OXIDATION OF SULFHYDRYL GROUPS TO DISULFIDES BY SULFOXIDES

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SUMMARY: Oxidation rates of SH groups in penicillamine, cysteine, and glutathione to the corresponding SS forms by dimethyl sulfoxide and other sulfoxides as a function of pH of the solvent and structure of reactants were measured by NMR spectroscopy. The observed second-order rate constant showed a biphasic pH dependence. A mechanism which rationalizes this result is proposed. These oxidations are proposed to have synthetic utility with biochemical implications.

Various oxidizing agents are known to oxidize biologically important SH groups to the disulfide and higher oxidation states (1). While examining addition reactions of mercaptide ions to vinyl compounds (2, 3), we noted precipitation of cystine when cysteine was treated with N-acetyl dehydroalanine in the presence of dimethyl sulfoxide (DMSO). Since SH groups and SS bonds are important in foods, enzymes, and other proteins and because DMSO is of biomedical interest, we examined the course and mechanism of this reaction with suitable model compounds.

MATERIALS AND METHODS [2]

N-Acetyl-methionine sulfoxide was prepared from methionine sulfoxide by a published procedure (4). Other chemicals were obtained from commercial sources.

Rate Studies: Rate constants were determined by NMR with a Varian A-60 spectrometer. Peak heights were measured as a function of time showing the disappearance of starting material and the concurrent appearance of product. For oxidation of penicillamine by DMSO, the shift of one of the methyl groups was monitored for the conversion of penicillamine to penicillamine disulfide. A typical example is shown in Figure 1.

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[2] Mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

For all other reactions, the shift of the S-methyl group was observed as the sulfoxide was reduced to the thioether. Solutions were prepared by dissolving 1.0 mmole of sulfur amino acid in 2.0 ml of 0.50*M* sulfoxide-D_2O solution. pH was measured and varied by adding F_3CCOOH, $(HOCH_2)_3CNH_2$(Tris), and NaOD, as needed. An aliquot of this solution, in a 5-mm NMR tube, was scanned at regular intervals. Peak heights were assumed to be directly proportional to concentration. Pseudo-first-order rate constants (k_1) and second-order rate constants (k_2) were determined from plots of time *versus* log concentration or *versus* reciprocal of the concentration of thiol, respectively.

Preparation of Cystine: L-Cysteine (6.05 g, 0.05 mole) was dissolved in 70 ml of H_2O and 4.2 g (0.05 mole) of DMSO added. After 12 hrs at 30°, the precipitate was collected by filtration, washed with ethanol, and dried under vacuum to yield 4.9 g (82%) of cystine. The material had spectral properties (IR and NMR) and retention time [amino acid analyzer (5)] identical to an authentic sample and was free of cysteine and cysteic acid.

RESULTS AND DISCUSSION

Penicillamine was selected as a model for study because the reactions of less sterically hindered sulfur amino acids (e.g., cysteine) were too fast to measure conveniently by NMR. Reaction kinetics established the stoichiometry of sulfhydryl group oxidations by DMSO. When sufficient excess of sulfoxide over thiol was used, the graph of thiol concentration plotted

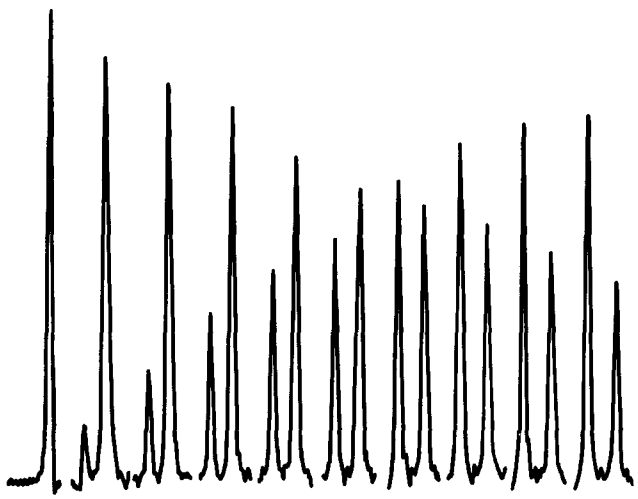


Figure 1. Decrease in the NMR signal of the CH_3 group of $(CH_3)_2CSH$-$CH(NH_2)COOH$ at τ 8.20 and concurrent increase of the corresponding signal for the CH_3 group in $HOOCCH(NH_2)$-$C(CH_3)_2SSC(CH_3)_2CH(NH_2)COOH$ at τ 8.01 as a function of time at 37°. The spectra were recorded at 2.0-minute intervals.

