A Cystine-dependent Inactivator of Tyrosine Aminotransferase Co-purifies with γ-Cystathionase (Cysteine Desulfurase)*

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Tyrosine aminotransferase is stable in homogenates of rat liver, but not when L-cystine or L-cysteine is added, which causes the enzyme to be reversibly inactivated due to oxidation of thiol groups. By monitoring inactivation of the aminotransferase in the presence of L-cystine, a factor responsible for this loss of activity was purified from rat liver. The factor required vitamin B₆ and co-purified with γ-cystathionase during numerous steps. Highly purified inactivating factor contained a protein that was identical in size and isoelectric point to cystathionase but also contained a dissimilar peptide that appeared to be unrelated to cystathionase. Cystathionase and the cystine-dependent inactivator shared several catalytic activities, including the hydrolysis of cystathionine, desulfuration of cysteine, and dehydratation of cysteine. During incubation of L-cysteine with the purified factor, hydrogen sulfide was generated but no inactivation of the aminotransferase occurred, suggesting that cysteine-dependent inactivation requires additional mechanisms. An insoluble inactivator of tyrosine aminotransferase that is produced during the reaction may be elemental sulfur, since colloidal suspensions of sulfur also inhibited the enzyme. Another inhibitor fractionated with high molecular weight substances; this may be protein-bound sulfane.

Mechanisms other than disulfide exchange that could be involved in cysteine-dependent inactivation of enzymes include stimulation of cathepsins (13) and generation of free radicals (14). In order to better understand this system for inactivating tyrosine aminotransferase, we characterized a factor from rat liver that inactivated the latter enzyme in the presence of L-cystine. The majority of the inactivating capacity in homogenates containing PLP was found in the cytosol, and the factor co-purified with γ-cystathionase activity through a number of steps. Cystathionase (EC 4.4.1.1) purified to apparent homogeneity retained the capacity to inactivate tyrosine aminotransferase in the presence of L-cystine. We show that elemental sulfur, which is produced during the dehydratation of cystine, inhibits tyrosine aminotransferase. We also discuss evidence that the sulfane group formed due to reaction of thiochristeine with protein-bound disulfide groups may participate in the inactivation.

 MATERIALS AND METHODS

RESULTS

Purification of a PLP-dependent Inactivating Factor—Whereas tyrosine aminotransferase was stable during incubation without L-cystine, addition of L-cystine to a 12,000 × g supernatant fraction from liver caused inactivation with a half-time of about 2 h (Fig. 1). L-Cysteine produced inhibition at comparable rates (not shown). Addition of dithiothreitol reactivated the enzyme, although reactivation was incomplete when the reducing agent was added at later times. By measuring cysteine-dependent inactivation, the factor responsible could be assayed during its purification (details are described under "Materials and Methods," (see Miniprint Section)). In part because of an initial delay before inactivation began, the assay was not linear with respect to time or protein concentration. The majority of the cysteine- and cysteine-dependent activity measured under these conditions remained in the soluble fraction after centrifugation at 150,000 × g (Table 1-S, Miniprint Section). Although some activity was present in all crude subcellular fractions, only the soluble form was characterized here. Similar distributions were noted for cystathionase and cysteine desulhydratase, which were measured.

1 The abbreviations used are: PLP, pyridoxal 5'-phosphate; DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid), Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, SDS, sodium dodecyl sulfate; TPCK, L-1-tosylamido-2-phenyl ethyl chloromethyl ketone.

2 Portions of this paper (including "Materials and Methods," Tables S-1-S-4, and Figs. S-1 and S-2) 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. 86M-3684, cite the authors, and include a check or money order for $4.00 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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Inactivator of Tyrosine Aminotransferase

Fig. 1. Cystine-dependent inactivation of tyrosine aminotransferase in a supernatant fraction from rat liver. Inactivation of tyrosine aminotransferase was studied in a 0.25-ml reaction mixture containing 0.1 M Hepes, pH 7.0, 1 mM EDTA, about 0.12 unit of tyrosine aminotransferase, and 0.75 mg of protein from a 12,000 × g supernatant fraction from rat liver. Aliquots initially containing about 3 milliliters of enzyme were taken at intervals and mixed with a diluent for assay as described under “Materials and Methods.” Tyrosine aminotransferase was stable in the absence of 1 mM L-cystine (open triangles), but lost activity rapidly when this amino acid was present (open circles). Addition of dithiothreitol to the aliquots at a final concentration of 40 mM (arrows) reactivated the enzyme during incubation at 37 °C (closed circles). Although most of the activity lost during a 2-h incubation with cystine was recovered upon addition of dithiothreitol, the inactivation was only partially reversed at 3 h (second arrow).

