solved. The ether solution was evaporated and the oil was dissolved in petroleum ether and cooled to yield 28 g. or 59% of ethyl phenylthiocarbamate, m.p. 70° (lit. 71–72°).[18]

Reactions of Triethyl Orthoformate with 1,1-Disubstituted Ureas. N-(Morpholine-4-methylene)-morpholine-4-carboxamide.—Two hundred and twenty grams (1.67 moles) of morpholine-4-carboxamide was added to 400 ml. (2.4 moles) of triethyl orthoformate and refluxed for 7 days. The solution was cooled in the refrigerator and 114 g. of solid crystallized. The remaining oil was allowed to stand and another 59 g. of solid was obtained. The combined solids were recrystallized from ethyl acetate to yield 149 g. (77%) of N-(morpholine-4-methylene)-morpholine-4-carboxamide, m.p. 126–127°.

Anal. Calcd. for $C_{10}H_{17}N_3O_3$: C, 52.80; H, 7.55; N, 18.52. Found: C, 52.50; H, 7.28; N, 18.92.

The oil remaining (51 g.) in the filtrate was distilled through a Widmer column to yield 20 g. (27%) of urethan, b.p. 52° at 0.4 mm., m.p. 47.5–48.5°. A mixed melting of the latter and an authentic sample of urethan was not depressed.

One and eight-tenths grams (0.01 mole) of morpholine-4-(ethoxymethylenecarboxamide) was allowed to stand for 3 days with 0.87 g. (0.01 mole) of morpholine. The solid was recrystallized from ethyl acetate to yield 1.5 g. (65%) of N-(morpholine-4-methylene)-morpholine-4-carboxamide, m.p. 126°.

1-(N,N-Diphenylcarbamyl)-3,3-diphenylformamidine.—Thirty grams (0.14 mole) of 1,1-diphenylurea, 30 g. (0.30 mole) of acetic anhydride and 200 ml. of triethyl orthoformate were refluxed for 12 hours. The solution was concentrated at 15 mm. on the steam-bath. Ether was added to the residue and 9 g. or 34% of 1-(N,N-diphenylcarbamyl)-3-diphenylformamidine remained undissolved. This was recrystallized from ethyl acetate, m.p. 174°.

Anal. Calcd. for $C_{26}H_{20}N_3O$: C, 79.95; H, 5.15; N, 10.75. Found: C, 80.49; H, 5.23; N, 10.58.

The ether solution was concentrated and allowed to stand. 1,1-Diphenyl-3-formylurea (3 g. or 8.8%) separated and was recrystallized from ethyl acetate, m.p. 148°.

Anal. Calcd. for $C_{14}H_{12}N_2O_2$: C, 69.78; H, 5.01; N, 11.62. Found: C, 70.02; H, 4.79; N, 11.52.

The remaining oil was distilled through a Widmer column to yield 5 g. (40%) of ethyl urethan, b.p. 68° at 0.5 mm., and 5 g. or 16.4% of diphenylurethan, b.p. 145–160° at 1–2 mm. The latter crystallized from a mixture of ether and petroleum ether, m.p. 72° (lit. 72°).[19]

(18) C. Leibermann, *Ann.*, **207**, 145 (1881).

(19) O. Meister, *Ber.*, **5**, 284 (1872).

INDIANAPOLIS, INDIANA

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, STATE UNIVERSITY OF IOWA]

The Acid Strength of the -SH Group in Cysteine and Related Compounds

BY RUTH E. BENESCH AND REINHOLD BENESCH

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The ultraviolet absorption of the mercaptide ion has been utilized to determine the relative proton affinity of the sulfur and nitrogen functions in a series of aminothiols. For those compounds which show a break in the spectrophotometric titration curve, all four possible pK's were calculated. The heat of dissociation of the -SH group was found to be about 6.5 kcal. by two independent methods. A simple spectrophotometric assay for mixtures of aminothiols is described. The implications of these findings for the mechanism of oxidation of -SH groups to disulfides and for other reactions of biological interest are discussed.

