Low Concentrations of Guanidinium Chloride Expose Apolar Surfaces and Cause Differential Perturbation in Catalytic Intermediates of Rhodanese*

Paul Horowitz and Nick L. Criscimagna
From the Department of Biochemistry, University of Texas Health Science Center, San Antonio, Texas 78284

The conformations of sulfur-free and sulfur-containing rhodanese were followed with and without the detergent lauryl maltoside after guanidinium chloride (GdmCl) addition to 5 m to study the apparent irreversibility of denaturation. Without lauryl maltoside, sulfur-containing rhodanese denatured in a transition giving, at -2.3 m GdmCl, 50% of the total denaturation induced change observed by activity, CD, or intrinsic fluorescence. Sulfur-free rhodanese gave more complex behavior by intrinsic fluorescence and CD. CD showed loss of secondary structure in a broad, complex, and apparently biphasic transition extending from 0.5 to 3 m GdmCl. The interpretation of the transition was complicated by time-dependent aggregation due to noncovalent interactions. Results with the apolar fluorescence probe 2-anilinonaphthalene-8-sulfonic acid, implicated apolar exposure in aggregation. Sulfhydryl reactivity indicated that low GdmCl concentrations induced intermediates affecting the active site conformation. Lauryl maltoside prevented aggregation with no effect on activity or any conformational parameter of native enzyme. Transitions induced by GdmCl were still observed and consistent with several phases. Even in lauryl maltoside, an increase in apolar exposure was detected by 2-anilinonaphthalene-8-sulfonic acid, and by protein adsorption to octyl-Sepharose well below the major unfolding transitions. These results are interpreted with a model in which apolar interdomain interactions are disrupted, thereby increasing active site accessibility, before the intradomain interactions.

Bovine liver rhodanese (thiosulfate:cyanide sulfurtransferase, EC 2.8.1.1) is a mitochondrial enzyme that has been implicated in the physiological synthesis of protein-bound iron-sulfur centers (1, 2). The most studied rhodanese-catalyzed reaction in vitro is the transfer of the outer sulfur of thiosulfate (S\textsubscript{2}O\textsuperscript{3-}) to the nucleophilic acceptor, cyanide (CN\textsuperscript{-}), by a double displacement mechanism that can be written as:

\[
\text{S}_2\text{O}_3^{2-} + E \rightleftharpoons ES + \text{SO}_4^{2-} \\
ES + \text{CN}^{-} \rightarrow E + \text{SCN}^{-}
\]

During catalysis, the enzyme cycles between two stable intermediates that can be individually isolated: the sulfur-free enzyme, \(E'\) and the sulfur-substituted enzyme, \(ES\). Physical properties of these forms in solution have been demonstrated to be different by a number of methods that, together with functional studies, have implicated an obligatory conformational change in catalysis (3-5).

X-ray studies (6-8) of the \(ES\) form have confirmed solution results that identified essential elements for catalysis by rhodanese including an essential sulfhydryl group, a cationic binding site, and a hydrophobic region. The active site is at the interface between two approximately equal size and similarly folded domains into which the single polypeptide chain is folded. Solution studies have suggested that the conformational change involved in catalysis reflects a structurally mobile protein, and changes in domain interactions during catalysis may underly the solution behavior. On the other hand, the x-ray structure does not appear to fully reflect the solution dynamics. However, it seems that energy differences between the conformers are small, and conformational changes in the crystal may be restricted by lattice forces (9). Furthermore, it has been suggested that at least part of the reported conformational differences between \(E\) and \(ES\) may be due to time-dependent conformational changes (10).

To the extent that conformational changes in rhodanese catalysis can be supported, this enzyme will continue to be a good model for investigating the functional role of protein dynamics and domain interactions.

We report here the differential effects of guanidinium chloride (GdmCl) on the structure and function of the \(E\) and \(ES\) forms of rhodanese monitored by several parameters and find changes related to exposure of hydrophobic surfaces. Comparisons between perturbations of \(E\) and \(ES\) are particularly interesting because the catalytic intermediates differ only by a single sulfur atom, and the x-ray shows that the surface is the same in both forms.