against time gave a straight line, establishing that the reaction followed pseudo-first-order kinetics. When equimolar amounts of penicillamine and DMSO were used, the reaction followed good second-order kinetics; the reciprocal of thiol concentration increased linearly with time. Furthermore, k_2 ($0.0120\ M^{-1}min^{-1}$) calculated from k_1 was essentially the same as that ($0.0118\ M^{-1}min^{-1}$) determined directly.

We also observed that when equimolar amounts of thiol and sulfoxide were used, half of the sulfoxide and all of the thiol were consumed. The formation of disulfide was evidenced from the synthesis of cystine from cysteine in 82% yield. The isolated product was shown to be free of cysteic acid and starting material. These observations are all consistent with the reaction shown in equation 1. The same stoichiometry has been reported

$$2\ R'SH + 1\ R_2SO \longrightarrow R'SSR' + RSR + H_2O \qquad (1)$$

by Wallace (6, 7) for oxidation of aromatic thiols by sulfoxides at 90-100° in the absence of solvents. Under more severe conditions, DMSO converts cysteine and cystine to cysteic acid (8, 9).

The effect of pH on rates of oxidation of penicillamine by DMSO is shown in Figure 2. The unexpected biphasic relationship suggests a complex reaction mechanism. The following reaction (equation 2) is consistent with our results (7). In this proposed reaction R'SH combines with the sulfoxide in the rate-

$$R'SH + (CH_3)_2SO \xrightarrow{k_1} \left[R_2\overset{OH}{\underset{|}{S}}\text{-}SR'\right] \xrightarrow[R'SH]{k_2} R'SSR' + CH_3SCH_3 + H_2O \qquad (2)$$

determining step to form a sulfoxide-thiol adduct. The adduct then reacts more rapidly with another molecule of thiol to form the observed products (i.e., $k_1 >> k_2$). The rate increase with increasing pH in the range 4-7 may be attributed to ionization of R'SH to the more reactive $R'S^{\ominus}$ which combines with DMSO in the rate-determining step (k_1). In aqueous solutions the pK value for the SH group in penicillamine is 7.90 (10). However, added DMSO lowers the pK of thiols (11). Accordingly, DMSO is expected to assist the ionization of penicillamine in the cited pH range. The decreasing rate in

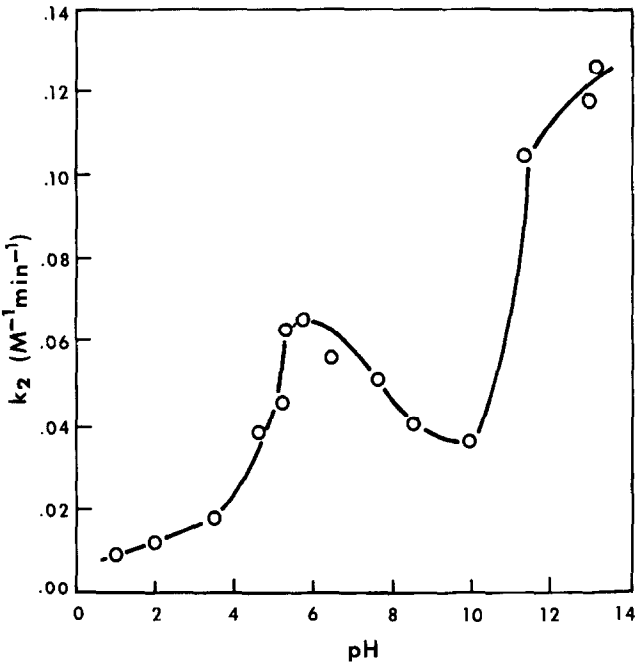


Figure 2. pH-rate profile for the oxidation of the SH group of pennicillamine to the SS form by DMSO in D_2O at 37°.