Introduction

The ionic equilibria of compounds which contain both a sulfhydryl and an ammonium group have been the subject of much discussion, since the relative contribution of each of these groups to the dissociation of the two protons could not be determined unequivocally. Reasoning mainly by analogy, three main viewpoints have been put forward: (1) The higher pK was assigned to the -SH group.[1] (2) Peters[2] and more recently Calvin[3] have reversed this assignment. (3) Edsall, as cited by Ryklan and Schmidt,[4] considered both pK values to be mixed ones, since he assumed that the intrinsic proton affinities of the sulfur and nitrogen are about the same.

A decision between these alternatives is difficult in the absence of a method which would permit a distinction between the ionized and un-ionized form of either of the two ionizing groups. The finding by Noda, *et al.*,[5] that butyl mercaptan absorbs ultraviolet light in strongly alkaline, but not in strongly acid, solution suggested a means of measuring the concentration of the RS^- form spectrophotometrically. This property was therefore used in the work presented here to determine the acid strength of the thiol group and its relation to the ammonium group in a series of aminothiols.

(1) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, p. 84.

(2) L. Peters, Thesis, University of Leeds, 1947.

(3) M. Calvin in "Glutathione," Academic Press, Inc., New York, N. Y., 1954, p. 9.

(4) L. R. Ryklan and C. L. A. Schmidt, *Arch. Biochem.*, **5**, 89 (1944).

(5) L. H. Noda, S. A. Kuby and H. A. Lardy, THIS JOURNAL, **75**, 913 (1953).

Experimental

All spectrophotometric measurements were made with a Beckman Model DU spectrophotometer equipped with "Thermospacers" for measurements at constant temperature (±0.5°). The pH measurements were carried out with a Beckman Model G, and in some cases a Beckman Model GS, pH meter, using a Beckman Type E glass electrode because of its high sensitivity in alkaline solution. All pH measurements were made at the same temperature as the corresponding spectrophotometric readings. The buffer system used for this investigation was a mixture of phosphoric and boric acids which was titrated to the required pH with NaOH and diluted to a final concentration of 0.02 M in each acid. This buffer was sufficient to cover the entire pH range used. All solutions were made up with distilled water which had been passed through Amberlite MB1 mixed anion- and cation-exchange resin.

For spectrophotometric measurements 0.10 ml. of a 1.5 to 2×10^{-2} M solution of the thiol was added from a Syringe Microburet (Micro-Metric Instrument Co., Cleveland, Ohio) to 10 ml. of the buffer solution. The absorption spectrum was determined immediately after mixing, using the corresponding buffer as the blank. All experiments were carried out at 23° unless otherwise stated.

The thiols used were obtained from the following sources: glutathione and L-cysteinyl-glycine, Schwarz Laboratories, Mount Vernon, N. Y.; L-cysteine hydrochloride, Nutritional Biochemicals Corporation, Cleveland, Ohio; DL-homocysteine, General Biochemicals Inc., Chagrin Falls, Ohio;

thioglycolic acid and β-mercaptoethylamine hydrochloride, Evans Chemetics, Inc., New York, N. Y.; L-γ-glutamyl-L-cysteine was a gift from Dr. John Snoke; L-cysteine ethyl ester hydrochloride was kindly donated by Dr. H. A. Lardy; the two isomeric L-cysteinyl-valines were synthesized in collaboration with Dr. L. C. King.

Results

General Characteristics of the Spectra.—The absorption spectra of cysteine, shown in Fig. 1, are representative of the spectra of the aminothiols investigated. The molar extinction coefficients of these compounds *in the completely ionized form* range from 4 to 6 × 10^3 and were found to be independent of concentration over the range used.

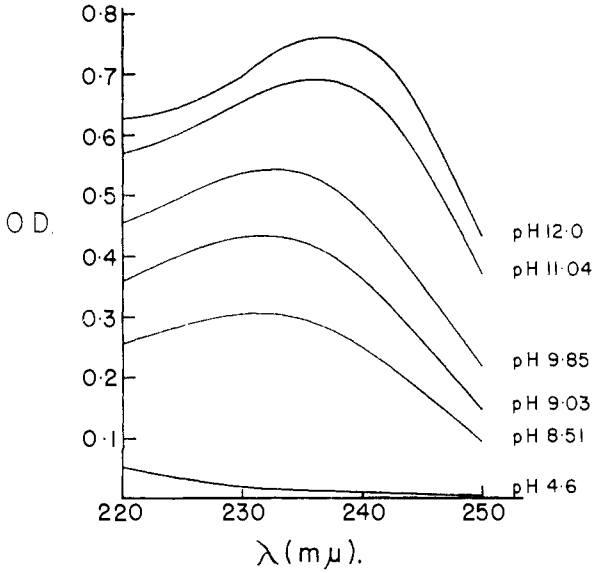

Fig. 1.—Absorption spectra of cysteine; concentration of cysteine 1.70 × 10^{-4} *M*.

