Inactivation of γ-Glutamylcysteine Synthetase, but Not of Glutamine Synthetase, by S-Sulfo cysteine and S-Sulfohomocysteine*

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The reactions catalyzed by γ-glutamylcysteine synthetase and glutamine synthetase are thought to proceed via enzyme-bound γ-glutamyl phosphate intermediates. We investigated the possibility that S-sulfo cysteine and S-sulfohomocysteine might act as analogs of γ-glutamyl phosphate or of the associated putative tetrahedral intermediates. The D- and L-enantiomers of S-sulfo cysteine and S-sulfohomocysteine were found to rapidly inactivate rat kidney γ-glutamylcysteine synthetase but to be reversible inhibitors of sheep brain glutamine synthetase. Inactivation of γ-glutamylcysteine synthetase does not require ATP and is associated with noncovalent binding of close to 1 mol of inactivator/mol of enzyme. The findings indicate that the S-sulfo amino acids are transition-state analogs, and that binding of S-sulfo amino acid to the enzyme induces formation of a very stable enzyme-inactivator complex. The data suggest that stabilization of the enzyme-inactivator complex results from interactions involving the sulfenyl sulfur atom of the S-sulfo amino acid and the active site thiol group of the enzyme.

γ-Glutamylcysteine synthetase and glutamine synthetase catalyze similar reactions in which enzyme-bound γ-glutamyl phosphate (Fig. 1, I) is formed as an intermediate (reaction 1 and 2) (1, 2):

\[ \text{Enzyme} + \text{L-glutamate} + \text{ATP} \rightarrow \text{enzyme}[\gamma\text{-Glu-P}] + \text{ADP} \]  

Enzyme[\gamma\text{-Glu-P}] + NH₃ + enzyme +\text{enzyme}[\gamma\text{-Glu-NHR}] + ADP + P₄

In glutamine synthetase, R = H (NH₃ may be replaced by NH₂OH).

In γ-glutamylcysteine synthetase, R = HSC₆H₂CHCOO⁻ (cysteine may be replaced by α-aminobutyrate). (Both reactions require divalent metal ions (Mg²⁺, Mn²⁺)).

The evidence for intermediate formation of enzyme-bound γ-glutamyl phosphate in the glutamine synthetase reaction 2 includes the findings that this enzyme catalyzes (a) cyclization of D- and L-glutamate to 5-oxoproline (reaction 3 (3, 4), (5),

\[ \text{Enzyme} + \text{L-glutamine} + \text{ADP} \rightarrow \text{enzyme}[\text{L-Glu-P}] + \text{ATP} \]

for ATP formation (reaction 4 (5)), and (c) synthesis of cycloglutamate (cis-L-amino-1,3-dicarboxycyclohexane phosphate (reaction 5) (6, 7)). The enzyme is inactivated by incubation with L-methionine-S-sulfoximine and ATP (reaction 6 (8-10)); in this reaction, the sulfoximine nitrogen atom is phosphorylated and the methionine sulfoximine phosphate formed (Fig. 1, II) binds tightly but noncovalently to the enzyme thus inhibiting it.

γ-Glutamyl phosphate formation is consistent with early ¹⁸O tracer studies (11, 12), and is supported by kinetic data from ¹⁸O scrambling experiments (13).

That the reaction catalyzed by γ-glutamylcysteine synthetase follows a similar pathway is consistent with ¹⁸O studies (14, 15) and in accord with the finding that this enzyme also catalyzes reaction 3 and is inactivated by L-methionine-S-sulfoximine (16, 17) (reaction 6).

It has been concluded that both reactions involve formation of a tetrahedral intermediate by interaction of NH₃ (glutamine synthetase) or the α-amino group of cysteine (γ-glutamylcysteine synthetase) with enzyme-bound γ-glutamyl phosphate and that L-methionine-S-sulfoximine phosphate formation thus reflects an aspect of the normal catalytic mechanisms (10). L-Methionine-S-sulfoximine phosphate may be viewed as an analog of the tetrahedral transition-state intermediate; it is bound noncovalently to the enzymes and dissociates so slowly that inhibition is essentially irreversible.

