

carbons (as indicated by NOE and line-width data) and two nonprotonated carbons. The resonance at 182 ppm is assigned to the carbonyl group while the nonprotonated ¹³C signals at 127 and 120 ppm are assigned to carbon 5 and the C≡N function, respectively.¹³ The ¹H NMR spectra are also consistent with 2; the chemical shift at 9.7 ppm arises from the aldehyde while δ 6.8 is assigned to the proton at C₄, with δ 5.0 for the proton at C₃. The large coupling constant between H₃ and H₄ (J_{cis} = 12.6 Hz) and longer range couplings to the aldehyde proton have ample literature precedent.¹⁴ The IR frequencies for the C≡N and CHO groups are a bit low compared to standard values.¹⁵ Bellamy suggests that conjugation lowers the C≡N and CHO stretching frequencies and our assignment of 1640 cm⁻¹ to the CHO stretch and 2200 cm⁻¹ to the C≡N stretch are in reasonable agreement with Bellamy's values of 1664 and 2218 cm⁻¹ for conjugated molecules.¹⁵

Intermediate 2 should be extensively stabilized by electron delocalization and the presence of the nitro group in the conjugated system accounts for the strong yellow color of the reaction mixtures. The driving force for decomposition of 2 to product presumably is the formation of the anion of the product, 2-hydroxy-5-nitropyridine.¹⁶

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Scheme I is also consistent with our observations regarding attack of hydroxide on 2-chloro-3-methyl-5-nitropyridine. Formation of an intermediate analogous to 2 in this reaction should not be particularly affected by the presence of the methyl group. The methyl group at position 3 does influence the reclosure of the intermediate to the pyridine nucleus, possibly by a conformation biasing effect at the C₃-C₄ bond.

We have therefore shown that a stable intermediate intervenes in the process which converts 2-halo-5-nitropyridines and hydroxide to 2-hydroxy-5-nitropyridine, at least in mixed organic solvent/water systems. A substantial body of evidence points to 2 as the structure of this intermediate.

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Registry No. 2-Chloro-3-methyl-5-nitropyridine, 22280-56-4; 2-chloro-5-nitropyridine, 4548-45-2; 2-hydroxy-5-nitropyridine, 6191-11-3.

(16) A reviewer has suggested that the conversion of 2 to 6 is more complex than necessary. The steps to form 3, 4, and 5 are chemically reasonable, but we have no additional evidence to indicate whether these steps actually occur.

Effect of Charged Substituents on Rates of the Thiol-Disulfide Interchange Reaction

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Rate constants in aqueous solution at 25 °C are reported for the production of *p*-nitrothiophenol from the mixed disulfides RSSC₆H₄NO₂ upon reaction with the thiol anions R'S⁻. The R' groups used varied in charge and include ⁻O₂CCH₂CH₂, ⁺H₃NCH₂CH₂, and HOCH₂CH₂. The R groups used were these same three in addition to ⁻O₂CCH₂CH₂CH₂. The solutions of RSSC₆H₄NO₂ were prepared by allowing O₂NC₆H₄S⁻ to react with an excess of RSSR, followed by rapidly mixing R'S⁻ and measuring the return of the absorption due to O₂NC₆H₄S⁻. The rates for reactions involving charged R and R' groups differ from those with uncharged groups of identical pK_a by factors of up to 2.5. Negatively charged groups, especially on the central thiol, slow the reaction and positively charged groups speed the reaction to a greater extent than that predicted by the calibrating ionization reaction. Ramifications of these observations are considered for the prediction of rate constants of thiol-disulfide interchange reactions and for the interpretation of β values when reactants of various charge types are used.

Introduction

We have demonstrated recently a simple method whereby the rates of reaction of disulfides with protein thiol groups may be measured.¹ Comparison of the rates of these reactions with the predicted rates based on structure-reactivity correlations²⁻⁵ provides useful infor-

mation about the site wherein the thiol group resides. The thiol-disulfide interchange reaction is important in cellular redox chemistry, as in the mechanism of glutathione reductase⁶ or dihydrolipoamide dehydrogenase,⁷ and is also important in the generation of protein tertiary structure.⁸

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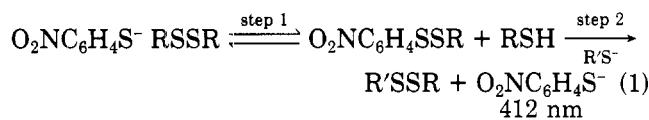
In order to understand complex processes of this type, it is mandatory to determine those factors which influence the rates of these reactions in aqueous solution, and this paper continues our efforts toward that end.