The position of the absorption maximum of these compounds does not remain constant, but shifts from 236–238 mμ to 230–232 mμ with decreasing *p*H (Fig. 1). The conclusion that this shift is due to the fact that the $S^-RNH_3^+$ form has a maximum absorption at somewhat shorter wave lengths than the S^-RNH_2 form is borne out by the following observations:

(1) No shift is observed in the case of thioglycolic acid.

(2) At constant *p*H the position of the maximum is independent of the concentration of aminothiol.

(3) The shift is related to the change in the ratio of the two RS^- forms with *p*H. Thus in the case of β-mercaptoethylamine, for example, the major shift in absorption maximum (from 236 to 232 mμ) occurs between *p*H 12 and 9.9. This is the region, as will be shown below, where nearly all the change from the S^-RNH_2 to the $S^-RNH_3^+$ form occurs. Analogous correlations apply to the other aminothiols.

The residual absorption in acid solution was determined for each aminothiol. This absorption, which was substantial only in the case of glutathione and γ-glutamyl-cysteine, was subtracted from the observed optical densities in order to obtain the "RS^- spectrum." The fraction of RS^- at each *p*H was determined from the maximum absorption of the corresponding "RS^- spectrum" divided by the maximum absorption of the "RS^- spectrum" in the completely dissociated state (*p*H 12 or 13). This involves, of course, the assumption that the molar extinction coefficients of the $S^-RNH_3^+$ and the S^-RNH_2 forms are identical, despite the difference in the position of the absorption maximum. The validity of this assumption is borne out most clearly by the fact that no significant change in the molar extinction coefficient of β-mercaptoethylamine occurs between *p*H 9.9 and 12. It may be added that alanine shows a negligible absorption both at *p*H 7 and at *p*H 12.

Thioglycolic Acid.—Results with this compound confirm the assumption that the intensity of absorption is directly proportional to the RS^- concentration. Plots of % RS^- *versus* *p*H at 24.5 and 38° follow the equation $pH = pK + \log \%\ RS^-/(100 - \%\ RS^-)$ from 10 to 90% dissociation. The *pK*'s were found to be 10.32 at 24.5° and 10.10 at 38°. This difference corresponds to $\Delta H_{31}^\circ = 6.9$ kcal. The heat of dissociation was also determined by measuring the *p*H of an approximately 0.1 *M* solution of thioglycolic acid to which 1.5 equivalents of NaOH had been added ([RSH] = [RS^-]). The *p*H was found to be 10.52 at 11.5°, 10.34 at 23.5° and 10.10 at 38°. This corresponds to a heat of dissociation of 7.0 kcal. at 31°.

The Δ*H* values obtained by the spectrophotometric and the electrometric method over the same temperature interval are therefore in excellent agreement. This relatively high heat of dissociation seems reasonable in view of its similarity to that of phenol (6 kcal.[6]). Surprisingly enough no experimental data on the heat of dissociation of an –SH group seem to have been published, but our results clearly contradict the assumption repeatedly made in the literature[7-9] and attributed originally to Cohn,[10] that the heat of dissociation of an –SH group is negligible. The interpolated value of 10.23 for the *pK* at 30° is identical with the value reported by Cannan and Knight[11] at this temperature.

Cysteine.—Cysteine has three dissociable protons. The dissociation of the proton from the carboxyl group occurs at such low *p*H values that, in the *p*H range studied here, this group is always fully ionized. The dissociation of the other two protons from the –SH and $-NH_3^+$ groups may be represented by the steps shown below[4]

$$\begin{array}{ccc} (a)\ HSRNH_3^+ & \overset{K_A}{\rightleftharpoons} & S^-RNH_3^+\ (b) \\ \downarrow\uparrow K_B & & \downarrow\uparrow K_C \\ (c)\ HSRNH_2 & \underset{K_D}{\rightleftharpoons} & S^-RNH_2\ (d) \end{array}$$

It is thus clear that for each of the two protons two dissociation steps are involved, one from the

(6) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, p. 82.