The soluble factor was stable during heat treatment at 65 °C and remained soluble when a 12,000 × g supernatant was acidified to pH 5.2. It did not bind to columns of DEAE-cellulose when applied in buffers with pH values below 8.2. Since tyrosine aminotransferase precipitates from 0.25 M sucrose at pH 5.2 and binds to DEAE-cellulose, these steps provided an effective means of separating the two activities and were employed to provide fractions of each activity for initial characterization. A point that became important in retrospect was the routine inclusion of 0.1 mM PLP in many initial supernatant fractions and in all preparations of tyrosine aminotransferase used to assay the factor. The minimum concentration of PLP was 4 mM after dilution into the incubation mixture. Subsequent tests also demonstrated that inactivation was not due to thiazolidine formation between amino acids and PLP.

Purification of the factor was begun using material that had been partially purified by heat treatment, acidification, and chromatography on DEAE-cellulose. The protein responsible for the activity precipitated between 45 and 75% saturation in ammonium sulfate. During prolonged dialysis of the precipitated material or chromatography on DEAE columns, however, most of the capacity to inactivate tyrosine aminotransferase was lost. On the supposition that these losses were due to removal of a cofactor, the inactivator was purified by a series of steps in which dialysis was minimized. Chromatography on hydroxylapatite and CM-Sephadex C-50 yielded a major peak of activity that coincided imperfectly with an abundant hemoprotein (as judged by the presence of a 410 nm chromophore). Although hematin mimicked the factor by causing inactivation of the aminotransferase during incubation with L-cystine, it did not reactivate the dialyzed factor.

Subsequent gel filtration using Sephacryl S-200 separated the activity from the heme-binding protein (Fig. 2) and resolved it from glutathione S-transferase activity.

Fig. 2. Chromatography of the inactivating factor on Sephacryl S-200. After it was eluted from hydroxylapatite, the factor was purified further by chromatography on CM-Sephadex C-50 (not shown), concentrated to a 15-ml volume with an Amicon pressure cell, and applied to a 2.5 × 80-cm column of Sephacryl S-200 equilibrated with 50 mM ammonium bicarbonate, pH 7.6, containing 1 mM EDTA, 1 mM dithiothreitol, and 0.1 mM PLP. The inactivating factor eluted as a single peak (peak 1, open circles) with absorbance at 280 nm (dashed line) and an approximate molecular weight of 100,000 (not shown). The remaining hemoprotein (peak 2, closed triangles) was well separated from the factor and eluted at a position corresponding to an Mr of about 45,000.

The absorption spectrum of the factor purified through the gel filtration step is shown in Fig. 3, trace 1. It is unremarkable, with a single major peak at 280 nm. Since most activity had been lost by this step and also is lacking in livers from pyridoxine-deficient animals (12), PLP was added to the material and a new peak appeared at about 420 nm, indicating formation of a Schiff base. Reduction with borohydride caused the expected shift to 320 nm (Fig. 3, traces 2 and 3). However, gel filtration of the unreduced material on Sephadex G-50 removed the 420 nm chromophore, indicating that the missing cofactor was not heme, but PLP (discussed below).

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A requirement for PLP was not obvious at first because the preparations of tyrosine aminotransferase and the crude factor contained this cofactor. To test whether PLP acted as a cofactor for the cystine-dependent inactivation, incubations were carried out with a dialyzed, inactive fraction obtained by precipitation with ammonium sulfate, chromatography on DEAE-cellulose, and gel filtration. By preincubating this inactive material with cystine in the presence or absence of PLP, it was shown that inactivation of tyrosine aminotransferase occurred more rapidly in the presence of PLP (Fig. 4).

A summary of the initial purification of the inactivating factor is shown in Table 2-S (Miniprint Section). The association of the factor with cystathionase activity was noted after the initial purification was complete. Cystathionase activity was then measured in all fractions that had been taken and was shown to be present at each stage of purification (Table 2-S). Note that the ability of the factor to inactivate tyrosine aminotransferase increased less than the de-

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3 James L. Hargrove, unpublished data.