Despite these similarities, there are some notable differences between the two enzymes; these include differences in the rates and extents of the cyclization reactions (reaction 3), and different effects of higher homologs of methionine sulfoximine on the two synthetases (10, 18, 19, 41). Several observations indicate that rat kidney γ-glutamylcysteine synthetase (in contrast to glutamine synthetase) has a thiol group at or close to its binding site for glutamate (20-24). This is considered further below.

In the present studies we have examined the effects of a series of Bunte salts, i.e., the S-sulfo derivatives of the L- and D-isomers of cysteine and homocysteine (III) on the two synthetase reactions. These compounds were studied because it was thought that they might act as analogs of γ-glutamyl phosphate (I) or of the putative tetrahedral intermediates (IV) involved in these reactions. We found that the S-sulfo...
Amino acids, which are reversible inhibitors of glutamine synthetase, markedly inactivate rat kidney γ-glutamylcysteine synthetase. Inactivation, which does not require ATP, is associated with stoichiometric binding of the inactivator to the enzyme. The inactivators are bound noncovalently and may be released intact from the enzyme.

**Experimental Procedures**

**Results**

Inactivation of γ-Glutamylcysteine Synthetase by S-Sulfo Derivatives of Cysteine and Homocysteine—The S-sulfo compounds were found not to be substrates (in place of L-glutamate or L-cysteine), as determined by amino acid analysis. When added to the standard reaction mixtures, the S-sulfo compounds were inhibitory; the corresponding apparent Ki values are given in Table I (Miniprint). Inhibition by S-sulfo-L-cysteine was essentially competitive with respect to L-glutamate (Fig. 2, Miniprint), whereas inhibition by S-sulfo-L-homocysteine (Fig. 3, Miniprint) and the corresponding D-isomers was mixed. The apparent Ki value for L-glutamate was found to be 1.6 mM, in agreement with earlier findings (24, 33). The data (Table I) suggest that the affinity of the enzyme for the S-sulfo-L-amino acids is somewhat less than that for L-glutamate. In agreement with earlier studies (20), DL-2-amino-4-phosphonobutyrate was found to inhibit, as also did L-homocysteate and L-homocysteine sulfinate (Table I).

When the enzyme was preincubated with the S-sulfo amino compounds it was rapidly inactivated. Inactivation was time-dependent (Fig. 4). Surprisingly, the initial rates of inactivation were not markedly different for the four S-sulfo compounds; as shown in Fig. 4, the initial rates of inactivation were in the decreasing order S-sulfo-L-cysteine ≥ S-sulfo-L-homocysteine > S-sulfo-D-homocysteine ≥ S-sulfo-D-homocysteine. The extent of inactivation, after prolonged incubation, was about the same with the four S-sulfo compounds. Notably, preincubation under the same conditions with L-homocysteate, L-homocysteine sulfinate, and DL-2-amino-4-phosphonobutyrate did not produce inactivation.

Inactivation by the S-sulfo compounds was found to be dependent upon the concentration of the inactivator. For example, after preincubation for 1 min with S-sulfo-D-homocysteine at concentrations of 0.1, 0.5, and 5 mM, the respective activities were 87, 72, and 11% of the untreated controls. The effects of preincubation of the enzyme with S-sulfo-D-homocysteine are given in Fig. 5 (Miniprint). The rates of inactivation were much less (<10%) in the absence of added divalent metal ion, and substantially greater in the presence of added Mn²⁺ than with Mg²⁺, although the extent of inactivation (>90%) was about the same. In the presence of 10 mM S-sulfo-L-homocysteine and 0.25 mM divalent metal ion, complete inactivation required 1 and 60 min, respectively, with Mn²⁺ and Mg²⁺.