(7) H. Borsook, E. L. Ellis and H. M. Huffman, *J. Biol. Chem.*, **117**, 281 (1937).

(8) W. Stricks and I. M. Kolthoff, THIS JOURNAL, **73**, 4569 (1951).

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(10) E. J. Cohn, *Ergeb. Physiol.*, **33**, 781 (1931).

(11) R. K. Cannan and B. C. J. G. Knight, *Biochem. J.*, **21**, 1384 (1927).

ammonium and one from the sulfhydryl group. In all cases where the acid strengths of the two groups are of similar magnitude, four different forms of the compound can exist and four dissociation constants describe their interrelationship. The limitation of hydrogen ion concentration measurements for this purpose is that the ratio of K_A/K_B (which is, of course, equal to K_D/K_C), *i.e.*, the relative tendency of each of the protons to dissociate from the ammonium and the sulfhydryl group, respectively, cannot be determined in this way.

The curve relating % RS^- to *p*H for cysteine is shown in Fig. 2. The break in the curve shows that $^2/_3$ of the -SH group has ionized as a result of the removal of the first proton. Hence only $^1/_3$ of the ammonium group could have ionized and the acid strength of the -SH group is therefore about twice as great as that of the $-NH_3^+$ group. For the calculation of the dissociation constants the following equation was used

$$\frac{[RS^-]}{[RS^-]_{max.}} = \frac{b+d}{a+b+c+d} = \frac{K_A/K_B + K_D/[H^+]}{[H^+]/K_B + K_A/K_B + K_D/[H^+] + 1}$$

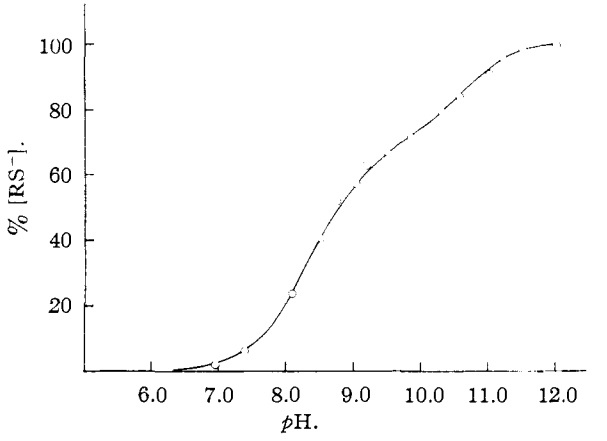

Fig. 2.—Spectrophotometric titration curve of cysteine, concentration of cysteine = 1.70×10^{-4} *M*. The points are experimental, the line is calculated from the constants given in Table I.

This was solved for K_A, K_B and K_D by substitution of the experimental values into three simultaneous equations. The constants thus obtained (Table I) were used to construct a theoretical curve which closely follows the experimental points (Fig. 2). From the four dissociation constants the relative concentration of each form of cysteine can be calculated as a function of *p*H as shown in Fig. 3.

TABLE I

DISSOCIATION CONSTANTS OF AMINOTHIOLS AT 23°

Compound	pK_A	pK_B	pK_C	pK_D	$pH_{[RSH]=[RS^-]}$
L-Cysteine	8.53	8.86	10.36	10.03	
L-Cysteine ethyl ester	7.45	6.77	8.41	9.09	
L-Cysteinyl-glycine	7.87	7.14	8.75	9.48	
β-Mercapto ethylamine	8.35	..	...	...	
Glutathione					9.20
L-γ-Glutamylcysteine					9.90
DL-Homocysteine					10.00