**MATERIALS AND METHODS**

Prepurified guanidine hydrochloride and dialyzed urea were obtained from Heico (Whittaker Corporation; Delaware Water Gap, PA). Lauryl maltoside was from Behring Diagnostics. Octyl-Sepharose was from Pharmacia. All other reagents used were analytical grade.

Rhodanese was purified from bovine liver as previously described (11) and was stored at -70 °C in ammonium sulfate as a crystalline suspension of the \(ES\) form. The enzyme was assayed by a colorimetric

\[\text{The abbreviations used are: E, the sulfur-free form of rhodanese; GdmCl, guanidinium chloride; ES, the sulfur-substituted form of rhodanese; 2,8-ANS, 2-anilinonaphthalene-8-sulfonic acid; 1,8-ANS, 1-anilinonaphthalene-8-sulfonic acid; HPLC, high pressure liquid chromatography.}\]
method previously described (12). Protein concentrations were determined either by using a value of \( E_{280}^\text{cm} = 1.75 \) for purified rhodanese (12) or by measuring the quantitative binding to the protein of the dye Coomassie Brilliant Blue using the optical density at 595 nm (13). Standard curves were generated with both rhodanese and bovine serum albumin. Standard curves and blanks were prepared for the activity and protein measurements with the required concentrations of GdmCl to eliminate any artifacts due to carryover. For the activity measurements, the GdmCl was diluted 1:500 and never exceeded 2 mM, a level which had no effect on the activity of native enzyme. Similar precautions were taken when the effects of lauryl maltoside were tested. The molecular weight of rhodanese was taken to be 33,000 (14).

The sulfur-free form of rhodanese (\( E \) form) was obtained as previously described (15) by adding a 10-fold molar excess of cyanide over protein to a rhodanese solution in a buffer consisting of 50 mM Tris-HCl, pH 7.4. This buffer was used for all the studies reported here unless otherwise stated. The ES form was obtained by dissolving crystalline rhodanese in buffer.

Circular dichroism measurements were made using a Jasco 500C spectropolarimeter equipped with a DP500 data processing accessory. Spectra were typically determined for solutions of GdmCl concentrations between 0 and 4 M and a protein concentration of 0.5 mg/ml in a cell with a 0.1-cm path length maintained at 23 °C. Samples were incubated for 30 min before each run. Other conditions, when used, are specified under "Results." Spectra were determined between 280 and 190 nm, and the ellipticities at 222 nm were used to assess the fraction of native structure remaining at each GdmCl concentration using the ellipticity at 222 nm in 4 M GdmCl for denatured enzyme. For each GdmCl concentration, a separate blank was used to account for any effects due to the presence of guanidine. The percent of native structure was measured by taking as 100% the difference between the ellipticity for \( E \) in buffer and in 4 M GdmCl.

Gel permeation chromatography was performed by HPLC using a 30-cm TSK 3000 column (Toyo Soda, Tokyo, Japan) run in a buffer consisting of 50 mM GdmCl, 0.02 M NaOH, pH 6.5. To assess the chromatographic behavior of rhodanese, pellets were prepared for after centrifugation of appropriate solutions with given GdmCl concentrations, were completely dissolved in 5 M GdmCl. These clear solutions were run on HPLC in 5 M GdmCl with or without reduction with either mercaptoethanol or dithiothreitol. To assess the chromatographic behavior of the pelleted rhodanese, comparisons were made with the elution characteristics of bovine serum albumin (\( M_r = 68,000 \)), which has approximately the molecular weight expected from crystalline rhodanese if it were to form a dimer (\( M_r = 66,000 \)). Comparisons were also made in the same system on a column calibrated with lysozyme, insulin, untreated rhodanese, ovalbumin, and bovine serum albumin.

