Isolation and Characterization of a Prokaryotic Sulfurtransferase*

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A sulfurtransferase has been purified to apparent homogeneity from the prokaryote Acinetobacter calcoaceticus luwofi by conventional protein fractionation techniques. Steady-state kinetic studies of the enzyme revealed that its formal mechanism varies with the acceptor substrate employed. With inorganic thiosulfate as the sulfane sulfur-donor substrate and cyanide anion as the acceptor, the enzyme was shown to catalyze the reaction by a double displacement mechanism like that of mammalian rhodanese, whereas (thiosulfate-cyanide sulfurtransferase, EC 2.8.1.1). In contrast, with a thiol as the acceptor substrate at relatively high concentrations, the reaction proceeds by a single displacement mechanism, reminiscent of catalysis by another sulfurtransferase, thiosulfate reductase, glutathione-dependent (EC 2.8.1.3). When dithiothreitol is the acceptor substrate, the enzyme cycles through both the single and double displacement pathways, with the flux through each depending differentially on the concentration of dithiothreitol employed. In view of both the relaxed acceptor substrate specificity and the corresponding variability of formal mechanism, the more general name of sulfane sulfurtransferase is proposed for this bacterial enzyme.

All proteins in the class of enzymes designated sulfurtransferases catalyze reactions in which a sulfane sulfur atom is transferred from a donor molecule to a thiolic acceptor substrate. Rhodanese (thiosulfate-cyanide sulfurtransferase, EC 2.8.1.1) performs this reaction by a double displacement formal mechanism, using cyanide anion or a dithiol such as dihydroxipolate or 2,3-dihioiopanol as acceptor substrate (1-3). Monothiols are poor acceptor substrates for this enzyme (4). In contrast, thiosulfate reductase, glutathione-dependent (EC 2.8.1.3) transfers sulfur only to thiolic acceptor substrates, and the reaction proceeds by an ordered single displacement formal mechanism (5). The difference in formal mechanism of these two enzymes is related, in part, to the source of the nucleophile that cleaves the sulfur-sulfur bond of the donor substrate. Both enzymes use a sulfhydril group for this nucleophile, but, in rhodanese, the thiol is supplied by an active site cysteine residue, while thiosulfate reductase uses the sulfhydril group of the acceptor substrate (5-8).

In the present work, a sulfurtransferase from the soil bacterium, Acinetobacter calcoaceticus luwofi, has been purified to homogeneity. This enzyme is capable of transferring sulfur to cyanide, monothiols, and dithiols. Steady-state kinetic studies of the mechanism of this enzyme show that catalysis can proceed by either a single or a double displacement pathway. With at least one acceptor substrate, both formal mechanisms are operative, the total flux in each pathway being determined by the concentration of that substrate. The wide range of acceptor substrates utilized makes this the most general of the known sulfurtransferases.1

**EXPERIMENTAL PROCEDURES**

**Materials**—The bacterium, Acinetobacter calcoaceticus luwofi, was obtained as an environmental isolate and identified by extensive testing in the Clinical Microbiology laboratory at Bernard Mitchell Hospital. Cell culture media, Bacto-yeast extract, and Bacto-trypotone extract were obtained from Difco. Hydroxylapatite (Bio-Gel HTP), the polyacrylamide gel matrix P-30, sodium dodecyl sulfate, acrylamide, bisacrylamide, bromophenol blue dye, N,N,N',N'-tetramethylethylene diamine, ammonium persulfate, and 0.7 × 15-cm columns were purchased from Bio-Rad Laboratories, DEAE-Sepharose was purchased from Pharmacia. Phenylmethylafoxil fluoride, 2-iodoacetic acid, and Coomassie Brilliant Blue R-250 were purchased from Sigma. Dithiothreitol (DTT)2 was obtained from Boehringer Mannheim Biochemicals. Analytical grade ammonium sulfate was purchased from Fisher Scientific. Pararosaniline acetate was bought from E. Merck. All other chemicals were of the highest reagent grade available. All water used was deionized or glass-distilled.

**Growth of Cells**—The Acinetobacter cell line was maintained in freezer cultures at ~20 °C in 50% horse serum and 50% LB broth (20 g of Bacto-tryptone, 10 g of Bacto-yeast, 20 g of NaCl per liter of H2O). Cells were grown aerobically at 30 °C for 24 h in 500-ml cultures of LB broth. Cultures were grown in 8-liter batches and harvested by centrifugation for 5 min at 10,000 × g, 4 °C.