Data on protection of the enzyme against inactivation by S-sulfo-L-cysteine by various substrates and products are given in Table II (Miniprint). Complete protection was observed with L-γ-glutamyl-L-α-aminobutyrate, L-glutamate + ATP, and ADP; similar results were obtained in studies on S-sulfo-D-cysteine and the L- and D-isomers of S-sulfo-homocysteine.

Previous studies showed that treatment of the enzyme with cysteamine leads to formation of a mixed disulfide between cysteamine and a thiol at or close to the binding site for glutamate (21). This leads to inactivation of the enzyme, which is readily reversible by treatment with dithiothreitol. Prior treatment of the enzyme with cysteamine was found to prevent interaction of the enzyme with chloroacetone inactiv-
Inhibitors bind to the active center of the enzyme in the regions of the glutamate and ATP-binding sites.

**Stoichiometry and Nature of Binding—** When γ-glutamylcysteine synthetase was incubated with [35S]sulfenyl-labeled sulfo-L-cys-teine and then subjected to gel filtration (Fig. 6, Miniprint), the enzyme fraction (completely separated from the low molecular weight fraction) was found to be enzymatically inactive and to contain radioactivity equivalent to 1.1 mol of S-sulfo-L-cysteine/mol of enzyme. In a duplicate experiment, a value of 0.93 mol/mol of enzyme was found. In an analogous experiment in which the sulfonyl sulfur atom of S-sulfo-L-cysteine was labeled with 35S, the binding of inhibitor to the enzyme was found to be equivalent to that found in the experiments with the sulfenyl 35S-labeled compound. The findings therefore suggest that the entire S-sulfo-L-cysteine molecule binds to the enzyme. The findings argue strongly against mixed disulfide formation between inhibitor and an enzyme thiol; this is also in accord with the finding that dithiothreitol does not reactivate the enzyme.

To further explore the nature of the bound inhibitor, the enzyme was inactivated by incubation with a preparation of S-sulfo-L-cysteine in which the sulfenyl and sulfonyl sulfur atoms were equivalently labeled with 35S. The enzyme-inhibitor complex was isolated by gel filtration as described above. Attempts to remove the labeled inhibitor from the enzyme by incubation in 50 mM Tris (pH 8.2) and MgCl₂ (5 mM) at 37 °C were unsuccessful. However, when the labeled enzyme was incubated at 37 °C in Tris (50 mM) in the presence of L-glutamate (10 mM), L-α-aminobutyrate (10 mM), EDTA (5 mM), ATP (5 mM), and MgCl₂ (20 mM), a slow return of enzyme activity was observed. After incubation for 20 h about 13% of the original enzyme activity returned. The enzyme could not be further reactivated by prolonging the incubation time because of substantial denaturation.

The 35S label could readily be removed from the enzyme under denaturing conditions; for example, by placing the enzyme solution at 100 °C for 5 min or by treating it with 18% trichloroacetic acid. After denaturation, all of the radioactivity was readily separated from the protein by gel filtration on Sephadex G-50 or by use of a Centricon-10 microconcentrator. The radioactive material was shown to be S-sulfohomocysteine by several procedures. (a) The radioactive material comigrated with authentic S-sulfohomocysteine on thin layer chromatography. (b) The radioactive material did not bind to a column of AG-50 (H⁺). (c) After treatment of the radioactive material with NaBH₄, followed by its application to a column of AG-50 (H⁺), half of the radioactivity applied to the column washed through the column on elution with water. The other half of the radioactivity eluted with 2 M NH₄OH. The findings are in accord with the predicted (34) reduction of S-sulfohomocysteine to sulfite and cysteine; this was confirmed in the present studies in experiments in which authentic S-sulfohomocysteine was treated with NaBH₄.