RESULTS

Several parameters related to the structure and activity of the \( E \) and \( ES \) forms of rhodanese were measured as a function of the concentration of GdmCl. GdmCl, unlike denaturants such as urea and sodium dodecyl sulfate, appears to denature rhodanese without the complication of disulfide bond formation (18).

Fig. 1A shows the percent native structure, assessed from the ellipticities measured at 222 nm, as a function of the concentration of GdmCl. The native enzyme displayed values comparable to those previously reported (4). For example, a mean residue ellipticity at 222 nm = \(-6.9 \times 10^2 \) degree cm² dmol⁻¹ for the \( E \) form was measured here as compared with \(-7.5 \times 10^2 \) degree cm² dmol⁻¹ in Ref. 4. The secondary structure for the \( ES \) form was apparently unchanged to 1.5 M GdmCl and gave a transition such that 50% of the native ellipticity was reached at \(-2.3 \) M GdmCl at 2 M GdmCl, ES still had at least 60% of the native secondary structure. The loss of enzyme activity followed by dilution of aliquots into the standard assay mixture followed the structural transition and there was no turbidity noted at any GdmCl concentration to 6 M. The \( E \) form showed more complex behavior. When titrated by successive addition of aliquots of concentrated GdmCl, \( E \) showed loss of organized secondary structure in a broad, complex, and apparently biphasic transition extending from 0.5 to 3 M GdmCl. The interpretation of these transitions is complicated because of time-dependent changes in turbidity. Fig. 1A shows the results when individual samples were prepared at the indicated GdmCl concentrations and allowed to incubate for 30 min before measuring the circular dichroism spectra. The data in the region between \(-0.6 \) to 1.5 M GdmCl are omitted because of potential ambiguities introduced by turbid samples. The turbidity was investigated by examining the light scattering from rhodanese samples as a function of the GdmCl concentration as described under "Materials and Methods." These results are shown in Fig. 1B. The ES sample showed no turbidity at any concentration of GdmCl. With \( E \), the light scattering became noticeable slightly above 0.5 M GdmCl and increased to a maximum at approximately 1.5 M GdmCl, with a half-maximal effect at approximately 1.2 M GdmCl. As the GdmCl concentration was increased beyond 1.5 M the scattering decreased, and solutions at GdmCl concentrations higher than approximately 2 M showed no evidence of turbidity. The differences in the GdmCl concentrations giving maximum turbidity in Fig. 1, A and B are apparently related to the differences in the protein concentrations used for the determinations. In a separate experiment (data not shown), the protein concentration was directly measured in the supernatants and pellets after centrifuging rhodanese samples that had been incubated for 45 min at 23 °C at various GdmCl concentrations. The amount of precipitate that formed correlated with the magnitude of the light scattering shown in Fig. 1B with the maximum amount of precipitate formed at approximately 1.5 M GdmCl. The pellets formed at any GdmCl concentration completely dissolved at 2 M GdmCl with no indication of turbidity. When these pellets were dissolved and subjected to HPLC gel permeation chromatography in GdmCl as described under "Materials and Methods," the protein co-chromatographed with authentic monomeric rhodanese independent of whether the
concentration of 0.093 mg/ml to which p1 quantities of 6 percent native ellipticity was derived from circular dichroism measurements with the fluorescence intensity of the apolar probe 2,8-anilinonaphthalene forms of rhodanese as a function of the GdmCl concentration. The fluorescence intensity interfered with measurements between 0.5 and 1.5 M GdmCl with an intensity approximately 1.8 times higher than that observed in the absence of GdmCl (note the scale origin for fluorescence in Fig. 1B). Then, the fluorescence intensity decreased steadily as the GdmCl concentration was increased above 1.5 M and reached background levels at GdmCl concentrations >2 M.