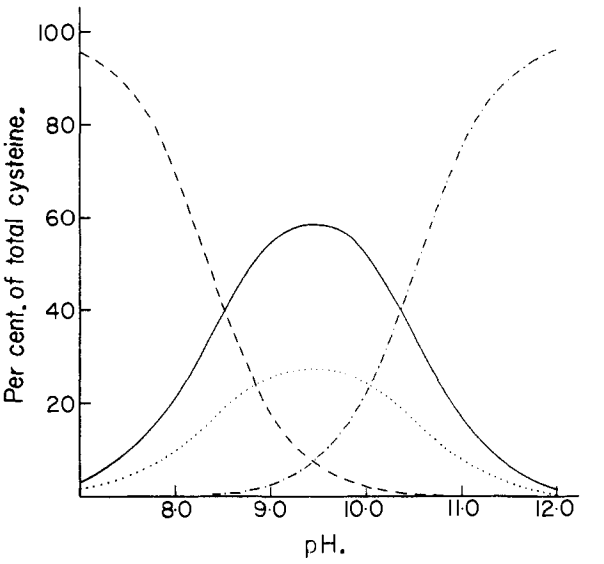

Fig. 3.—Concentration of the different ionic forms of cysteine as a function of *p*H, calculated from the constants in Table I: ------, $HS\text{-}R\text{-}NH_3^+$; ——, $^-S\text{-}R\text{-}NH_3^+$; ...., $HS\text{-}R\text{-}NH_2$; -·-·-, $^-S\text{-}R\text{-}NH_2$.

An evaluation of the dissociation constants for cysteine given in the literature seems pertinent. Since the acid strengths of the ammonium and sulfhydryl group in this compound are not sufficiently different, an assignment of a single pK_{SH} and a single $pK_{NH_3^+}$ is obviously untenable. This criticism therefore applies both to the values given in Cohn and Edsall[1] as well as to those assumed by Calvin, although his assumption[3] that the -SH group ionizes completely before the NH_3^+ group begins to dissociate is more nearly correct in this case.

Ryklan and Schmidt[4] as well as Stricks and Kolthoff[8] recognized that all four constants must be considered in the ionization of cysteine. However, on the basis of the assumption that the effect of an -SH group on the ionization of neighboring group is the same as that of an $-SC_2H_5$ group, they concluded that the ratio of the acid strength of the -SH to that of the $-NH_3^+$ group is 0.9 instead of 2.1 as found here. Their values for the four constants are therefore incorrect. Stricks and Kolthoff in addition neglected the heat of dissociation of the -SH group, so that their values involve another error depending on the temperature.

Other Aminothiols.—Titration curves of four derivatives of cysteine in which the carboxyl group is modified, *i.e.*, L-cysteine ethyl ester, L-cysteinyl-glycine, L-cysteinyl-L-valine and L-cysteinyl-D-valine, differ from that of the parent compound in two major respects: (1) The position of the break and therefore the ratio of K_A/K_B is much lower. (2) The absolute values of all four pK's are also lower (*cf.* Table I).

In all four compounds the negative charge of the carboxyl group is either eliminated (cysteine ester) or its distance from the other two ionizing groups is increased (cysteine peptides). As a result the acid

strength of the ammonium and, to a lesser extent, that of the –SH group is increased.

β-Mercaptoethylamine (Fig. 4, curve A) is the only example among the aminothiols investigated where the acid strength of the –SH group is so much greater than that of the ammonium group that the dissociation of the first proton is confined almost entirely to the former group. K_B and K_D may therefore be neglected compared to K_A and K_C. On this assumption pK_A for this compound is 8.35. Calvin reports[3] a pK_{SH} of 8.6 for this compound based on electrometric titration.

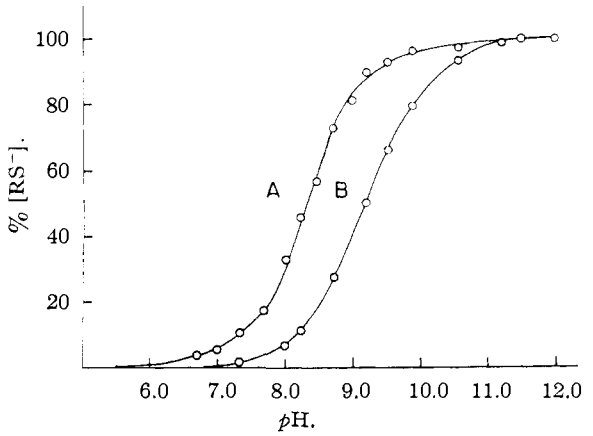

Fig. 4.—Spectrophotometric titration curves of: A. β-mercaptoethylamine; B, glutathione.