Previous studies have shown that changing the pK_a of the attacking thiol anion ($\beta_{nuc} = 0.50$)² or the central thiol ($\beta_{CT} = -0.40$)⁴ has a substantial effect on the rate constants measured. The studies done in determining these structure-reactivity correlations were carried out with uncharged R groups on the attacking nucleophile. It was not clear that the data obtained would be applicable to those cases in which charged groups were present.

It is reasonable to presume initially that points for charged entities would fall on correlation lines defined by uncharged entities. This requires only that the ratio of the effect on transition-state energy to that for the calibrating ionization reaction be the same for uncharged and charged substituents. There are reactions, nucleophilic attack on esters of different charges, for example,⁹ in which charge changes the reactivity more than that expected on the basis of the calibrating ionization reaction. On the other hand, Brønsted plots are often determined by using groups of reactants of various charge types which inherently presumes that no deviation depending on charge type exists. This last practice presents a real problem since the plot of rate vs. pK_a is likely to have negatively charged entities at the high pK_a end and positively charged entities at the low pK_a end. Oppositely directed deviations for positively and negatively charged groups would therefore tend to tilt the plot and indicate a substantially different degree of bond formation than would be indicated by uncharged groups.

For these reasons, we wished to measure the rates of reaction of variously charged thiol anions with variously charged disulfides and compare them with the rates predicted for uncharged reactants with identical pK_a values. There are several technical problems that make these measurements difficult. The reactions must be designed so that a chromophore is generated and so that there is no ambiguity arising because of the possibility of attacking two different sulfur atoms of a mixed disulfide to produce two different products. Another problem arises because of the difficulty in synthesizing pure samples of mixed disulfides.

As shown in eq 1, we planned to surmount these diffi-



culties by allowing the disulfide $\text{O}_2\text{NC}_6\text{H}_4\text{SSR}$ to be generated in situ in step 1 by using an excess of RSSR. Rapid mixing in of R'S⁻ in step 2 would then give an easily observed return of the chromophoric *p*-nitrothiophenol anion as it reacted with $\text{O}_2\text{NC}_6\text{H}_4\text{SSR}$. All other entities present in the equilibrium mixture would cause no change in absorbance. By varying the charge on R and R', it would be possible to assess their effect on the reaction rate.

Experimental Section

The rates of reaction of the thiol anions with mixed disulfides were done in a manner analogous to that described below for a typical example and as shown in Figure 1.

The following solutions were prepared in argon flushed flasks, using freshly boiled doubly distilled water. A 7.64×10^{-4} M solution of *p*-nitrothiophenol was prepared which also contained 10^{-3} M imidazole adjusted to pH 6.0 with chlorine-free HCl. The

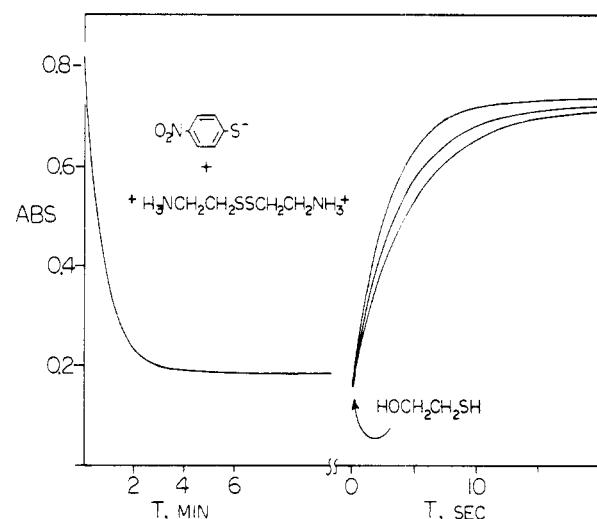


Figure 1. Plot of absorbance vs. time for the experiment in which the rate constant for the reaction of $\text{HOCH}_2\text{CH}_2\text{S}^-$ with $^+\text{H}_3\text{NCH}_2\text{CH}_2\text{SSC}_6\text{H}_4\text{NO}_2$ was determined at pH 6.0 and $\mu = 1.0$ M at 25°C . As described in the Experimental Section, the mixed disulfide is formed in situ and then the attacking thiol is added rapidly and mixed. The curves shown resulted from having concentrations of $\text{HOCH}_2\text{CH}_2\text{SH}$ of 0.0203, 0.0162, and 0.0142 M. Because of dilution, the final A_∞ value is smaller than the initial $\text{O}_2\text{NC}_6\text{H}_4\text{S}^-$ absorbance.

