines can likewise be established. Correspondingly, enzymic hydrolysis of *meso*-lanthionine might be employed for the preparation of D-cysteine. The rapid hydrolysis of the dicysteine derivative of β,β-dithiolisobutyric acid to the free dithiol (which occurs in asparagus[19]) suggests a possible pathway of biosynthesis of this and other naturally occurring dithiols such as lipoic acid. MELVILLE *et al.*[20], showed that only the sulfur of cysteine is incorporated into ergothioneine, a finding which is consonant with the possibility of C-S-lyase action in the biosynthesis of this compound.

ACKNOWLEDGEMENT

One of us (S.S.) is a senior Postdoctoral Fellow, National Science Foundation, U.S.A., on leave from the Western Regional Research Laboratory, U.S. Department of Agriculture, Albany, Calif. (U.S.A.)

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