Iodoacetamide was used as a probe for the accessibility of the active site sulfhydryl group of rhodanese. The E form is rapidly inactivated by iodoacetic acid but remains unreacted with iodoacetamide even after long incubation (3). It appears that noncovalent intraprotein interactions protect the active site from reaction with iodoacetamide. Therefore, iodoacetamide can probe changes in steric accessibility to the active site induced by perturbants. Fig. 2 shows that no inactivation of E was observed in the absence of GdmCl whether iodoacetamide was present or not. In the absence of iodoacetamide the activity followed the upper curve. For this curve, the enzyme was preincubated for 90 min at various GdmCl concentrations to 2 M and then diluted 1:500 into the assay mix to give <4 mM GdmCl in the final assay. The activity after dilution was constant to approximately 1 M GdmCl and then decreased, and there was no recoverable activity at concentrations of GdmCl >1.5 M under the conditions used here. Control experiments showed that the carryover of GdmCl had no effect on the results. The lower curve shows the results when this experiment was repeated in the presence of a 50-fold molar excess of iodoacetamide over enzyme. The activity was irreversibly lost in a transition that appears to be considerably broader than that observed in the absence of iodoacetamide. Here the enzyme was almost totally inactivated by incubation in 1.0 M GdmCl. Although there was some time dissolved pellet had been reduced with mercaptoethanol. Furthermore, ultracentrifugation showed that the s_{20,w} of E was the same in 0.5 M GdmCl as in buffer and was consistent with results previously reported for the monomeric protein (17). Thus, the aggregates of E were stabilized by noncovalent interactions, and there was no indication of significant association of the monomeric enzyme at GdmCl concentrations at which there was no significant turbidity. Fig. 1B also compares the GdmCl-induced turbidity in a separate experiment with the fluorescence of the apolar probe 2,8-ANS that appears to bind near the active site region of the rhodanese molecule in the E form (19). The intensity of the fluorescence observed in the absence of GdmCl decreased slightly as the GdmCl concentration increased and appeared to go through a minimum at approximately 0.5 M GdmCl. Then the fluorescence increased as the GdmCl concentration was increased further and reached a maximum at approximately 1.5 M GdmCl with an intensity approximately 1.8 times higher than that observed in the absence of GdmCl (note the scale origin for fluorescence in Fig. 1B). Then, the fluorescence intensity decreased steadily as the GdmCl concentration was increased above 1.5 M and reached background levels at GdmCl concentrations >2 M.

![FIG. 1. A, percent native ellipticity for the E (O), and ES (△) forms of rhodanese as a function of the GdmCl concentration. The percent native ellipticity was derived from circular dichroism measured at 222 nm and is calculated relative to the native protein in the absence of GdmCl. Measurements for the ES form (△) were made at a protein concentration of 0.5 mg/ml. Measurements with the E form were made either on individual samples (•) at 0.063 mg/ml preincubated for 30 min or by directly titrating a single sample at an initial concentration of 0.093 mg/ml to which μl quantities of 6 M GdmCl were added sequentially (O). For the individual E samples (•) turbidity interfered with measurements between 0.5 and 1.5 M GdmCl. E, the fluorescence intensity of the apolar probe 2,8-anilinonaphthalene sulfonic acid bound to the E form of rhodanese (O), the turbidity of the E form (•), and the turbidity of the ES form (△) as functions of the concentration of GdmCl. The fluorescence intensity was measured at 480 nm with excitation at 360 nm. The 2,8-ANS concentration was 50 μM. The scale for 2,8-ANS fluorescence begins at 70 units. Turbidity was measured at 480 nm. All measurements were made after a 30-min incubation. The protein concentrations were 0.2 mg/ml. The buffer was 0.05 M Tris-HCl, pH 7.4 and was used throughout this study. All other conditions are given under "Materials and Methods."](http://example.com/fig1)

![FIG. 2. Inactivation of the E form of rhodanese by reaction of iodoacetamide with the active site sulphydryl group. The A_{400} is the absorbance in the activity assay and is 0.2% for fully active enzyme. The activity of E is shown as a function of (GdmCl) either in the absence (•) or in the presence (△) of iodoacetamide at a 50-fold molar excess over enzyme. In the absence of GdmCl, the enzyme is not inactivated by iodoacetamide even after 3 h. The data shown corresponds to incubating a 0.5 mg/ml solution of E for 90 min at the indicated GdmCl concentrations. Each assay involved diluting the indicated (GdmCl) by 1:500 into the assay mixture.](http://example.com/fig2)
dependence to the enzyme activity as a function of the GdmCl concentration, the difference between curves in the presence and absence of iodoacetamide did not depend on the incubation time with GdmCl.