As can be seen from Fig. 4, curve B, the spectrophotometric titration curve of glutathione shows no evidence of a break. This is to be expected from the electrometric titration curve of this compound[12] which shows that the points corresponding to 50% dissociation of each proton are only one *p*H unit apart. By comparing the electrometric titration curve of Pirie and Pinhey with the spectrophotometric curve shown in Fig. 4, curve B, it can be estimated that the contributions of the –SH and the $-NH_3^+$ groups to the ionization of each proton are about equal. Thus, according to Pirie and Pinhey, glutathione has lost one proton at *p*H 9.2 (neglecting the carboxyl groups). This corresponds *exactly* to the *p*H at which the concentrations of RS^- and RSH are equal (see Fig. 4, curve B). It may be mentioned that the spectrophotometric titration curve of L-γ-glutamyl-L-cysteine has the same shape as that of glutathione, except that the *p*H at which the –SH group is half ionized is 9.9.

The plot of % RS^- as a function of *p*H for homocysteine is flatter than in the case of glutathione, indicating a greater difference between the ionizing regions of the two protons, although it is not sufficient for a break to occur. Comparison with the electrometric data of Ryklan and Schmidt[4] again permits the conclusion that the proton affinities of the sulfur and nitrogen functions in this compound are about equal.

Spectrophotometric Analysis of Mixtures of Aminothiols.—The differences in the shapes of the titration curves of different aminothiols make it possible to devise a simple spectrophotometric assay for the analysis of mixtures of some of these compounds. This is well illustrated by considering a mixture of glutathione and cysteine. Inspection of Figs. 2 and 4 shows that the ratio of the optical density at *p*H 10 to that at *p*H 8 is about 7 for glutathione but less than 3 for cysteine. The ratio of the optical densities at these two *p*H values for a mixture of the two compounds therefore should be proportional to the composition of the mixture but independent of the total concentration. The results of such an experiment are shown in Table II. Several other pairs of aminothiols could, of course, be analyzed in an analogous fashion.

TABLE II

ASSAY OF CYSTEINE-GLUTATHIONE MIXTURES

Cysteine, %	O.D. *p*H9.58/ O.D. *p*H8.06	Cysteine, %	O.D. *p*H9.58/ O.D. *p*H8.06
0	6.99	58.6	4.01
19.1	5.73	79.0	3.43
28.8	5.20	100.0	2.82
38.6	4.82		

Discussion

The main conclusion which may be drawn from these results is that none of the generalizations, referred to in the Introduction, can be valid for all aminothiols. Each particular compound must be considered separately in assessing (a) the relative affinity of the sulfur and nitrogen function for each proton and (b) the separation between the two buffering regions also differs from compound to compound. The reasons which govern the variation of these two factors in the different aminothiols studied here may now be outlined.

The simplest case is represented by β-mercaptoethylamine. In agreement with the interpretation of Calvin,[3] the pK of the –SH group in this compound is very much lower than that in ethyl mercaptan, owing to the positive charge of the ammonium group. The pK of this group, in turn, is higher than that in ethylamine because of the negative charge on the sulfur atom.

The introduction of an α-carboxyl group in cysteine results in an increase in the acid strength of the ammonium group because the inductive effect of the carbonyl group outweighs the electrostatic effect of the negative charge. This therefore brings the ionization of the ammonium group into the range of that of the –SH group, leading to "mixed pK's," although the ammonium group still remains the weaker acid.

As the carboxyl group is further modified, as in cysteine ester and the cysteinyl peptides, its acid-strengthening effect (particularly on the ammonium group) becomes even greater since only the electrostatic, but not the inductive, effect is modified. As a result the acid strength of the ammonium group becomes actually greater than that of the –SH group.

Finally, in homocysteine, γ-glutamyl-cysteine and glutathione the greater distance between the –SH and $-NH_3^+$ groups diminishes their effect on each other, so that the two buffering regions overlap very closely and each proton has an approximately equal chance to ionize from either group.

