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Cysteine Conjugate β-Lyase in the Gastrointestinal Bacterium *Eubacterium limosum*

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SUMMARY

A cysteine conjugate β-lyase (β-lyase) from the gastrointestinal bacterium *Eubacterium limosum* has been isolated and characterized. This organism has the highest specific activity for cysteine conjugate β-lyase of the gastrointestinal bacteria studied. The β-lyase was found to cleave the thioether linkage of *S*-alkyl- and *S*-aryl-L-cysteine conjugates. Stoichiometric amounts of 2-mercaptobenzothiazole, pyruvic acid, and ammonia were produced from the β-lyase cleavage of *S*-(2-benzothiazolyl)-L-cysteine. The enzyme activity was inhibited by hydroxylamine, iodoacetic acid, or KCN. The enzyme appears to be a 75,000-Da dimer of two 38,000-Da subunits. A natural substrate, cystathionine, was cleaved by this enzyme, indicating that this β-lyase has β-cystathionase activity. These data suggest that a β-cystathionase from *E. limosum* may be an important enzyme in the metabolism of a wide range of cysteine conjugates of xenobiotics to thiol-containing products.

During the past five years, a novel pathway (Fig. 1) has been described in pig and rat for the introduction of a thiol, methylthio, methylsulfinyl, or methylsulfonyl moiety into a xenobiotic by a process which involves mammalian mercapturic acid synthesis and the enteric-bacterial enzyme cysteine conjugate β-lyase [E.C. 4.4.1.13] (1–6). Sulfur from glutathione is introduced at electrophilic centers in xenobiotics by glutathione-*S*-transferase. The resulting glutathione conjugate undergoes sequential enzymatic hydrolysis (7, 8) to yield the *S*-cysteine conjugate which, in turn, is acetylated to form a mercapturic acid (9). These metabolites (termed the mercapturic acid pathway metabolites) are excreted in the bile and can be converted to their corresponding cysteine conjugate in the gastrointestinal tract (3). The β-lyase in the gastrointestinal tract is a key enzyme in the methylthiolation pathway because it converts *S*-cysteine conjugates of xenobiotics to thiol-containing metabolites. Thiols are generally considered to be toxic (10) and are thought to be precursors for nonextractable residues in feces. These thiols are also precursors for *S*-glucuronide and for methylthio-, methylsulfinyl-, and methylsulfonyl-containing xenobiotics (10–12).

β-Lyase enzymes have been isolated, characterized, and partially purified from *Fusobacterium necrophorum* (4) and *Fusobacterium varium* (5). In a recent study, β-lyase enzyme was detected in 27 of 43 of the intestinal bacteria tested, indicating a general distribution of β-lyase among gastrointestinal bacteria. β-Lyases isolated from these bacterial sources catalyzed the cleavage of *S*-aryl- or *S*-alkyl-linked cysteine conjugates of xenobiotics (6). One of the bacteria in the distribution study, *Eubacterium limosum*, had considerably higher specific activity (16-fold) than did other gastrointestinal bacteria. *E. limosum* occurs in the normal flora of man, poultry, and ruminants (13). This report describes isolation and characterization of the β-lyase from *E. limosum*. This enzyme cleaves *S*-alkyl- and *S*-aryl-L-cysteine conjugates of xenobiotics, as well as the naturally occurring substance cystathionine.

Materials and Methods

Eubacterium limosum (VPI strain 54) was obtained from the culture collection of Dr. Milton J. Allison of the National Animal Disease Laboratory (Ames, IA) and was used as the source for the β-lyase. The bacterium was grown at 37° under anaerobic conditions (100% CO_2 gas phase) in 3 liters of peptone, yeast extract, glucose broth (13). Growth was determined by optical density measurements in 18 × 150 mm tubes at 600 nm (Bausch and Lomb Spectronic 20). Cells were harvested at the end of the exponential growth phase (0.6% inoculum, 18–20 hr incubation, absorbance ~0.50) with a continuous flow centrifuge (Sorval model RC-2). The bacteria were washed with 2 liters of deionized water and frozen with liquid nitrogen. The wet weight yield was about 2 g of cells/liter of culture medium.

The synthesis, purification, and purity of the cysteine conjugate of propachlor (2-*S*-cysteinyl-*N*-isopropylacetanilide), hereafter referred to as the propachlor conjugate, has been described previously (4). The *S*-oxide of the propachlor conjugate (L-alanine-3-[[2[(1-methylethyl)phenylamino]-2-oxoethyl]-sulfinyl]) was synthesized by mixing

[1] No warranties are herein implied by the U. S. Department of Agriculture.

ABBREVIATIONS: FAB, fast atom bombardment; CBZ, *S*-2-(benzothiazolyl)cysteine; SDS, sodium dodecyl sulfate; TLC, thin layer chromatography.