4 James L. Hargrove, manuscript in preparation.
Is Cystathionase the Cystine-dependent Inactivator?—One of the few known mammalian enzymes that hydrolyzes cystine and has the set of properties described above is γ-cystathionase (26). To test this possibility, highly purified material was passed over Sephacryl S-200 (Fig. 1-S, Miniprint Section) and CM-Sephadex C-50, and in both cases cystathionase activity co-chromatographed with cystine-dependent inactivation of tyrosine aminotransferase. Cystathionase was purified as described under "Materials and Methods" in order to compare the two proteins with respect to cystathionine hydrolysis, cysteine desulfhydration, and inactivation of tyrosine aminotransferase. As shown in Table 3-S (Miniprint Section), both fractions contained all these activities, but the cystathionase had a higher specific activity with respect to cystathionine hydrolysis and cysteine desulfhydration. All fractions that inactivated the aminotransferase also generated hydrogen sulfide from cysteine in a PLP-requiring reaction (much less was formed from cystine) and produced an opalescent turbidity that is probably due to elemental sulfur produced during desulfuration of cystine (19, 27).

Several further experiments confirmed the similarity of purified cystathionase and preparations of the inactivating factor. The smaller (43 kDa) subunit was present in both preparations, migrated to identical positions during isoelectric focusing, and produced an activity stain during incubation containing cystathionine. However, the larger (45 kDa) band (trace 1) is probably not related, since it contains different peptides than authentic cystathionase, migrates to a different position during isoelectric focusing, and produced an inactive and colorless, and showed no evidence of having bound cofactor, as judged by the absence of a chromophore at 420 nm (trace 1). Upon addition of 0.1 mM PLP (final concentration) to the fraction, however, such a chromophore formed (trace 2), and after reduction with sodium borohydride, the peak at 420 nm was replaced by one at 320 nm (trace 3). About 1 mg of protein was employed to obtain each tracing. These data suggest that the inactivating factor binds PLP weakly.

**Mechanism of Inactivation**—The hydrolysis of cystine by cystathionase produces three reactive, sulfur-containing compounds including thiocysteine, elemental sulfur, and an en-
zyme-bound sulfane (19, 28). To determine which of these compounds might inactivate tyrosine aminotransferase, reaction mixtures in which the inhibitor had been produced were fractionated as described under "Materials and Methods." The turbid material that formed during the reaction contains atomic sulfur and denatured protein; it was precipitated by centrifugation and washed with buffer to separate it from soluble components. After being resuspended in the Hepes buffer used for the reaction, the washed precipitate still inactivated tyrosine aminotransferase (Table 4-S, Mini-print Section). To confirm that this result could be due to sulfur, tyrosine aminotransferase was incubated with a suspension of colloidal sulfur, which inactivated the enzyme in a time- and concentration-dependent fashion (Fig. 2-S, Mini-print Section). Dithiothreitol prevented this inactivation and partially reactivated the enzyme after the incubation (not shown).

The accumulation of thiocysteine can be prevented by including 5,5'-dithiobis-(2-nitrobenzoic acid) in the reaction mixture, which generates an arylalkyl disulfide and releases sulfur (19). The turbid, inhibitory material was still formed in the presence of 5,5'-dithiobis-(2-nitrobenzoic acid) (not shown).

The sulfane that is produced due to nonenzymatic interaction of thiocysteine with disulfide bonds in cystathionase and other proteins (28) could also inhibit tyrosine aminotransferase. To test whether this reactive species was present, the reaction mixture was fractionated by gel filtration and ion exchange chromatography. Tyrosine aminotransferase was inactivated by the high molecular weight fraction obtained by gel filtration of a reaction mixture in which the inhibitor was generated with cytosolic protein (Table 4-S). This inhibitory material also bound to DEAE-cellulose (not shown), and adding dithiothreitol to the fraction that eluted in 1.0 M NaCl from the DEAE column generated H$_2$S. Inhibitory material was not present in the high molecular weight fraction of a reaction mixture that contained only purified cystathionase (Table 4-S).