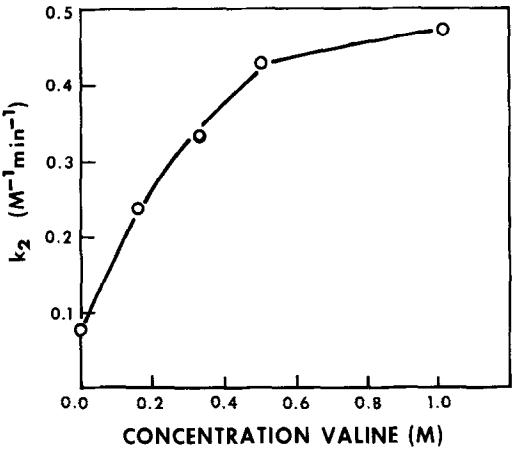
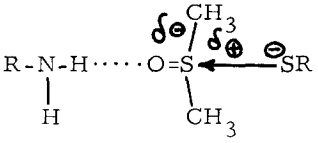


Figure 3. Effect of added valine in increasing the rate of oxidation of the SH group in penicillamine to the SS form by DMSO in D_2O at 37° and pH 11.0. Concentrations of penicillamine and valine are 0.5

the pH range 6-10 is understandable if the protonated form of the sulfoxide is a catalyst or preferred reactant. Protonation is expected to vary inversely with pH. It is noteworthy that no oxidation was observed in the absence of DMSO. The observed base-catalyzed rate increase of oxidation above pH 10 is, therefore, attributed to amine catalysis. For instance, the increasing rapid oxidation of penicillamine as valine is added (Figure 3) supports this conclusion (12). This catalytic effect is presumably due to hydrogen bonding between the NH_2 group of valine and the S=O group of DMSO so that attack by $RS^{\ominus}$ on the S=O is favored, as illustrated:



The postulated mechanism is supported by the observed lack of catalysis by a tertiary amine [e.g., $(C_2H_5)_3N$] and by the fact that valine does not affect the oxidation rate under acidic conditions; presumably protonated valine is not the strongest acid under these conditions. The observed catalytic effect of valine implies that ε-NH_2 groups of lysine side chains in proteins will exert an analogous catalytic effect on the oxidation of protein SH groups by sulfoxides under alkaline conditions.

The effect of reactant structure on the oxidation of sulfhydryl groups to disulfides by sulfoxides, in acidic media, is shown in Table 1. At pH 1.5, oxidation of penicillamine decreased in the order DMSO > N-acetyl-methionine sulfoxide > methionine sulfoxide > S-methyl cysteine sulfoxide, suggesting that sterically hindered sulfoxides react more slowly. A similar trend was shown by the oxidation of several sulfur amino acids by methionine sulfoxide: cysteine > N-acetyl-cysteine > glutathione > penicillamine. Accordingly, oxidation rates depend strongly on the steric environment of both the SH and the S=O groups.

At pH 12.0, DMSO was the only sulfoxide in Table 1 that reacted. This result may be explained as an effect of charge repulsion between the carbohydrate

Table 1. Relative Rates of Oxidation of SH Groups by Sulfoxides at 37° and pH 1.5

Amino Acid	Sulfoxide	k_2 ($M^{-1} min^{-1}$)	Rate Ratio
Penicillamine	Dimethyl	1.08×10^{-2}	30.68
Penicillamine	N-Acetyl Methionine	1.99×10^{-3}	5.65
Penicillamine	Methionine	8.73×10^{-4}	2.48
Penicillamine	S-Methyl Cysteine	3.52×10^{-4}	1.00
Cysteine	Methionine	6.83×10^{-3}	19.40
N-Acetyl Cysteine	Methionine	4.82×10^{-3}	13.69
Glutathione	Methionine	3.42×10^{-3}	9.72
Penicillamine	Methionine	8.73×10^{-4}	2.48

anion ($RCOO^{\ominus}$) of the sulfoxide amino acid and the sulfhydryl anion ($RS^{\ominus}$).

In conclusion, these observations are relevant to several pharmacologically and biochemically important problems: a) the action and mechanism of DMSO *in vivo*; b) the effect of DMSO, methionine sulfoxide, and other naturally occurring sulfoxides on: sulfhydryl-disulfide interchange in proteins and dough-systems, the mechanical behavior of keratins and related phenomena; and, c) the effect of methionine sulfoxide on the nutritional quality of food proteins. In this last instance, our results suggest that it may be possible to improve the nutritional quality of food proteins by transforming protein-bound methionine sulfoxide to methionine residues with the aid of cysteine or other thiol derivatives. Some of these problems are currently under investigation.

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