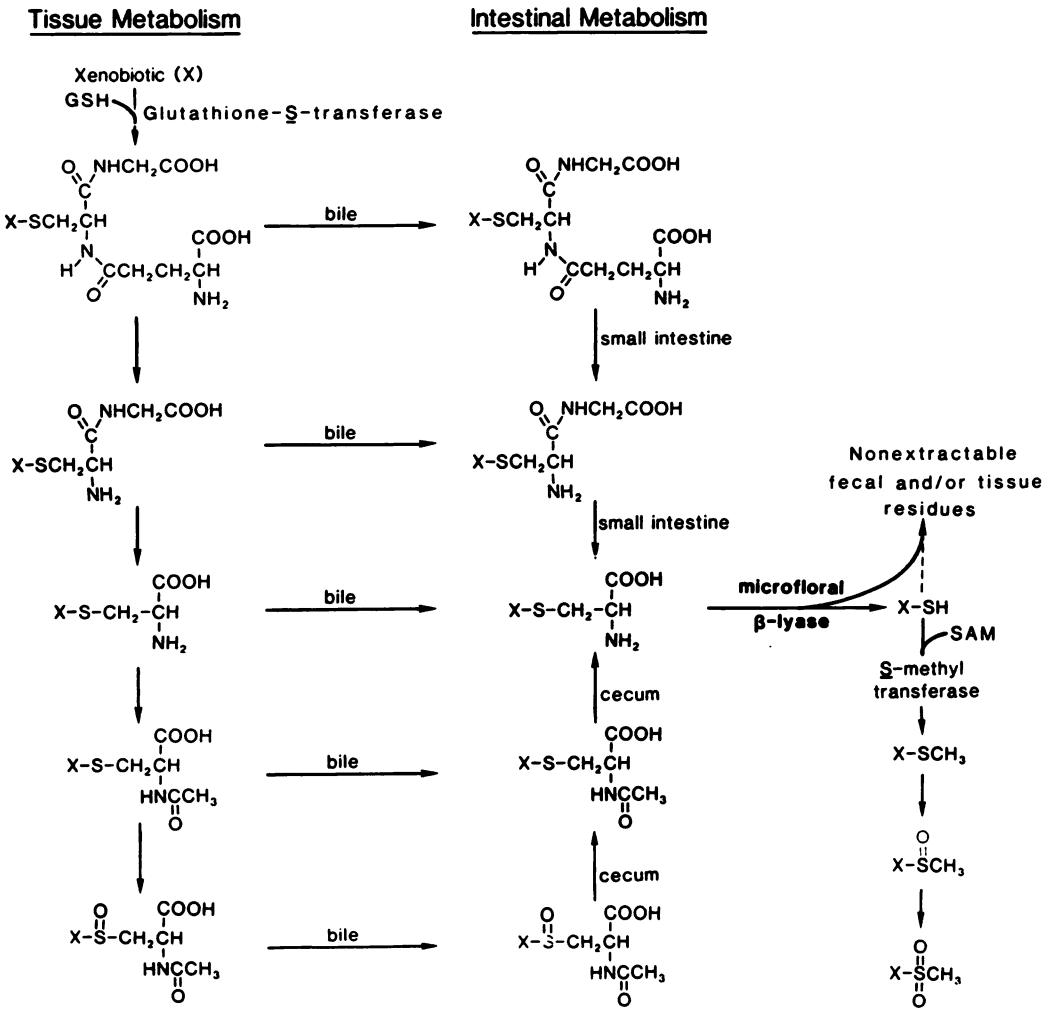

Fig. 1. Pathway leading to the formation of thiol-containing xenobiotics in the gastrointestinal tract and subsequent formation of methylthio-containing xenobiotics from the mercapturic acid pathway.

0.1 mmol of the propachlor conjugate with 3 ml of 30% (w/v) H_2O_2 in 66% acetone (total volume of 46 ml) and reacted for 18 hr at room temperature. The *S*-oxide of the propachlor conjugate was purified by chromatography on Sephadex LH-20 (Pharmacia) with water (3). FAB mass spectra on the *S*-oxide of the propachlor conjugate were obtained using a Varian Mat CH-5 DF mass spectrometer equipped with an FAB gas gun (saddle-field ion source B12b; Ion Tech Ltd., Middlesex, England). The sample (5 μg) was dissolved in a thin layer of glycerol (0.2 μl) on the probe tip and introduced into the mass spectrometer source. The major ions observed in the negative FAB mass spectrum were at m/z = 134 (100% relative abundance), 139 (75), 112 (45), 118 (38), 238 (30), 123 (30), 198 (21), and 311 (10, M − H). In the positive FAB mass spectrum, major ions were observed at m/z = 313 (100% relative abundance, M + H), 226 (89), 174 (88), 135 (47), 120 (43), 335 (20, M + Na), 162 (12), 297 (11), and 206 (8.5). Synthesis and purification of CBZ has been described previously (14). *trans*-9-Hydroxy-10-[*S*-(L-cysteinyl)]-9,10-dihydrophenanthrene was synthesized by the reaction of phenanthrene-9,10-oxide with cysteine using a modification of the procedure of Hylarides *et al.* (15) and purified using high pressure liquid chromatography.