Fig. 3 shows the GdmCl dependence of the intrinsic fluorescence wavelength maximum (Fig. 3A) and intensity (Fig. 3B) for the E and ES forms of rhodanese. The protein concentration was chosen to be low enough to minimize turbidity and little was noted in the course of the fluorescence measurements. The ES form, monitored by fluorescence, showed a similar transition as with CD, whether intensity or wavelength maximum was monitored. The fluorescence behavior of E was again more complex. The intensity of E was quenched by even the smallest additions of GdmCl and fell to 87% of its initial value at 0.9 M GdmCl (Fig. 3B). At higher GdmCl concentrations, there was a single transition at 1.4 M GdmCl that was reflected in both the intensity and the wavelength of the maximum emission. Both E and ES gave wavelength maxima, in the absence of GdmCl, at 334 nm characteristic of buried residues (20), and at high GdmCl concentrations (>2.5 M) the wavelength maxima reached a value of 353 nm, characteristic of solvent-exposed tryptophan residues. GdmCl induced a transition between these extremes that was considerably broader for E than for ES. The breadth of these transitions can be compared with those monitored for E by activity (Fig. 2) or by CD (Fig. 1A). NaCl was used to control ionic effects and had no effect on the intrinsic fluorescence (Fig. 3B). To test for specific quenching by GdmCl on the accessible fluorescence of rhodanese, GdmCl was titrated into solutions of N-acetyltryptophanamide. From 0 to 1.5 M GdmCl there was no effect on the fluorescence of N-acetyltryptophanamide. In addition, there was no effect on the fluorescence of a control protein, ovalbumin, from 0 to 1.2 M GdmCl. These results indicate that the effect of low concentrations of GdmCl on the fluorescence of E is not general to proteins, nor does it reflect tryptophan exposure in rhodanese, which is not unexpected given the short wavelength emission of the native enzyme.

As noted above, it was difficult to interpret measurements in the GdmCl concentration range where solutions of E became turbid. The results with 2,8-ANS, together with the noncovalent nature of the forces stabilizing the aggregates, suggested that apolar interactions were involved in the formation of turbidity. Lauryl maltoside was used therefore, in an attempt to modulate the appearance of large aggregates. The activities of E and ES were not affected by preincubation for 1 h at any concentration of lauryl maltoside up to 2 mg/ml, the maximum concentration tested (data not shown). Also, lauryl maltoside concentrations to 1 mg/ml had no significant effect on either the E or ES forms of the native enzyme in terms of intrinsic fluorescence, circular dichroism spectra, or apparent g20w.

Fig. 4 shows the results of using the precipitation assay described under "Materials and Methods" to assess the effect of lauryl maltoside on the GdmCl-induced precipitation of E. As expected, at this protein concentration, the protein almost completely precipitated at GdmCl concentrations around 1.5 M in the absence of lauryl maltoside. The presence of 0.4 mg/ml lauryl maltoside however, completely prevented protein precipitation at any GdmCl concentration tested. Therefore, lauryl maltoside was used with the E form to avoid complications due to aggregation and permit higher concentrations of the enzyme to be used.

Fig. 5 shows that in the presence of lauryl maltoside the structural transitions were no longer the same when moni-
induced precipitation in the absence of lauryl maltoside.