**FIG. 5. Comparison of purified inactivating factor and purified cystathionase.** Cystathionase was purified to apparent homogeneity as described under "Materials and Methods" and compared with the inactivating factor by several techniques. Electrophoresis in sodium dodecyl sulfate-polyacrylamide gels (Laemmli system, 10% monomer) showed that the inactivating factor (lane 1, 20 µg) and cystathionase (lanes 3 and 4, about 10 µg) both contained a subunit of about 43 kDa; a 45-kDa peptide was also present in the preparation of inactivating factor. Lane 2 contained 4 µg of a fraction from a less purified source of cystathionase. Lanes 5-8 show electrophoresis in a nondenaturing gel system with staining for cystathionase activity as described under "Materials and Methods." Lanes 5 and 6 contained 30 and 60 µg, respectively, of the inactivating factor, and lanes 7 and 8 had 10 and 20 µg, respectively, of the most purified cystathionase (post-Sephacyl S-200). Lanes 9-12 show tryptic peptides from the factor and cystathionase. Samples in lanes 9 and 12, respectively, were inactivating factor (30 µg) and cystathionase (15 µg) after treatment with 1.2 or 0.6 µg of L-tosylamido-2-phenylethyl-chloromethyl ketone-trypsin for 15 min. Lanes 10 and 11 contained the same amounts of untreated samples. Arrows on right show peptides shared by both preparations; arrows on left show unshared peptides.

**FIG. 6. Comparison of cystathionase and inactivating factor by isoelectric focusing.** Two halves of an isoelectric focusing gel that contain identical samples are shown, with one stained for cystathionase activity (left panel) and the other stained for protein with Serva blue R250 (right panel) as described under "Materials and Methods." Samples applied to each side were as follows: lanes 1 and 6, 10 µg of cystathionase (post-Sephacryl S-200); lanes 2 and 7, 5 µg of cystathionase; lanes 3 and 8, 5 µg of cystathionase (post-hydroxyapatite); lanes 4 and 9, 10 µg of inactivating factor; lanes 5 and 10, 5 µg of inactivating factor (post-CM-Sephadex II). At these protein concentrations, only the authentic cystathionase gave an activity stain (lanes 1-3). The major bands of protein in cystathionase migrated to a slightly more alkaline position than the major bands of protein in the inactivating factor (arrows on right). The two preparations of protein, however, contained bands that overlapped and may be identical. The gel contained amphotolites ranging from pH 3 to pH 10; the positions of standards of known isoelectric points (Serva pI markers) are designated in the center.

**DISCUSSION**

This paper shows that a previously unidentified factor that inactivates tyrosine aminotransferase in the presence of PLP and L-cysteine co-purifies with a second vitamin B₆-dependent enzyme, γ-cystathionase. The two proteins share several properties and catalytic activities, and antisera produced against each one inhibits the cystathionase activity of the other. Cystathionase hydrolyzes cysteine and yields products that inhibit some enzymes (27); this inhibition is probably due to reversible modification of exposed thiol groups. Furthermore, the hemoprotein that separated from the inactivating factor (cystathionase) during gel filtration (Fig. 2) oxidizes cysteine to cystine; by combining this protein with cystathionase, one can duplicate the phenomenon of cysteine-dependent enzyme inactivation. Thus, cystathionase and the hemoprotein together permit the reconstitution in vitro of cystine- and cysteine-dependent inactivation of enzymes, a phenomenon
that has often been observed in unpurified fractions of liver (4-13).

Cystathionase is a multifunctional lyase that eliminates substrates at the α, β, and γ carbon atoms of its substrates. It catalyzes the hydrolysis of cystathionine and homoserine, desulfuration of cystine, and desulfhydration of cysteine (26). The reaction that inactivates tyrosine aminotransferase is cystine desulfuration, as shown by the requirement for cystine and production of elemental sulfur by fractions that cause inactivation. Direct assays for sulfur have not been done, but addition of dithiothreitol to the reaction vessels releases hydrogen sulfide and the turbidity disappears. No other substrates tested, including cysteine, cystathionine, homoserine, and homocysteine, caused inactivation. Since cysteine, homoserine, and homocysteine all form adducts with PLP (29), whereas disulfides do not, this mechanism can be excluded as the basis for the inactivation.

The phenomenon of cystine-dependent inactivation of tyrosine aminotransferase was discovered in the cytosolic fraction by Holten et al. (4). However, later investigators identified an activity associated with membrane fractions that is capable of inactivating tyrosine aminotransferase and several other enzymes (6, 10, 11). The membrane-bound factor also uses cysteine but can only be solubilized by use of detergents and proteases (10, 11). These results suggest the presence of a second inactivating activity. Cystathionase, which is cytosolic, may have been identified here because PLP had been added to the homogenization buffers routinely, whereas this had not been done in the earlier studies.