S-Benzyl-L-(*N*-acetyl)cysteine was synthesized and purified by the procedure of van Bladeren *et al.* (16), and *S*-1,2-dichlorovinyl-D-cysteine and *S*-1,2-dichlorovinyl-L-cysteine were synthesized and purified as described previously (14).

Other compounds used in this study were purchased as follows: L-cystathionine, L-cystine, and gabaculine from Calbiochem (La Jolla, CA); β-chloro-D-alanine from Vega-Biochemical (Tucson, AZ); β-chloro-L-alanine, L-homoserine, L-kynurenine, DL-lanthionine, *S*-ethyl-L-cysteine, DL-vinylglycine, DL-*C*-proparagylglycine, *O*-phospho-L-serine, *O*-acetyl-L-serine, L-serine-*O*-sulfate, α-ketobutyric acid, L-histidine monohydrochloride monohydrate, and cysteine from Sigma Chemical Co (St. Louis, MO); and *S*-benzylcysteine from Aldrich Chemical Co. (Milwaukee, WI).

Enzyme Assays

Cysteine conjugate β-lyase activity was measured by one of two methods. The standard assay for the enzyme utilized the procedure of Stevens and Jakoby (14) in which CBZ was used as substrate and product was measured by the appearance of 2-mercaptobenzothiazole absorbing at 316 nm with a dual-beam Cary 219 spectrophotometer operated at 30° (CBZ assay). Under the conditions described, the reaction was linear for 3 min with respect to time and protein concentration when less than 20 nmol of product was formed per min.

All other substrates were assayed for β-lyase activity by measuring the rate of pyruvate formation (Cary 219, at 340 nm) at 30° using the procedure of Guggenheim (17) (NADH assay). Specific activity is defined in terms of units of activity per mg of protein.

Ammonia was determined using Nessler's reagent, as described previously (4). Kynureninase activity was assayed by the method of Takeuchi *et al.* (18) using 50 mM potassium phosphate (pH 7.8) rather than Tris buffer.

Gel filtration determination of molecular weight. The molecular weight of the enzyme was estimated using a Sephacryl S-200 column (2.2 × 85 cm) eluted with 0.1 M potassium phosphate buffer (pH 7.4) containing 50 μM pyridoxal 5-phosphate and calibrated with thyroglobulin (669,000), ferritin (440,000), aldolase (158,000), bovine serum albumin (67,000), and chymotrypsinogen A (25,000) as standards under identical conditions.

Electrophoresis. Nondenaturing-discontinuous gel electrophoresis was performed in tube gels, 7.5% acrylamide, in Tris-glycine, pH 8.3 (19). SDS gels were run under reducing conditions. Trypsin inhibitor (21,500), carbonic anhydrase (31,000), ovalbumin (45,000), bovine serum albumin (67,000), and phosphorylase B (93,000) served as standard globular proteins for the estimation of molecular weight. Both nondenaturing gels and SDS gels were stained with Coomassie blue as described by Maizel (19).

After nondenaturing electrophoresis, activity of the protein was assessed by cutting the cylindrical polyacrylamide gels into 1.5-mm slices which were then crushed in tubes containing 1 ml of 50 μmol potassium phosphate (pH 7.8), 50 nmol pyridoxal 5-phosphate and 1 μmol CBZ. Absorbance at 316 nm was used as a function of product formation.

Isoelectrofocusing

Isoelectrofocusing was conducted using an LKB 8100-1 apparatus in a 0–60% glycerol gradient of 2.5% ampholine (54%, LKB Ampholine 3.5–5.0; 46%, LKB Ampholine 5.0–8.0) as described previously by the manufacturer. Cathode solution was 0.04 M histidine (pH 7.6) and the anode solution was 0.1 M glycylglycine (pH 4.1) in 60% glycerol.

Thin Layer Chromatography

Silica gel TLC plates (0.25 mm thickness; MCB Manufacturing Chemists, Inc.) were used with two solvent systems: (a) ethylacetate:xylene:water:formic acid (35:1:1:0.25), and (b) ethylacetate:water:formic acid (35:1:0.05), for identification of the dinitrophenylhydrazine derivatives of α-keto acids.

Isolation of the thiols, high pressure liquid chromatography, derivatization, gas-liquid chromatography, and instrumentation have all been described previously (4).

Purification of Cysteine Conjugate β-Lyase

A typical purification of the β-lyase is shown in Table 1. Frozen *E. limosum* cells (70 g) were suspended in 0.05 M potassium phosphate buffer (1 g wet weight cells to 4 ml of buffer, pH 7.4). All procedures were carried out at 5° or in an ice bath. Because previous reports demonstrated that the addition of pyridoxal 5-phosphate during purification was required for stability of bacterial and mammalian β-lyases (4, 5, 14), pyridoxal 5-phosphate was included in all buffers unless specifically indicated.