**Protection of γ- Glutamylcysteine Synthetase against inactivation by S-sulfo compounds by prior treatment with cystamine**

The reaction mixtures (final volume, 100 µl) contained Tris/HCl buffer (pH 8.2, 50 mM), MnCl₂ (0.25 mM), enzyme (10 units), and added compounds as indicated. The solutions, prior to mixing, were placed at 37 °C for 10 min. Cystamine (0.1 mM), S-sulfo-L-cysteine (10 mM) and S-sulfo-DL-homocysteine (1 mM) were added as stated. In experiment 4, cystamine was added 2 min prior to addition of S-sulfohomocysteine. In experiment 5, cystamine was added 2 min prior to addition of S-sulfohomocysteine. After incubation of the complete reaction mixtures for 120 min at 37 °C, a portion (10 µl) was removed and assayed for enzyme activity in the presence and absence of 1 mM dithiothreitol.

| Experiment | Compounds added | % Activity remaining |
|------------|------------------|----------------------|
|            | Without dithiothreitol | With dithiothreitol |
| 1          | None             | 100                  | 98                |
| 2          | Cystamine        | 100                  | 98                |
| 3          | S-Sulfohomo cysteine | 10                  | 10                |
| 4          | Cystamine + S-sulfohomo cysteine | 1 | 100                |
| 5          | S-Sulfohomo cysteine | 5                   | 5                 |
| 6          | Cystamine + S-sulfohomo cysteine | 2 | 59                 |
synthetase and γ-glutamylcysteine synthetase. The potential in vivo usefulness of S-sulfo amino acids as selective inactivators of γ-glutamylcysteine synthetase is noteworthy; studies on this possibility are in progress. Other in vivo interactions are suggested by the reports that S-sulfocysteine is found in the urine of patients with sulfite oxidase deficiency (35) and that this compound may serve as an excitatory neurotransmitter (36, 37).

Inactivation of γ-glutamylcysteine synthetase is time-dependent and is also dependent upon concentration of inactivator. The possibility that inactivation is due to slow formation of an enzyme-inhibitor complex (i.e. EI formation is slower than ES formation) is unlikely because in such a mechanism the initial velocity of the enzyme-catalyzed reaction would be expected to be independent of concentration of inactivator. The experimental findings seem to support a mechanism of inactivation in which EI is formed rapidly and then converted to a different form (E'1). According to this mechanism (Scheme 1), which has been termed “slow-binding inhibition” (38-40), an initial complex (EI) is rapidly formed, and undergoes slow (relative to the rate of EI formation) isomerization to a more stable enzyme-inhibitor complex (E'1), from which the inhibitor may be released, but at a very slow rate. In this scheme k2 and k3 are much smaller than k1, k4, or the rate constants involved in the catalytic reaction; for inactivation of the enzyme to occur, k2 must be much smaller than k4. The Ks value determined from initial velocity studies in the presence of inhibitor is k2/k1. Ks, the overall dissociation constant of inhibitor from EI*, is Ks(k2/k4). The rate constant (k3) for the isomerization of EI to E'1 can be determined by measuring the effect of varying inhibitor concentration on the rate of inactivation. From such data it is possible to estimate k4, by extrapolation (Fig. 7, Miniprint); a value of 2.5 min⁻¹ was obtained. An estimate of k3 may be obtained by following the regain of enzyme activity from EI*. Thus, enzyme which had been inactivated by S-sulfo-L-cysteine was reactivated by incubation with substrates; about 13% of the initial enzyme activity returned after 20 h from which k4 may be estimated to be about 1 × 10⁻⁴ min⁻¹. This corresponds to a half-life of about 4 days for the S-sulfo-L-cysteine-enzyme complex. Ks* for this complex is thus estimated to be about 1 × 10⁻⁷ M.

Such slow binding inhibition of enzymes has been reported with adenosine deaminase (42), ribulosebisphosphate carboxylate-oxygenase (43), and alanine racemase (44). It has been postulated that in each instance, the initially formed EI complex in some manner perturbs the enzyme sufficiently to produce an E'1 complex that binds to the enzyme more tightly than EI. The chemical nature of these interactions is not yet clear.