The involvement of cystathionase in cystine-dependent enzyme inactivation was first noted during a study of sulfur metabolism by Kato et al. (30). Serine dehydratase was thought at the time to be an activity associated with membrane fractions that is capable of inactivating tyrosine aminotransferase and several other enzymes (6, 10, 11). The membrane-bound factor also uses cysteine but can only be solubilized by use of detergents and proteases (10, 11). These results suggest the presence of a second inactivating activity. Cystathionase, which is cytosolic, may have been identified here because PLP had been added to the homogenization buffers routinely, whereas this had not been done in the earlier studies.

The mechanism by which cystine desulfuration occurs suggests that three intermediates or products could be involved. These include the persulfide, thiocysteine, which is the product formed enzymatically; a sulfane (trisulfide), which is then produced by reaction of thiocysteine with a disulfide bond present in cystathionase; and elemental sulfur, which is released when the trisulfide decomposes (29). The concentration of elemental sulfur that would be produced under the conditions tested here do inactivate tyrosine aminotransferase (Fig. 2-S). Since 5,5'-dithiobis-(2-nitrobenzoic acid) prevents the accumulation of thiocysteine but does not reduce the accumulation of sulfur (19) nor affect the production of an inhibitor of tyrosine aminotransferase, the persulfide may not be directly involved. Sulfur may not be the only potential inhibitor formed, however, since a reactive species that behaves as if it were associated with proteins is produced during the metabolism of cystine by a cytosolic fraction from liver (Table 4-S). This high molecular weight material may contain enzyme-bound sulfane. We are now testing the possibility that other proteins in the cytosol modify the inactivation of tyrosine aminotransferase, since such an interaction might explain the poor ability of purified cystathionase to inactivate the enzyme. The thiocysteine produced by γ-cystathionase during the desulfuration of cystine reacts with mitochondrial proteins to generate sulfanes (32).

Sulfane groups generated by δ-cystathionase may regulate δ-aminolevulinate synthetase in the bacterium Rhodopseudomonas spheroides. δ-Aminolevulinate synthetase occurs in an inactive, reduced form in that organism during growth under anaerobic conditions (33-35), and activation takes place upon addition of t-cystine to cellular extracts. The activation requires two proteins, one of which is δ-cystathionase; the other protein forms a trisulfide in response to cystathionase-catalyzed cystine desulfuration. This sulfane oxidizes thiol groups in aminolevulinate synthetase, thereby activating it (34).

Mammalian γ-cystathionase also forms an intra-enzymic sulfane and activates bacterial aminolevulinate synthetase (33).

Mammalian cystathionase may interact with PLP-dependent enzymes in other ways than the one described here for tyrosine aminotransferase. It forms complexes with cytosolic aspartate aminotransferase in vitro and will donate PLP to aminolevulinate synthetase as a consequence of its relatively weak affinity for the cofactor (36, 37). We have not tested whether direct interactions occur between cystathionase and tyrosine aminotransferase. Since hepatic PLP content regulates the rate of synthesis of tyrosine aminotransferase, but not its rate of degradation (4, 38), no physiological significance can yet be ascribed to the system described here.

Acknowledgments—J. L. H. thanks Samy Ashkar for discussions of the phenomenon of disulfide exchange, Dr. Dean P. Jones for helpful suggestions and assistance during this project, and the Emory University Research Fund for continued support.

REFERENCES
Inactivator of Aminotransferase


SUPPLEMENT TO A CaTion-dependent Inactivator of Tyrosine Aminotransferase

By James L. Harpe and R. Douglas Winnick

MATERIALS AND METHODS

The animals used as sources for enzymes in this study were obtained from Sprague-Dawley and Wistar rats of the Sprague-Dawley strain. The cats were maintained on a 12 h light:12 h dark cycle with free access to water and food. The tissues used for enzyme purification were purchased from Sigma Chemical Company. The enzymes used in this study were purified by the methods described in the literature. The protocols are given in the following references:


Assay and Purification of Cystathionase

Cystathionase activity was assayed in the presence of cysteine and by measuring the production of cystathionine by the production of glutathione from cysteine by the production of cystathionine from cysteine and cysteine. The assay was conducted at 25° in 0.1 M EDTA, 0.1% Triton X-100, 0.2 mg/ml cystathionase, 1 mM EDTA, 0.1 mM MgCl₂, and 0.1% ascorbic acid. The reaction was started by adding 4.1 ml of 0.5% 200, 0.2 ml of 0.5% 200, and 0.1 mM cystathionase to the reaction mixture. The mixture was incubated for 30 min at 37°. The reaction was stopped by adding 10% trichloroacetic acid to the reaction mixture and the reaction was continued at 37° for 10 min. The samples were then treated with 10% trichloroacetic acid and then were stained in Serva blue R 2.50, 1% Serva blue B 0.50, and 3% Serva blue 0.50. The stained gels were then washed with 10% Tris-HCl, pH 7.6. The absorbance was measured at 560 nm using a spectrophotometer.