Steps 1 and 2: Homogenization and extraction. The suspended bacterial cells were passed through a French pressure cell (55,200 kPa, two passes) and centrifuged at 10,000 × *g* for 15 min. The supernatant fluid was collected and centrifuged at 100,000 × *g* for 60 min. Little β-lyase activity was found in the 10,000 × *g* or 100,000 × *g* pellets. The 100,000 × *g* supernatant was used for further purification.

Step 3: Ammonium sulfate precipitation. Solid ammonium sulfate was added to the 100,000 × *g* supernatant with constant stirring to 0.55 saturation, and then was centrifuged at 22,000 × *g* for 15 min. The pellet was discarded and the supernatant was adjusted to 0.65 saturation with addition of ammonium sulfate and centrifugation as above. The pellet contained active β-lyase and was dialyzed overnight against 1 liter of 0.1 M potassium phosphate buffer (pH 7.4) containing 50 μM pyridoxal 5-phosphate (buffer A).

Step 4: DEAE-Sephacel. A column of DEAE-Sephacel (2.4 × 40 cm) was equilibrated with buffer A and charged with the active fraction from step 3. After washing with 150 ml of buffer A, a 500-ml linear gradient from 0.1 to 0.5 M potassium phosphate buffer (pH 7.4) containing 50 μM pyridoxal 5-phosphate (buffer B) was applied, followed by a 150-ml buffer B wash, and 8-ml fractions were collected (Fig. 2A).

TABLE 1

Purification of cysteine conjugate β-lyase from 70 g of *Eubacterium limosum*

Step	Procedure	Vol	Protein	Enzyme activity	Specific activity	Fold purified
		ml	*mg*	*units*[a]	*units/mg*	
1	Homogenate	464	13,000	1,100,000	86.2	
2	Supernatant ($10^5 \times g$)	432	8,530	1,210,000	142	1.6
3	$(NH_4)_2SO_4$ (55–65%)	21.2	515	646,000	575	6.7
4	DEAE-Sephacel	53.2	238	443,000	1,900	21.6
5	Sephacryl S-300	26.4	22.8	428,000	18,800	218
6	Hydroxyl apatite	2.4	2.10	93,700	46,600	540

[a] One unit equals 1 nmol of 2-mercaptobenzothiazole produced per min in 1.0 ml under the assay conditions described in Materials and Methods.