**Electrophoresis Techniques**—Denaturing and non-denaturing polyacrylamide gel electrophoresis techniques employed during this work were both modifications of the original Laemmli protocol for discontinuous gel electrophoresis (9). The stacking gel contained 125 mM Tris-chloride buffer, pH 6.80, with the acrylamide concentration at 4.5% (w/v). The separation gel contained 90 mM Tris-chloride buffer, pH 8.80, and acrylamide at 15% (w/v). The electrode buffer used was 25 mM Tris-chloride, pH 8.30, containing 50 mM glycin. Polymerization of the gels was initiated by the addition of N,N,N',N'-tetramethylethylenediamine and ammonium persulfate. When denaturing conditions were desired, all of these buffer systems were made 0.1% in sodium dodecyl sulfate. With non-denaturing gels, 5 mM sodium thiosulfate was added to the electrode buffer, and the gels were pre-electrophoresed for 60 min to dispose of the free radicals left behind by the polymerization process. Gels were subjected to a constant current of 20 mA at 4 °C until the bromphenol blue tracking dye was within a few millimeters of the bottom of the gel. All gels reported here had dimensions of 80 × 105 × 1 mm.

Staining of the gels was done by a modified Coomassie Blue protocol. Gels were placed in fixative (12.5% trichloroacetic acid (v/v), 4% sulfosalicylic acid (w/v)) and left on a reciprocating shaker for 60 to 90 min. After transfer to a staining solution (27% 2-propanol)

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1 The more general name sulfane sulfurtransferase is here proposed for the Acinetobacter enzyme because of its more inclusive specificity, utilizing the acceptor substrates and the formal mechanisms characteristic of both rhodanese and thiosulfate reductase catalysis.

2 The abbreviation used is: DTT, dithiothreitol.
Isoelectric Focusing—Isoelectric focusing was conducted in a \textit{miniaturized} system. A standard Curie point to be taken as evidence that supports the proposed reaction mechanism.

\textbf{RESULTS}\textsuperscript{5}

\textit{Initial Velocity Studies}—When thiocyanate and cyanide were the varied substrates, the initial velocity patterns in Fig. 4 were generated. These lines were obtained through hyperbolic fitting of the data with ENZKIN (see "Experimental Procedures"). With either \textit{S}_{2}O_{5}^{2-} \text{ or KCN} \text{ as the varied substrate, the double reciprocal pattern consisted basically of parallel lines with significantly different intercepts on the } 1/ v_{o} \text{ axis. The thiosulfate primary plots exhibited strong upward curvatures at high thiosulfate concentrations, and this effect was exacerbated by low cyanide concentrations. The same phenomenon is seen in the cyanide primary plot as an increased slope at high thiosulfate concentrations. This behavior is indicative of substrate inhibition by thiosulfate. Both the parallel line pattern and the competitive substrate inhibition are typical of double displacement formal mechanisms (15, 16). The secondary plot of [KCN] \textsuperscript{-1} \text{ versus intercepts of the thiosulfate primary plots is linear and has a non-zero ordinate intercept which provides a value for } k_{2}, \text{ the rate constant for the rate-limiting step of the enzyme-catalyzed reaction at substrate saturation. The kinetic parameters corresponding to these primary and secondary plots are summarized in Table II. The similarities between the values for these kinetic coefficients and those established for bovine liver rhodanese (17) under similar conditions should be noted.}

Bovine liver rhodanese uses only anionic species as donor substrates (18) and is susceptible to competitive inhibition by anions that are not substrates. This phenomenon was explored with sulfane sulfurtransferase using sulfate as the anion. This experiment produced the inhibition pattern presented in Fig. 6. There was no significant difference in the intercepts of the double reciprocal plots. The secondary plot of ammonium sulfate concentration \textit{versus} primary plot slopes was linear. These data show that sulfate anion is a linear competitive inhibitor of sulfate sulfurtransferase with respect to thiosulfate as a substrate. For comparison, the same experiment was performed with bovine liver rhodanese, with very similar results (not shown). Sulfate is not a particularly potent inhibitor of either enzyme, giving } K_{i} \text{ values of } 118 \pm 10 \text{ mM and } 258 \pm 9 \text{ mM for sulfane sulfurtransferase and rhodanese, respectively.}

\textit{Initial Velocity Experiments with Dithiothreitol}—Initial velocity with thiolsulfate and dithiothreitol as the varied substrates were performed. The double reciprocal primary plots of these data exhibit a pattern of straight lines with significantly different slopes converging toward a common point to the left of the ordinate (Fig. 8). This pattern is characteristic of a single displacement type of formal mechanism (15, 16), an inference supported by the secondary plots of the slopes and intercepts (Fig. 8). The two intercept replots have the same intercept, and the two slope replots have approximately the same slope.