Biological Implications.—It has been realized for a long time that the rate of oxidation of thiols is

(12) N. W. Pirie and K. G. Pinhey, *J. Biol. Chem.*, **84**, 321 (1929).

highly *p*H dependent. Despite its great biological importance the mechanism of this reaction is still not understood. The simple case of thioglycolic acid shows that its rate of oxidation increases systematically with increasing *p*H,[13] supporting the view that the oxidation proceeds *via* the mercaptide ion. It is therefore surprising at first sight that the rates of oxidation of cysteine and glutathione first increase and then decrease again with increasing *p*H.[13,14] If the oxidation is assumed to take place in two steps, either of which may be rate determining, then the "*p*H optimum" observed with these two aminothiols may be understood in terms of their ionic equilibria

$$RS^- \longrightarrow RS\cdot + e^- \quad (1)$$

$$RS\cdot + RS\cdot \longrightarrow RSSR \quad (2)$$

The rate of reaction (1) would be expected to increase with increasing *p*H and the same will be true of the over-all rate of oxidation as long as (1) is rate limiting. It seems reasonable to suppose, however, that as the total RS^- concentration and therefore the rate of step (1) increases, step (2) will become rate determining. The rate of *this* reaction will undoubtedly depend on the charge of the RS· free radicals. In the case of cysteine the radicals derived from the $^-OOCRS^-NH_3^+$ form have zero net charge, whereas those derived from the $^-OOCRS^-NH_2$ species have a negative charge. Therefore reaction (2) would be expected to be faster for the former than for the latter. As can be seen from Fig. 3, the concentration of $^-OOCRS^-NH_3^+$ goes through a maximum as a function of *p*H and the rate of oxidation would therefore also be expected to show a maximum. An analogous argument would apply to glutathione, except that in this case the charge of the two forms of radicals would be -1 and -2, respectively. The actual *p*H values at which Dixon and Tunnicliffe and Mathews and Walker found the rate of oxidation to be maximal are, however, lower than would be predicted from the *pK*'s found here. A probable explanation of this discrepancy is contamination of their admittedly impure thiols with metals which are known to catalyze the oxidation, particularly at lower hydrogen ion concentrations.

Another reaction which is known to proceed through the mercaptide ion[3] is the reduction of disulfide bonds by -SH groups. This reaction is of great importance in protein chemistry since it can lead to a drastic rearrangement of the disulfide bonds.[15-17] The effectiveness of different thiols in initiating this reaction at physiological *p*H would therefore be expected to be in the order given in Table III subject, of course, to other factors such as over-all charge on the protein, etc.

TABLE III

IONIZATION OF THIOL GROUPS AT PHYSIOLOGICAL *p*H

Compound	% RS^- at *p*H 7.4	Compound	% RS^- at *p*H 7.4
L-Cysteine ethyl ester	16	Glutathione	1
L-Cysteinyl-glycine	11	DL-Homocysteine	1
β-Mercaptoethylamine	11	L-γ-Glutamyl-cysteine	1
L-Cysteine	6	Thioglycolic acid	0

Undoubtedly many other correlations between the extent of ionization in the physiological *p*H range and the biological activity of different thiols could be found. One such example is the rate of inactivation of penicillin by thiols. This was found by Cavallito[18] to increase with increasing *p*H and, at constant *p*H, to vary with the structure of the thiol in the order: thioglycolic acid < N-acetyl-L-cysteine < glutathione < glycyl-L-cysteine < L-cysteine and L-cysteinyl-glycine. This is the precise order which would be expected on the basis of the assumption that the inactivation is caused by the mercaptide ion.

In conclusion, some comments on glutathione seem pertinent. Much work and even more speculation has centered on the role of this compound in biological systems.[19] The two main functions which have suggested themselves for this compound have been (1) that of a reducing agent and (2) that of a γ-glutamyl transfering agent in view of the well-known lability of this linkage. However, the remarkable stability of glutathione toward oxidation, already deplored by its discoverer,[20] would seem to disqualify it as a significant biological reducing agent. Its γ-glutamyl transfering function in peptide synthesis also has been questioned since this linkage has not been found in proteins. One might therefore speculate[21] that the function of the γ-glutamyl transferase enzyme may be the transformation of glutathione into a product with a much more reactive thiol group (compare glutathione with cysteinyl-glycine in Tables I and III).

Acknowledgment.—This investigation was supported by grants from the National Heart Institute of the National Institutes of Health, Public Health Service, and from the Division of Molecular Biology, National Science Foundation.

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(21) We are indebted to David R. Schwarz for this intriguing suggestion.