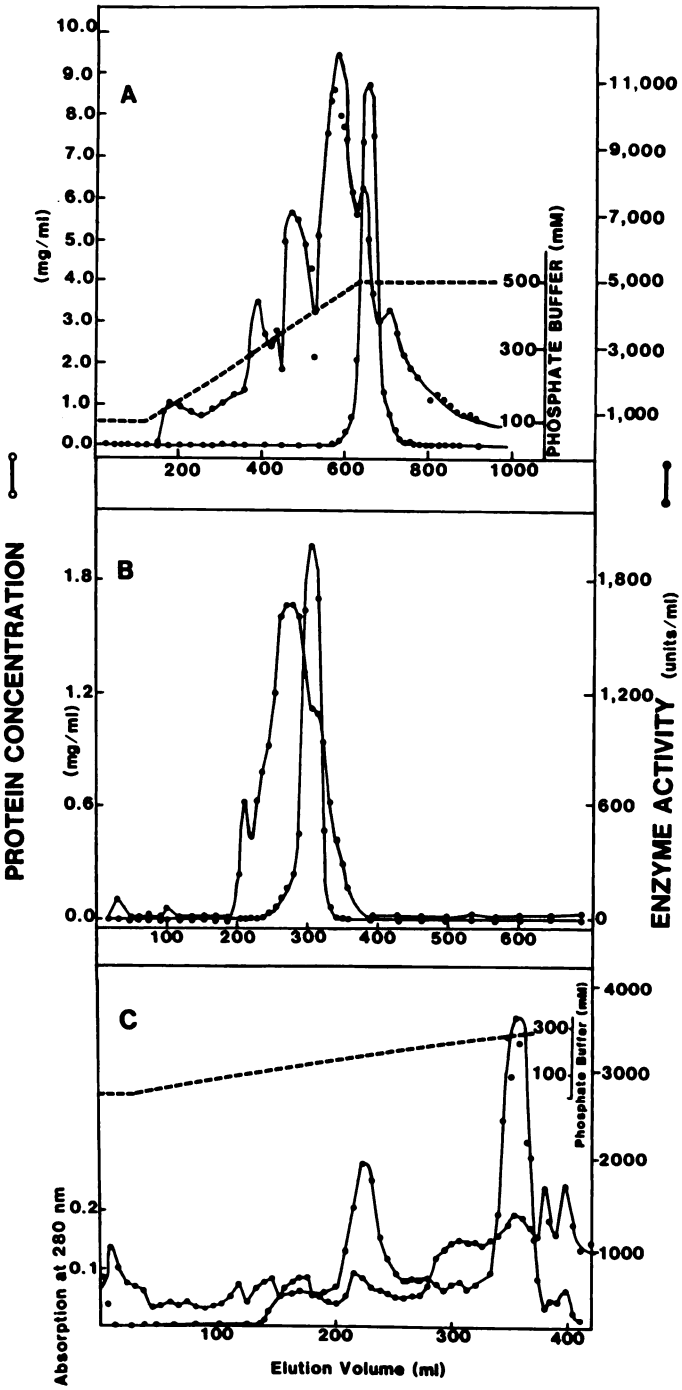

Fig. 2. Elution patterns upon chromatography from the DEAE-Sephacel (A), Sephacryl S-300 (B), and hydroxylapatite (C) in the purification of cysteine conjugate β-lyase. ●, enzyme activity; ○, protein concentration; – – –, phosphate buffer concentrations.

Active fractions eluted at the end of the linear gradient (0.5 M potassium phosphate concentration). Fractions containing β-lyase activity were pooled and concentrated to about 2.5 ml with an Amicon ultrafiltration apparatus and a Diaflow PM-10 membrane.

Step 5: Sephacryl S-300. The concentrated β-lyase solution from step 4 was applied to a column of Sephacryl S-300 (2.4 × 100 cm) equilibrated with buffer A and eluted with the same buffer, and 8-ml fractions were collected. A single peak of β-lyase activity eluted on the tailing side of the major protein peak (Fig. 2B). Fractions containing β-lyase activity were pooled, concentrated, pH adjusted to 6.25 with 0.01 M potassium phosphate containing 50 μM pyridoxal 5-phosphate (buffer C), and concentrated to 2 ml with an Amicon ultrafiltration apparatus.

Step 6: Hydroxylapatite. A column of hydroxylapatite (1.5 × 7.5 cm) was equilibrated with buffer C and the concentrated β-lyase solution from step 5 was applied to the column. The column was washed with 40 ml of buffer C, and a 380-ml linear gradient of buffer C (0.01–0.3 M potassium phosphate at the same pH) was applied, followed by a final wash of 50 ml of buffer B. Two peaks of β-lyase activity eluted from the hydroxylapatite column (Fig. 2C). Fractions corresponding to these peaks were concentrated into two corresponding solutions of about 2 ml. The smaller initial peak had low specific activity, whereas the later peak had high specific activity (Table 1) and was used as the enzyme source.

Results

Purification resulted in a protein with a 540-fold increase in specific activity (Table 1). Polyacrylamide-discontinuous gel electrophoresis of this protein revealed a single concomitant band of protein and catalytic activity (Fig. 3A), but SDS-polyacrylamide gel electrophoresis of this protein (Fig. 3B) revealed several minor bands, and a single major band with molecular weight estimated at 38,000. Comparison of the β-lyase with globular protein standards gave an estimation of 75,000 for the molecular weight. These data indicate that the β-lyase exists as a dimer of identical 38,000-Da subunits.

Stoichiometry. Two peaks of activity eluted from the hydroxylapatite column (Fig. 2C). The nature of the two peaks will be discussed later in relation to the stability of the enzyme.

Thiol-containing products from cleavage of the propachlor conjugate and CBZ were isolated and were identified as 2-mercapto-*N*-isopropylacetanilide and 2-mercaptobenzothiazole, respectively, as previously reported (4).

To determine the stoichiometry of the β-lyase reaction, CBZ was incubated with β-lyase from step 6, using the standard assay mixture, except that pyridoxal 5-phosphate was not added. The amount of 2-mercaptobenzothiazole, pyruvate, and ammonia in the incubation mixture was quantified; the three products were formed in approximately equal molar ratios (1.0:0.93:0.92, respectively). No product formation was observed when a heat-denatured enzyme was used.

Substrates. Results summarized in Table 2 show K_m and V_m values of the β-lyase for various substrates. Cystathionine was cleaved by the β-lyase as measured by pyruvic acid forma-