With a single displacement mechanism, it is desirable to ascertain the order of substrate binding and product discharge. To this end, initial velocity experiments were performed with thiolsulfate and dithiothreitol as varied substrates and sulfite as product inhibitor (Fig. 9). The dashed lines in the inset inhibitor plots of Fig. 9A indicate that the formulation of these plots as linear is only one of the possible interpretations (see below). Nevertheless, the fact that both the slopes and the intercepts exhibit systematic change with sulfite concentration indicates that sulfite is a noncompetitive inhibitor with respect to thiolsulfate. With dithiothreitol as the varied substrate, the double reciprocal pattern is a set of

\textsuperscript{5} Portions of this paper (including portions of "Results," Figs. 1–4 and 7, and Tables I and III) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 87M-1805, cite the authors, and include a check or money order for $3.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
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**FIG. 5. Initial velocity patterns of sulfane sulfurtransferase.** A, thiosulfate varied. \([\text{S}_2\text{O}_3^{2-}]\) was varied at constant KCN concentrations of 5 mM (○), 10 mM (△), 20 mM (●), 50 mM (▲), and 100 mM (□). The secondary plot inset displays the line of primary plot intercepts versus reciprocal [KCN]. B, cyanide varied. [KCN] was varied at constant \([\text{S}_2\text{O}_3^{2-}]\) concentrations of 1 mM (○), 2 mM (●), 4 mM (▲), 10 mM (○), and 20 mM (□).

**TABLE II**

Comparison of kinetic parameters for Acinetobacter sulfane sulfurtransferase and bovine liver rhodanese

<table>
<thead>
<tr>
<th>Kinetic parameter</th>
<th>Acinetobacter sulfane sulfurtransferase</th>
<th>Bovine liver rhodanese</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>( k + 2 )</td>
<td>240 s(^{-1})</td>
<td>300 s(^{-1})</td>
<td>Intercepts of secondary intercept plots</td>
</tr>
<tr>
<td>( k + 3 )</td>
<td>7.9 ( \times 10^6 ) M(^{-1}) s(^{-1})</td>
<td>5.2 ( \times 10^6 ) M(^{-1}) s(^{-1})</td>
<td>Slope of KCN secondary intercept plot</td>
</tr>
<tr>
<td>( K_{m(SO_3^-)} )</td>
<td>3.2 mM</td>
<td>4.0 mM</td>
<td>Slope of ([\text{S}_2\text{O}<em>3^{2-}]) primary plot ( \times k</em>{c2} )</td>
</tr>
<tr>
<td>( K_{m(CN)} )</td>
<td>3.0 ( \times 10^{-5} ) M</td>
<td>6 ( \times 10^{-5} ) M</td>
<td>Ratio: ( k_{c1}/k_{c2} ) or slope of KCN primary plot ( \times k_{c2} )</td>
</tr>
</tbody>
</table>

Mixed Acceptor Substrate Studies—Some ambiguities remained after the sulfite inhibition studies. While it had been clearly established that sulfane sulfurtransferase changes formal mechanism in response to the acceptor substrate, it was still not certain which substrate is bound to the enzyme first.
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FIG. 8. Initial velocity patterns of sulfane sulfurtransferase. A, thiosulfate varied. [S$_2$O$_3^-$] varied at constant DTT concentrations of 10 mM (○), 15 mM (■), 20 mM (▲), 25 mM (●), 50 mM (△), and 100 mM (▲). Secondary plot insets show reciprocal [DTT] versus primary plot slopes and intercepts. B, dithiothreitol varied. [S$_2$O$_3^-$] varied at constant DTT concentrations of 1 mM (○), 1.5 mM (■), 2 mM (▲), 5 mM (●), 10 mM (△), and 20 mM (▲). Secondary plot insets show reciprocal [S$_2$O$_3^-$] versus primary plot slopes and intercepts.