The present studies offer an approach to a mechanistic understanding of the way in which S-sulfo compounds inhibit γ-glutamylcysteine synthetase. Thus, the S-sulfo amino acids appear to act as transition-state analogs which resemble the high-energy metastable γ-glutamyl phosphate intermediate. Such an analog might be expected to induce a conformational change in the enzyme (45, 46, 48). (Notably, the Ks* value for

\[
E + I \rightleftharpoons EI \rightleftharpoons EI^* \rightleftharpoons E + I + P
\]

\[
K_m = \frac{k_2}{k_1} = \frac{k_3}{k_4}
\]

\[k_3/k_4 \approx k_3/k_2 \approx K_s (k_2/k_4)
\]

\[K_{s*} = \frac{k_2}{k_4}
\]

\[K_i = \frac{k_4}{k_2}
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\]
### Materials

1. Glutathione synthetase was isolated from rat kidney as described (12). Glutathione synthetase, a relatively stable enzyme, is sensitive to chelators, such as cyanide, and requires a reducing agent for activity. Ammonium acetate (0.1 M) was employed to maintain a pH of 7.4. The enzyme was purified by a modification of the method of Pauling and Itano (20) described by Lerner and Lasker (21).

2. Phosphatase assay was performed using the method of Lowry et al. (22) described by Bradford (23).

### Methods

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2. Phosphatase assay was performed using the method of Lowry et al. (22) described by Bradford (23).

### Observations

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### Results

-For the determination of $V_0$, constant conditions were used, except that the concentration of the inorganic phosphate was varied. Initial velocities were determined by direct linear plots (12) and $V_0$ values were determined by a method of least squares (24).

### Discussion

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### Acknowledgments

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*References*

1. Pauling, L. (1940) *Proc. Natl. Acad. Sci. U. S. A.* 26, 157-166.

2. Folin, O. (1947) *Biochim. Biophys. Acta* 7, 95-108.

3. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* 193, 265-275.

4. Bradford, M. M. (1976) *Anal. Biochem.* 72, 248-254.

5. Swain, J. M. (1957) *Nature* 180, 643-645.

6. d'Uldineaud, V., and Patterson, W. I. (1956) *J. Biol. Chem.* 219, 719-725.

7. Orlowski, M., and Meister, A. (1971) *Biochemistry* 10, 185-199.

8. Rowe, W. B., Ronzio, R. A., Welker, V. P., and Meister, A. (1970) *Methods Enzymol.* 17, 900-910 (abstr.).

9. Esnault, R., and Cornish-Bowden, A. (1974) *Biochem. J.* 139, 155-170.

10. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* 193, 265-275.

11. Bradford, M. M. (1976) *Anal. Biochem.* 72, 248-254.

12. Swain, J. M. (1957) *Nature* 180, 643-645.

13. d'Uldineaud, V., and Patterson, W. I. (1956) *J. Biol. Chem.* 219, 719-725.

14. Orlowski, M., and Meister, A. (1971) *Biochemistry* 10, 378-382.

15. Beyer, R. E., Oberster, A. E., Hoffman, F., and Saret, L. H. (1960) *J. Am. Chem. Soc.* 82, 170-178.

16. Irirve, F., Rudd, S. H., Heizer, W. D., and Laster, L. (1967) *Biochem. Med.* 1, 187-217.

17. Mewett, K. N., Oakes, D. J., Olverman, H. J., Smith, D. A. S., and Watkins, J. C. (1983) *Adv. Biochem. Psychopharmacol.* 37, 163-164.