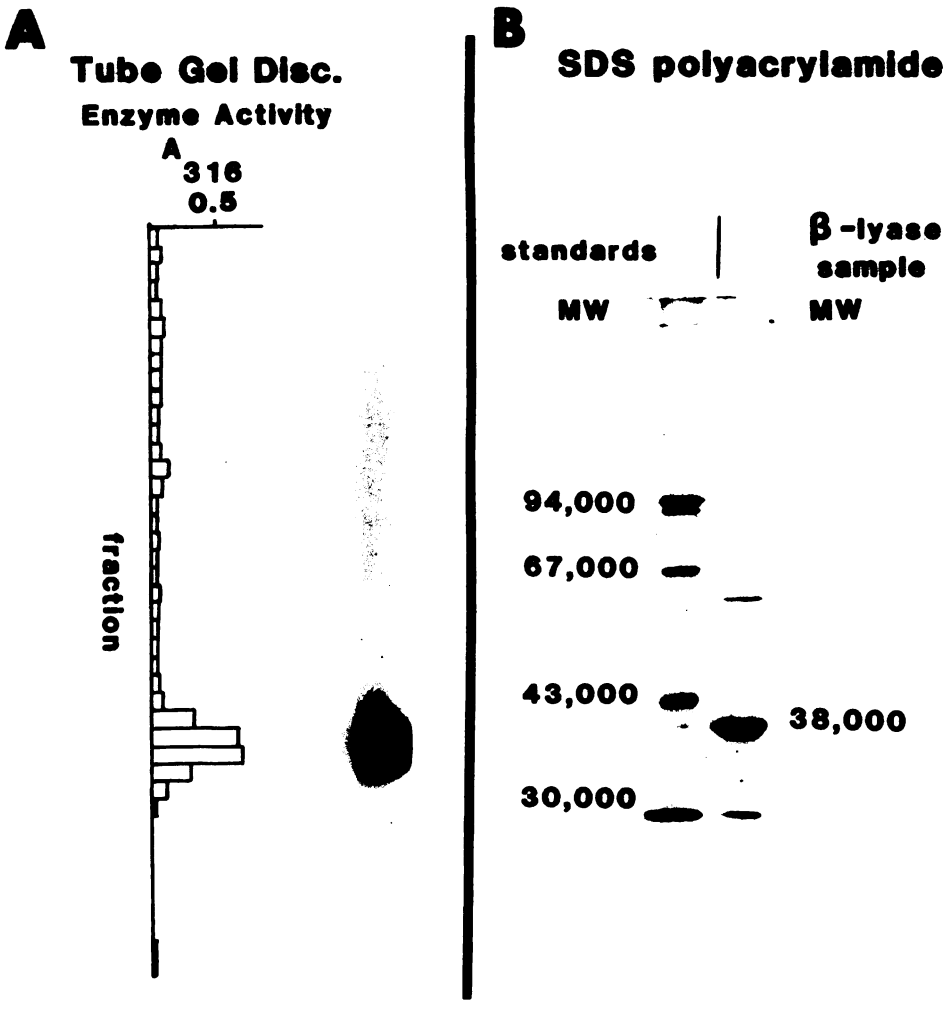

Fig. 3. Electrophoresis of purified enzyme. A, Photograph of a disc gel stained for protein; a duplicate gel, assayed for enzyme activity, is shown as described in Materials and Methods. B, Photograph of an SDS gel of the purified protein. *MW*, molecular weight.

TABLE 2
Apparent kinetic constants for cysteine conjugate β-lyase

Substrate	Activity	
	K_m	V_m
	mM	μmol/min·mg
Cysteine conjugate of propachlor[a]	1.49	20.0
S-Oxide of the cysteine conjugate of propachlor[a]	3.89	77.5
S-(2-Benzothiazolyl)cysteine[a]	0.73	30.0
S-Benzyl-L-cysteine[a]	1.05	15.0
S-Benzyl-D-cysteine	—[b]	—
L-Cystathionine[a]	1.19	10.0
Djenkolic acid[a]	2.10	49.3
DL-Lanthionine[a]	3.9	5.2
S-Ethyl-L-cysteine[a]	3.8	3.2
S-Ethyl-D-cysteine[a]	—	—
S-1,2-Dichlorovinyl-L-cysteine[a]	6.9	45.5
S-1,2-Dichlorovinyl-D-cysteine[a]	—	—
β-Chloro-L-alanine[a]	19.7	23.8
β-Chloro-D-alanine[a]	—	—
trans-9-Hydroxy-10-[*S*-(L-cysteinyl)]-9,10-dihydrophenanthrene[a]	0.99	83.1
L-Homoserine[a]	—	—
L-Cystine[a]	0.98	7.6
L-Serine-*O*-sulfate[a]	5.6	14.7
O-Phospho-L-serine[a]	—	—
O-Acetyl-L-serine[a]	2.05	0.99
L-Kynurenine[c]	—	—

[a] The reaction mixture contained 10–0.05 μg of purified enzyme (step 6), 50 μmol of potassium phosphate buffer (pH 7.8), 50 nmol of pyridoxal 5-phosphate, substrate (2-0.01 μmol), 0.3 μmol of NADH, and 5 units of lactate dehydrogenase. Incubation time was 1–3 min at 30° and each substrate level was run in triplicate.
[b] —, no activity.
[c] The reaction mixture contained 10–0.05 μg of purified enzyme (step 6), 50 μmol of potassium phosphate buffer (pH 7.8), 50 nmol of pyridoxal 5-phosphate, and 1 μmol of kynurenine in a 1.0-ml volume. Incubation time was 13 min at 25°.

tion in the NADH assay. Formation of pyruvic acid was verified by cochromatography of the 2,4-dinitrophenylhydrazone of the α-keto acid in the enzyme reaction mixture [TLC solvent system a, R_f = 0.78 and 0.60 (from E and Z isomers); TLC solvent system b, R_f = 0.44 and 0.22] and with the 2,4-dinitrophenylhydrazone of pyruvic acid. The 2,4-dinitrophenylhydrazone of α-ketobutyric acid chromatographed in these TLC solvent systems was as follows: TLC solvent system a, R_f = 0.80 and 0.74; and TLC solvent system b, R_f = 0.47 and 0.32. Formation of pyruvate by β-lyase cleavage of cystathionine shows that the β-lyase has β-cystathionase activity.