or whether the mechanism might be random. Also unexplained was the parabolic curvature of sulfite inhibition plots (Fig. 9). Furthermore, there remained the question of whether the double displacement mechanism with cyanide as acceptor substrate and the single displacement mechanism with dithiothreitol as acceptor substrate are both operative at the same active site. The latter question, in particular, is best addressed by assays in which more than one acceptor substrate is present (20). Accordingly, experiments varying the concentrations of both KCN and dithiothreitol in the presence of constant [S$_2$O$_3^-$] were undertaken. The thiocyanate detection system was chosen because it provided the best quantitation of all products of enzyme action. Thiocyanate is formed both by the reaction of CN$^-$ with the sulfur-substituted enzyme and also by the nonenzymic reaction of CN$^-$ with dithiothreitol persulfide, the product of the enzyme-catalyzed reaction with DTT. This uncatalyzed reaction was found to be adequately fast to keep pace with the enzymic reaction except in reaction mixtures with very low [KCN] and high [DTT].

The initial velocity measurements made with the mixed acceptor system are presented as double reciprocal patterns in Fig. 10. The KCN primary plots are a pattern of straight lines converging toward a common intercept on the 1/υ₀ axis. This pattern indicates that dithiothreitol behaves as a linear competitive inhibitor with respect to CN$^-$.

Thiocyanate is formed both by the reaction of CN$^-$ with the sulfur-substituted enzyme and also by the nonenzymic reaction of CN$^-$ with dithiothreitol persulfide, the product of the enzyme-catalyzed reaction with DTT. This uncatalyzed reaction was found to be adequately fast to keep pace with the enzymic reaction except in reaction mixtures with very low [KCN] and high [DTT].

Kinetic Characterization of the Mechanism—When cyanide is the only acceptor substrate provided, sulfane sulfurtransferase acts strictly as a traditional rhodanese. Both initial velocity patterns are composed basically of parallel lines (Fig. 5). These patterns are diagnostic of a double displacement formal mechanism (15, 16), and they strongly resemble the patterns observed with bovine liver rhodanese when thiosulfate and cyanide are the varied substrates (17). Rhodanese is known to have a cationic binding site in the region of the active site cleft. This site renders the enzyme subject to

DISCUSSION

Purification—The sulfane sulfurtransferase of Acinetobacter calcoaceticus lwoffi has been purified 700-fold from crude extracts, to apparent homogeneity. The enzyme migrates on nondenaturing gels as a tight double band. Both bands show sulfurtransferase activity as indicated by staining in situ with a fluorigenic substrate. No other protein species are detectable in these gels by the Coomassie Blue or silver staining technique. Additional evidence for homogeneity is provided by primary structural analysis currently in progress. When automated Edman degradation is performed on known amounts of native enzyme, there is quantitative agreement between the concentration of total protein as determined by amino acid analysis and the yield per cycle of phenylthiohydantoin amino acid derivatives. This purification represents a substantial improvement over the method reported by Vandenberg and Berk (21), who achieved a 40-fold purification of rhodanese from the same species of bacterium. Whereas they reported an apparent molecular weight of 35,000 for that rhodanese, the present sulfane sulfurtransferase, at 17,000, is much smaller.
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Fig. 9. Inhibition studies on sulfane sulfurtransferase with sulfite as inhibitor. A, primary plot of sulfane sulfurtransferase activity in the presence of inhibitory concentrations of sulfite. \([\text{SO}_3^-]\) was varied with \([\text{DTT}]\) held constant, at sulfite concentrations of 0 mM (△), 0.075 mM (□), 0.25 mM (●), and 0.3 mM (○). Secondary plot insets show \([\text{SO}_3^-]\) versus primary plot slopes and intercepts. Dotted lines in the secondary plots indicate that systematic curvature is an alternative interpretation to these data. B, primary plot of sulfane sulfurtransferase activity in the presence of inhibitory concentrations of sulfite. \([\text{DTT}]\) was varied with \([\text{SO}_3^-]\) held constant, at sulfite concentrations of 0 mM (△), 0.1 mM (□), 0.2 mM (△), 0.25 mM (○), and 0.3 mM (●). Secondary plot inset shows \([\text{SO}_3^-]\) versus primary plot slopes.