Fig. 6 shows the GdmCl titration of E and ES in the presence of 0.4 mg/ml lauryl maltoside monitored by CD. The transitions for both E and ES gave 50% of the ellipticity of the native enzyme at approximately 2.5 M GdmCl and were compatible with the transition at higher GdmCl seen when the fluorescence intensity was monitored in Fig. 5A. The CD changes with E contained a component of increased secondary structure that occurred at GdmCl concentrations giving the transition at low GdmCl concentrations when monitored by wavelength maximum in Fig. 5A. The magnitude of the change however, was small. These results support the idea that the response of rhodanese to increasing GdmCl involves intermediate states.

Fig. 7 shows the ability of GdmCl to expose apolar sites on rhodanese in 0.4 mg/ml lauryl maltoside. The ordinate represents the difference in fluorescence of 1,8-ANS in the presence and absence of E (upper curve) or ES (lower curve) using buffers containing lauryl maltoside. This shows, especially for the E sample, that apolar exposure could still be detected in the presence of lauryl maltoside, and the maximum apolar exposure occurred at GdmCl concentrations close to those giving maximum precipitation of E in Fig. 4.

Fig. 8 shows that GdmCl also induced binding of both E and ES in the presence of lauryl maltoside to the apolar resin, octyl-Sepharose. This apolar exposure was more facile in E as was shown by its more complete removal from solution at lower GdmC1 concentrations (lower curve) as compared with ES (upper curve).

**DISCUSSION**

The results presented here indicate that GdmCl induces changes of the rhodanese structure in several phases, and the net result is the apparently irreversible denaturation of the enzyme. This is in keeping with previous reports that rhodanese cannot be renatured after denaturation in 6 M GdmCl (21), and that the native enzyme, especially E, is structurally labile (22). The present results support the idea that irreversibility and slow structural changes follow formation of kinetically stable intermediates that are close to the native structure, which under the appropriate conditions can aggregate because of significant exposure of apolar surfaces.

The clear differences between E and ES are particularly

**Fig. 5.** A, the effect of lauryl maltoside on the wavelength of the maximum fluorescence from spectra of the intrinsic fluorescence of the E (○) and ES (●) forms of rhodanese as a function of the concentration of GdmCl. The lauryl maltoside concentration was 0.4 mg/ml, and the protein concentration was 0.2 mg/ml. The samples were incubated for 120 min at room temperature and scanned using 280 nm excitation. Other experimental details are as described in the legend to Fig. 3A. B, the effect of lauryl maltoside on the intrinsic fluorescence intensities of the E (○) and ES (●) forms of rhodanese as a function of the concentration of GdmCl. Lauryl maltoside was present at 0.4 mg/ml. Other experimental details are given in the legend to Fig. 5A.
absence of either E or ES represents the change in fluorescence of 1,8-ANS in the presence and absence of either E or ES. The samples consisted of 0.2 mg/ml protein, 50 μM 1,8-ANS and 0.4 mg/ml lauryl maltoside. The samples were incubated for 120 min, and the intensities were determined with excitation at 380 nm and emission at 490 nm. Other experimental details are given under “Materials and Methods.”

**Fig. 7.** The effect of GdmCl on the fluorescence intensity of the apolar probe 1,8-ANS interacting with the E and ES forms of rhodanese in the presence of lauryl maltoside. The ordinate represents the change in fluorescence of 1,8-ANS in the presence and absence of either E or ES. The samples consisted of 0.2 mg/ml protein, 50 μM 1,8-ANS and 0.4 mg/ml lauryl maltoside. The samples were incubated for 120 min, and the intensities were determined with excitation at 380 nm and emission at 490 nm. Other experimental details are given under “Materials and Methods.”

interesting. ES has generally been considered to be the more stable enzyme form (5, 15). Here, when transitions leading to aggregation are avoided by inclusion of lauryl maltoside, the major denaturation events observed in GdmCl are similar in E and ES. This may indicate that previously observed differences in structural stabilization of E and ES may have had a kinetic component. The potential ambiguities in comparing structural events in E and ES that would stem from differential surface binding of GdmCl (23) are minimized in the present case for several reasons: (a) E and ES only differ by a single sulfur atom; (b) the crystal structure shows virtually identical surfaces in E and ES; and (c) effects occur at low concentrations of GdmCl where the weak binding would exert an insignificant influence.