Six *S*-cysteine conjugates of xenobiotics including the *S*-oxide of the propachlor conjugate were found to be β-lyase substrates (Table 2). β-Chloro-L-alanine was cleaved by this β-lyase, although the K_m was approximately 24-fold higher than K_m values for cystathionine, the propachlor conjugate, or CBZ. L-Serine-*O*-sulfate and *O*-acetyl-L-serine were also substrates for this β-lyase. Cleavage of the *S*-oxide of the propachlor conjugate, β-chloro-L-alanine, L-serine-*O*-sulfate, and *O*-acetyl-L-serine by this enzyme shows that the enzyme is not specific for a thio-ether linkage in an *S*-cysteine conjugate when a good leaving group is present at the β-position.

pH Optimum. The pH optimum for enzyme activity was found to be 7.8 using the CBZ assay in 50 mM acetate, potassium phosphate, Tris-HCl, and borate buffers. A small increase (17%) in enzymatic activity was observed for potassium phosphate at pH 7.8 compared to Tris-HCl at the same pH. Tris buffers have been shown to be inhibitory to other β-lyase enzymes (14).

Isoelectrofocusing. Isoelectrofocusing of the enzyme gave a single isoelectric point at pH 4.9.

Stability. The enzyme was most stable when stored on ice in the presence of glycerol (20%) and increasing ionic strength (0.1 M potassium phosphate, pH 7.0). Bovine serum albumin increased the stability of the enzyme but was not used routinely. In the presence of 20% glycerol and phosphate buffer in excess of 0.1 M, dithiothreitol, ethylenediaminetetraacetate, and protease inhibitor *N*-*p*-tosyl-L-lysine chloromethyl ketone did not seem to increase stability. However, preincubation of the enzyme with the protease inhibitors phenylmethylsulfonylfluoride, 9,10-phenanthroline, or leupeptin stabilized the enzymatic activity 1.5–2.4-fold compared to controls to which no protease inhibitor was added. Therefore, a small amount of protease is thought to be present in the β-lyase resulting in the decrease in enzymatic activity on storage and the presence of minor protein bands in the SDS gel (Fig. 3B). The two peaks of β-lyase activity eluting from the hydroxylapatite column and the minor bands on the SDS gels may also be the result of proteolysis. The earlier eluting peak has a lower specific activity, consistent with partial proteolytic degradation of the β-lyase eluting in the later peak.

Even though other β-lyases which have been purified require pyridoxal 5-phosphate for stability, removal of pyridoxal 5-phosphate by dialysis resulted in no loss of enzymatic activity of the *E. limosum* β-lyase. In contrast, 80% of the enzymatic activity was lost when the β-lyase was isolated from *Fusobacterium necrophorum* in the absence of pyridoxal 5-phosphate.

Inhibitors. The presence of reagents which react with carbonyl groups (20) (e.g., hydroxylamine or cyanide) inactivated the enzyme (Table 3). Pyridoxal 5-phosphate inhibitors DL-vinylglycine (21), DL-*C*-propargylglycine (22), and gabaculine (23) did not inactivate the β-lyase. Inhibition of the enzyme by iodoacetic acid indicates that a sensitive sulfur is present on the enzyme. Cysteine has been shown to inhibit the hepatic β-lyase (14), but it does not inactivate the *E. limosum* enzyme. Cleavage of β-chloro-L-alanine by this β-lyase does not result

TABLE 3
Inhibition of cysteine conjugate β-lyase cleavage of *S*-(2-benzothiazolyl)cysteine
The reaction mixture contained 10–0.5 μg of purified enzyme (step 6), 50 μmol of potassium phosphate buffer (pH 7.8), 50 nmol of pyridoxal 5-phosphate, 1 μmol of CBZ, inhibitor (0–1.0 μmol), in 1.0 ml. Incubation time was 3 min at 37° and each value represents the average of duplicate measurements (numbers in parentheses).