inhibition from competing non-substrate anions. As shown under "Results," rhodanese and sulfane sulfurtransferase respond in a similar fashion to the presence of sulfate anion (Fig. 6 and Table III). The evidence indicates a formal mechanism such as that presented in Scheme 1. Step 4 represents substrate inhibition by thiosulfate. This kind of nonproductive binding of a substrate with the "wrong form" of the enzyme is also typical of double displacement mechanisms (22). Step 5 represents dead-end inhibition competitive with thiosulfate by a non-substrate anion. A summary of how the values for these microconstants were estimated from the experimental data is provided in Table II. The rate expression corresponding to Scheme 1 was entered into a BASIC program which stimulates velocity behavior in accordance with the rate equation and kinetic parameters supplied. When the experimental values found for these parameters from the studies on sulfane sulfurtransferase with thiosulfate and cyanide were inserted into the program, patterns that closely resembled the experimental double reciprocal plots were generated (Fig. 11). The closeness of the fit is evidence that a mechanism of this form could generate the observed behavior.

With thiol as acceptor substrates, sulfane sulfurtransferase exhibits different behavior. When a monothiol is the acceptor substrate, the enzyme yields nonlinear kinetics in initial velocity experiments, and steady-state velocity measurements are not possible. Hysteretic behavior is fairly common among enzymes in general, and there are a variety of mechanisms that can account for this type of kinetics (23, 24). In this case, it is likely that the hysteresis is caused by the formation of mixed disulfides with the active site sulfhydryl group at higher monothiol concentrations. Although readily reversible under these conditions, the formation of such disulfides has the effect of removing some of the enzyme from the catalytic cycle, thus lowering the overall rate of product formation.

When dithiothreitol is the acceptor substrate, steady-state experiments with sulfane sulfurtransferase produce a pattern of straight lines of differing slopes converging to a common point to the left of the ordinate (Fig. 8). This pattern is diagnostic of a single displacement formal mechanism (15, 16). Considered together with the inhibition by product sulfite (competitive with dithiothreitol and noncompetitive with respect to thiosulfate), these results might be taken to indicate either a simple ordered mechanism with dithiothreitol as leading substrate and sulfite the final product discharged, or a Theorell-Chance type mechanism with thiosulfate as the leading substrate and sulfite the first product discharged. However, neither of these forms accounts for the parabolic tendency of the sulfite inhibition plots. Similarly, neither explicitly addresses the relationship to the double displacement mechanism that occurs when cyanide is the acceptor substrate. These considerations led to experiments with a mixture of acceptor substrates.
When KCN and dithiothreitol were varied at a constant S\textsubscript{2}O\textsubscript{2}^- concentration, the double reciprocal plots of Fig. 10 were generated. The KCN primary plots present a pattern of lines of differing slopes converging to a common intercept on the ordinate. This pattern shows that DTT behaves as a competitive inhibitor with respect to CN\textsuperscript{-}, thereby indicating that both reaction pathways are operative at the same active site. The DTT primary plots exhibit a pattern of lines that curve downward at concentrations of DTT exceeding 70 mM. This curvature gradually lessens as the concentration of KCN is raised. This pattern indicates that dithiothreitol interacts with more than one form of the enzyme in the catalytic cycle. These results led to the formulation of the more comprehensive formal mechanism presented in Scheme 2. As before, dithiothreitol is represented in this scheme by a generic dithiol. The method of King and Altman (25) was used to derive the corresponding rate equation:

\[
v_0 = \frac{k_{+3}[\text{CN}^-][\text{ES}]}{k_{-3}[\text{CN}^-][\text{ES}]} + \frac{k_{+4}[\text{E-} \text{DTT persulfide}]}{k_{-4}[\text{E-} \text{DTT persulfide}]} + \frac{k_{+6}[\text{E-} \text{DTT persulfide}]}{k_{-6}[\text{E-} \text{DTT persulfide}]} + \frac{k_{+7}[\text{E-} \text{DTT persulfide}]}{k_{-7}[\text{E-} \text{DTT persulfide}]} + \frac{k_{+8}[\text{E-} \text{DTT persulfide}]}{k_{-8}[\text{E-} \text{DTT persulfide}]} + \frac{k_{+9}[\text{E-} \text{DTT persulfide}]}{k_{-9}[\text{E-} \text{DTT persulfide}]}
\]