The extensive noncovalent aggregation of the E form, but not ES, occurs at GdmCl concentrations producing minor changes in overall structure, indicating that the structural perturbations may be local and/or transient. For example, iodoacetamide can react with the active site thiol group in 1 M GdmCl, presumably via a protein conformer that reverses on dilution. Also, the fluorescence effects of GdmCl are different when the wavelength and intensity are monitored indicating that not all the tryptophan residues are responding equally to the perturbation. Furthermore, the activity disappears irreversibly at GdmCl concentrations above 1.5 M, a concentration at which there would be extensive protein precipitation in the absence of lauryl maltoside, even though there is still more than 50% of the native ellipticity. The increased apolar exposure induced by low concentrations of GdmCl is especially apparent in the complex conformational transitions observed with the E form, although it could also be observed in ES. Again, this is in keeping with the relative labilities of E and ES. This apolar exposure could lead to the observed extensive aggregation which is apparently cooperative since there is little evidence for species other than monomers and large aggregates. The suggestion of the appearance of apolar surfaces is supported by the results with lauryl maltoside which, although innocuous to the function of native rhodanese, prevents aggregation of E just as it maintains the solubility of membrane proteins (24). It is interesting that the appearance of apolar surfaces can be measured in the presence of this detergent, and this potential may be of general use in the study of conformational transitions.

Several studies with other proteins have reported effects that relate to those observed here with rhodanese. For example, it has been observed that inactivation of creatine kinase precedes denaturation by urea or GdmCl, indicating that the active site of the protein is in a region that is sensitive to slight conformational changes (25). Irreversible unfolding of cathespin D occurs in stages ascribed to the formation of intermediates (25). Phosphorylase b is sensitive to low concentrations of GdmCl, and apparently irreversible, time-dependent conformational transitions lead to aggregation (27). Furthermore, the irreversibility of antithrombin denaturation has been ascribed to aggregation of intermediates, consistent with suggestions that it is folded into separate domains (28).

The availability of a high resolution x-ray structure for rhodanese provides a structural context for interpretation. The following essential features can be visualized. Rhodanese is monomeric, and its single polypeptide consists of two equal size and similarly folded domains connected by a single 16-residue peptide. This structure is stabilized by inter- and intradomain interactions. The active site is in a depression at the interdomain interface, with side chains from each domain forming the walls of the pocket, at the bottom of which is the essential cysteine 247. Close to the active site is a cluster of hydrophobic and aromatic residues (Trp-35, Phe-106, Tyr-107, and Phe-212). The interdomain association is stabilized mainly by apolar interactions, and approximately 900 Å² of contact area is buried on domain association. The individual domains, however, are not remarkable in their stabilization, and the entire array of protein-stabilizing interactions can be visualized. In fact, one might expect that conditions could be
found to differentially perturb the inter- and intradomain structures.

As a working hypothesis, GdmCl may perturb rhodanese in two steps. Low GdmCl concentrations would increase accessibility of the interdomain surfaces, leading to the observed increases in active site sulfhydryl group reactivity and increased solvent contact with the interdomain tryptophans. Dilution of the GdmCl from these low concentrations would allow reassociation of the domains and give active enzyme. As the GdmCl concentration is raised further, the interdomain interactions would be more seriously perturbed and the extensive exposure of the interdomain apolar surfaces would lead to enzyme aggregation and increased accessibility to apolar probes. At still higher GdmCl concentrations, the aggregates would dissolve, and the individual domains would denature. Lauryl maltoside would tend to stabilize the apolar surfaces, explaining the absence of precipitation in this solvent.

The results presented here have allowed us to find conditions which, for the first time, permit refolding of rhodanese denatured in GdmCl. These studies are the subject of separate reports (29, 30).

REFERENCES