Results and Discussion

The results are presented in Table 1. Table 1 shows the purification of cystathionase from rat liver. The purification was performed by a combination of high-performance liquid chromatography and polyacrylamide gel electrophoresis. The enzyme activity was assayed by measuring the amount of cystathionine produced in the reaction mixture. The enzyme activity was determined by measuring the absorbance at 560 nm using a spectrophotometer. The results are presented in Table 1. Table 1 shows the purification of cystathionase from rat liver. The purification was performed by a combination of high-performance liquid chromatography and polyacrylamide gel electrophoresis. The enzyme activity was assayed by measuring the amount of cystathionine produced in the reaction mixture. The enzyme activity was determined by measuring the absorbance at 560 nm using a spectrophotometer.

Table 1: Purification of Cystathionase

<table>
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<tr>
<th>Step</th>
<th>Protein (mg)</th>
<th>Protein concentration (mg/ml)</th>
<th>Enzyme activity (U/mg)</th>
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<tr>
<td>1</td>
<td>79±3</td>
<td>37.4±5</td>
<td>31.3±17</td>
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<tr>
<td>2</td>
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<td>5</td>
<td>2.3±0.6</td>
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<td>0.98±0.05</td>
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</tbody>
</table>

Note: The enzyme activity was determined by measuring the absorbance at 560 nm using a spectrophotometer. The results are presented in Table 1. Table 1 shows the purification of cystathionase from rat liver. The purification was performed by a combination of high-performance liquid chromatography and polyacrylamide gel electrophoresis. The enzyme activity was assayed by measuring the amount of cystathionine produced in the reaction mixture. The enzyme activity was determined by measuring the absorbance at 560 nm using a spectrophotometer.

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factions from the first centrifugation were passed over 3.6 ml columns of Sephadex G50 that had been calibrated with myoglobin and bromophenol blue. The fractions that co-eluted with these two standards were saved at 4°C. All weight material obtained by gel filtration was applied to 1.0 ml columns of DEAE-cellulose or CM-cellulose G25. The void volume of these columns had been determined with multiple blue or bromophenol blue, respectively. Material in the flow-through was saved, the high-molecular weight material obtained by gel filtration with 1.5 M NaCl, and pellet fractions prepared from the reaction mixture. Inhibitor was produced by incubation with cysteine at 37°C for 0.1 ml of the insoluble material and 0.2 ml of the other fractions. No inactivation of tyrosine aminotransferase during incubation with sulfur was observed. Blank incubations with washed twice with 0.2 ml of 0.1 M Hepes, pH 7, containing 1 M EDTA, 0.1 M dithiothreitol, and 0.1 M PLP, and sulfur concentrations from 10⁻³ to 10⁻² M, ethanol did not inhibit the assay at concentrations unless PLP was omitted.

### Table 1-8

<table>
<thead>
<tr>
<th>Sample</th>
<th>Volume (ml)</th>
<th>Protein (mg/ml)</th>
<th>Total Protein (mg)</th>
<th>Cystathionase (unit/mg)</th>
<th>Tyrosine Aminotransferase Inactivated (unit/mg/2 h)</th>
<th>% Recovery</th>
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<td>3. Pent halves</td>
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<tr>
<td>9. On Sephadex G25</td>
<td>10.0</td>
<td>3.4</td>
<td>30</td>
<td>451</td>
<td>260</td>
<td>18</td>
</tr>
</tbody>
</table>

### Figure 1-8

The inactivating factor copurifies with cystathionase and cystathionase activities. Tyrosine aminotransferase was assayed in the presence of cysteine desulfhydratase activity during chromatography on Sephacryl S-300 as described in the Methods section. The product measured was actually N-ethylthiourea and not pyruvate. Cystathionase activity was assayed as described in the Methods section.