This expression holds whenever [CN\textsuperscript{-}] is high enough to render the nonenzymic reaction with dithiothreitol persulfide sufficiently fast not to be rate-limiting. The first term results from the formation of product in the typical double displacement mechanism with CN\textsuperscript{-} as the acceptor substrate. The behavior denoted by the term \(k_{+3}[\text{E-} \text{DTT persulfide}]\) is more complex because it represents the sum of two pathways. Both of these steps involve the entry of dithiothreitol, but they differ in their apparent \(K_m\) values for this substrate. Step 6 can utilize the dithiol effectively at much lower concentrations than can step 4. Thus, at low dithiothreitol concentration, the enzyme cycles primarily through the double displacement pathway with CN\textsuperscript{-} as the acceptor substrate, and the dithiol acts as a competitive inhibitor through step 6. As the concentration of dithiothreitol is raised, the magnitude of this inhibition increases, until the apparent \(K_m\) for dithiothreitol in the pathway through step 4 is exceeded, whereupon the enzyme begins to cycle primarily through this single displacement pathway, increasing in velocity with increasing dithiol concentration. As with all competitive inhibition, the effect
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Fig. 11. Modeling of sulfane sulfurtransferase as a double displacement enzyme. Computer-generated initial velocity pattern modeling the activity of sulfane sulfurtransferase as a double displacement enzyme, including substrate inhibition by thiosulfate. The pattern produced by this model represents a best fit for the entire range of substrate concentrations employed, and the quality of the fit of any portion of the pattern to a line of data at any fixed substrate concentration should be judged with this in mind.

The discharge of the product sulfite in two different branches of the pathway explains the parabolic curvature of the sulfite inhibition plots. Sulfite inhibition is competitive with respect to dithiothreitol in each branch, but just as the two enzyme forms vary in their sensitivity to [DTT], they also differ in their reactivity with sulfite. Thus, with dithiothreitol as the sole acceptor substrate, varying [DTT] and [SO\textsuperscript{3-}] produces a pattern of initial velocities that represents a combination of the two forward reactions with dithiothreitol, each inhibited to varying degrees in accordance with the concentration of sulfite.

The mechanism of Scheme 2 has been successfully modeled on the computer in a qualitative way. Owing to the complexity of this reaction mechanism, it is difficult to determine precise...
values for the various rate constants, and this hampers the modeling process. Nevertheless, it was possible to formulate a semiquantitative model based on estimates of these constants and using substrate concentrations within the range of the experiments. The model thus generated (Fig. 12) shows that a rate equation of this form is capable of producing the type of complex double reciprocal pattern observed in Fig. 10, thereby suggesting that this formal mechanism is a generally correct representation of the experimental system.

REFERENCES
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Figure 1. Hydroxyapatite Chromatography. The elution profile of a 3.5 x 4.5 cm Bio-Gel HTP hydroxyapatite column. Fractions of 4 ml were collected. Total protein was monitored by its absorption at 280 nm. As shown, sulfur sulfurtransferase activity was measured in 10-ml eluate at 15 μl of effluent. Thiopropionate was detected in its ferric complex by form by its absorption at 440 nm. -

Figure 2. P-30 Chromatography. The elution profile of a 2.5 x 40 cm Bio-Gel P-30 column. Fractions of 5 ml were collected. Total protein was monitored by its absorption at 280 nm. As shown, sulfur sulfurtransferase activity was measured in 5-ml eluate at 15 μl of effluent. Thiopropionate was detected in its ferric complex by form by its absorption at 440 nm.

Table I

<table>
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<th>Step</th>
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<td>10% ammonium</td>
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<td>17</td>
<td>8.9</td>
<td>1,170</td>
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</table>

* Unit = μmol SCN⁻/min in standard rhodanese assay

Table II

<table>
<thead>
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<th>Substrate</th>
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<td>l-aspartic acid</td>
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<td>dimethylsulfone</td>
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Figure 3. Sulfur Sulfurtransferase with Dimethylsulfone as Acceptor Substrate. A, varying incubation time. A plot of the amount of sulfone produced by sulfur sulfurtransferase with dimethylsulfone as the acceptor substrate versus time. B, varying concentration of the enzyme. The amount of sulfone produced by sulfur sulfurtransferase with dimethylsulfone as the acceptor substrate, varying the concentration of the enzyme.