18. Olney, J. N., Mi, R., and de Grubareff, T. (1975) *J. Neurochem. & Exp. Neurol.* 34, 167-177.

19. Johnson, F. J., and Walsh, C. T. (1987) *Adv. Enzymol.* 61, 291-301.

20. Williams, J. W., Morrison, J. F., and Duggleby, R. G. (1979) *Biochemistry* 18, 2567-2573.

21. Schloss, J. V., Porter, D. J. T., Bright, H. J., and Cleland, W. W. (1980) *Biochemistry* 19, 7828-7833.

22. Griffith, O. W. (1982) *J. Biol. Chem.* 257, 13704-13712.

23. Frieden, C., Kurz, L. C., and Gilbert, H. R. (1980) *Biochemistry* 19, 5303-5309.

24. Pierce, J., Tobert, N. B., and Barker, R. (1980) *Biochemistry* 19, 4324-4329.

25. Bodet, B., and Walsh, C. (1985) *Biochemistry* 24, 1333-1341.

26. Hammond, G. S. (1955) *J. Am. Chem. Soc.* 77, 334-338.

27. Pauling, L. (1946) *Chem. Eng. News* 24, 1375.

28. Huang, C. S., Moore, W. R., and Meister, A. (1987) *Fed. Proc.* 46, 1757.

29. Pauling, L. (1948) *Am. Sci.* 36, 51.
γ-Glutamylcysteine Synthetase Inactivation

D-α-aminoimidazole was prepared by reduction of D-α-aminomimidazole (900 g, 17.4 mmol) with sodium in liquid NH₃ (21). After removal of the N₂ by evaporation, the solids obtained were dissolved in water and concentrated to a viscous syrup. The syrup was then dried in vacuo and redissolved in a solution of 10% H₂O/H₂O to give a 0.25 M solution. The reaction mixture was stirred magnetically for 30 min and then the reaction was terminated by adding a solution (100 ml) of barium acetate (80.2 g, 300 mmol). Barium acetate was removed and the column chromatography steps were performed as described for the synthesis of δ-aminolysine. The product (85% yield, 2.35 mmol) was purified from water by precipitation with cold 0.1 M citrate. 

**Table 1**

| Inhibitor          | IC₅₀ (M) |
|--------------------|---------|
| D-α-aminoimidazole | 3.7     |
| D-α-aminoimidazole | 4.3     |
| D-α-aminoimidazole | 12     |
| D-α-aminoimidazole | 32     |
| D-α-aminoimidazole | 0.05   |
| D-α-aminoimidazole | 0.3    |
| D-α-aminoimidazole | 0.25   |

IC₅₀ values were determined by varying the concentrations of L-glutamylcysteine and inhibitor (see above). Complete inactivation was observed within 1 min at a concentration of 70 mM.
### Table II

Protection by Various Compounds Against Inactivation by S-Bufle-L-Cysteine

| Compound(s)         | % Activity Remaining |
|---------------------|----------------------|
| None                | 100                  |
| None                | 0                    |
| L-γ-Glutamyl-L-cysteine | 100              |
| ATP                 | 98                   |
| L-Glutamate         | 59                   |
| L-Glutamate + ATP   | 56                   |

The reaction mixtures (final volume, 0.1 ml) contained Tris/HCl buffer (pH 8.2, 50 mM), MgCl₂ (10 mM), enzymes (10 units), S-bufle-L-cysteine (10 mM) and added compounds (10 mM). The reactions were incubated for 60 min, at which time a portion (10 µl) was removed and assayed for ATP formation by the coupled enzyme assay.

### Table IV

$K_i$ Values for S-Bufle Compounds and Glutamyl Synthetase

| Inhibitor                  | $K_i$ (mM) |
|----------------------------|------------|
| S-bufle-L-cysteine         | 0.84       |
| S-bufle-L-homocysteine     | 1.4        |
| S-bufle-D-cysteine         | 4.2        |
| S-bufle-D-homocysteine     | 9.2        |

S-glutathione synthetase activity was determined by measuring ATP formation. Kinetic constants were calculated from direct linear plots. The $K_i$ value for L-glutamate is 5 mM. Inhibition by the L-isomer is competitive with respect to L-glutamate. Inhibition by the D-isomer is mixed with respect to L-glutamate. (See the text).