Inhibitor	Concentration	Inhibition
	mM	%
Hydroxylamine	0.001	30.8 (30.0–31.5)
	0.01	94.2 (93.6–95.4)
	0.1	86.2 (81.7–90.8)
Potassium cyanide	0.001	25.8 (25.4–26.2)
	0.01	75.2 (73.3–77.2)
	0.1	90.4 (89.3–91.6)
Iodoacetic acid	0.001	100.0[a]
Gabaculine	0.1	1.8 (1.1–2.6)
	1.0	4.1 (4.1–4.1)
10-min preincubation	1.0	10.8 (9.3–12.2)
DL-Vinylglycine	0.1	1.8 (1.1–2.6)
	1.0	1.25 (0–2.5)
10-min preincubation	1.0	19.4 (18.0–20.9)
DL-*C*-Propargylglycine	0.1	0
	1.0	0
Cysteine	0.1	1.8 (1.1–2.6)
	1.0	7.2 (5.0–9.3)
15-min preincubation	1.0	14.4 (13.6–15.1)

[a] Single value.

in inhibition, which occurs with the pyridoxal 5-phosphate-containing liver cysteine conjugate β-lyase (14) and kynureninase (24).

Discussion

Bacteria resident in the gut of mammals have been shown to play a major role in the biotransformation of xenobiotics (1, 25). Recently, Bakke *et al.* have shown that germ-free rats do not form novel thiol metabolites of propachlor and other xenobiotics (1). The thiol metabolites arise from the β-cleavage of cysteine conjugates of the parent xenobiotic by enzymes known as cysteine conjugate β-lyases (2–4, 14). Although cysteine conjugate β-lyases are present in mammalian tissues, they do not metabolize the cysteine conjugate of propachlor or some *S*-alkyl- and *S*-aryl-L-cysteine conjugates (14). Therefore, the formation of a wide variety of novel thiol-containing metabolites may depend largely on the β-lyase enzymes of the gut flora in agreement with the work of Bakke *et al.* (1).

The general distribution of β-lyases in enteric and environmental bacteria has been established by Larsen (6). In that study it was found that *E. limosum*, an enteric anaerobe, had much higher levels of β-lyase activity than did other bacteria tested. The purified cysteine conjugate β-lyase from *E. limosum* uses a broad spectrum of substrates including *S*-alkyl- and *S*-aryl-L-cysteine conjugates as well as a number of other β-substituted alanines. In addition, the enzyme metabolized several naturally occurring compounds which can be considered derivatives of cysteine. This broad specificity supports the hypothesis that the enteric bacteria may be the major source of β-cleavage reaction which is a key step in the introduction of sulfur into a wide variety of xenobiotics.

Rat hepatic cysteine conjugate β-lyase has been shown to be kynureninase, an enzyme which functions in tryptophan catabolism (26). The enzyme reported here did not use kynurenine as substrate but did cleave cystathionine to form pyruvate and, presumably, homocysteine. Therefore, two cysteine conjugate β-lyases have been shown to have a natural function other than cysteine conjugate metabolism. In addition, β-lyase from rat kidney differs from the rat liver enzyme, and the three β-lyases isolated from bacterial sources appear to have different characteristics. For example, β-lyases isolated from *Fusobacterium necrophorum* (4) and *Fusobacterium varium* (5) did not cleave cystathione, required pyridoxal 5-phosphate for stability, and had molecular weights of about 228,000 and 70,000 respectively. In contrast, the β-lyase reported in this paper has β-cystathionase activity, is stable in the absence of pyridoxal 5-phosphate, and has a molecular weight of about 75,000 with two subunits having molecular weights of about 38,000. Furthermore, the β-lyase isolated from *F. necrophorum* appears to have more stability to heat than do the other two bacterial β-lyases. It is possible that purification of other β-lyases and investigation of natural substrates will show that other β-lyases are also enzymes which metabolize endogenous compounds.

Gabaculine and propargylglycine are specific inhibitors for pyridoxal 5-phosphate enzymes (22, 23) which form a β-carbanion during catalysis (e.g., γ-cystathionase). Neither of these compounds inhibited this enzyme, supporting the conclusion that it has β- rather than γ-cystathionase activity. However, the lack of stabilization by pyridoxal 5-phosphate suggests that this enzyme may differ from previously reported β-cystathionase (E.C. 4.4.1.8) (27). DL-Vinylglycine and β-chloro-L-alanine did not inhibit enzymatic activity. Both of these compounds have been reported to be inhibitors of other pyridoxal 5-phosphate-dependent enzymes (21, 28). The carbonyl-reactive agents hydroxylamine and cyanide did inhibit the enzyme. Therefore, at present, we can only conclude that the enzyme contains an active carbonyl and that further work is necessary to establish the role of pyridoxal 5-phosphate as a cofactor in the enzyme.

Although mammalian cysteine conjugate β-lyase enzymes have been implicated in the toxicity of certain *S*-cysteine conjugates, little work has been done on the role of toxic thiols produced in the gut. Because the bile is a common excretory route for mercapturic acid pathway intermediates (1), the gut is exposed to a wide spectrum of cysteine and glutathione conjugatés. Whether the β-lyase enzymes in gut can play a role in the production of species which damage the gut and other organs is not known. However, the disposition of mercapturate intermediates and the broad spectrum of activity of the enzyme described here suggests that this possibility is worthy of further investigation.

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