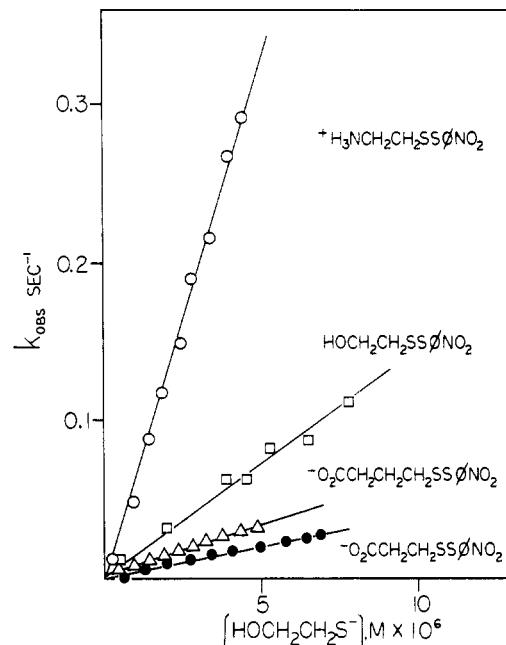


Figure 2. Plot of k_{obs} vs. concentration of $\text{HOCH}_2\text{CH}_2\text{S}^-$ for the reaction with the disulfides shown. The rate constants obtained from the slopes of such plots are listed in Table I. The concentration of $\text{HOCH}_2\text{CH}_2\text{S}^-$ was computed from the pK_a and the thiol concentration and the pH measured for that run.

solution also contained enough KCl so that the ionic strength was 1.0 M. A 1.0 M KCl solution containing 10^{-3} M imidazole buffer at pH 6.0 was made. A solution of 0.223 M mercaptoethanol was prepared also with 1.0 M KCl and 10^{-3} M imidazole buffer at pH 6.0. A solution containing 5.09×10^{-2} M $(^+\text{H}_3\text{NCH}_2\text{CH}_2\text{S})_2$ was prepared with 10^{-3} M imidazole and enough KCl to raise the ionic strength to 1.0 M.

Shown in Figure 1 is the change in absorbance at 412 nm that occurs with time at 25°C when 0.2 mL of the nitrothiophenol solution is added to a mixture of 1.30 mL of the KCl solution and 0.50 mL of the solution of the disulfide. After 10 min there is no further change in absorbance and most of the nitrothiophenol has been converted to the mixed disulfide. By use of a rapid mixing device previously described and a spring-loaded calibrated

Table I. Second-Order Rate Constants for the Reaction of Charged Thiol Anions with Charged Mixed Disulfides at 25 °C, $\mu = 1.0$ M, in Aqueous Solution

attacking thiol (pK_a)	$k_2, M^{-1} s^{-1} (r^2)$	disulfide			
		${}^+H_3NCH_2CH_2SSC_6H_4NO_2$	$HOCH_2CH_2SSC_6H_4NO_2$	${}^-O_2CCH_2CH_2SSC_6H_4NO_2$	${}^-O_2C(CH_2)_3SSC_6H_4NO_2$
${}^+H_3NCH_2CH_2SH$ (8.23)	$k_2, M^{-1} s^{-1} (r^2)$	0.804×10^4 (0.970)	0.331×10^4 (0.996)	0.115×10^4 (0.995)	0.170×10^4 (0.991)
	concn range, M	0.0046-0.0230	0.0057-0.0230	0.0023-0.0207	0.0023-0.0207
$HOCH_2CH_2SH$ (9.61)	pH (no. of runs)	5.94 (9)	5.97 (10)	5.88 (9)	5.82 (9)
	$k_2, M^{-1} s^{-1} (r^2)$	7.4×10^4 (0.996)	1.33×10^4 (0.986)	0.379×10^4 (0.998)	0.626×10^4 (0.996)
${}^-O_2CCH_2CH_2SH$ (10.05)	concn range	0.0010-0.0188	0.0053-0.0210	0.0026-0.0265	0.0025-0.0250
	pH (no. of runs)	5.99 (10)	6.16 (6)	6.03 (10)	5.90 (10)
	$k_2, M^{-1} s^{-1}, (r^2)$	9.51×10^4 (0.996)	2.36×10^4 (0.991)	0.660×10^4 (0.982)	0.981×10^4 (0.994)
	concn range	0.0012-0.0232	0.0022-0.0221	0.0132-0.0221	0.0092-0.0230
	pH (no. of runs)	6.03 (10)	5.99 (10)	5.98 (9)	5.85 (9)

Hamilton injection syringe, the reaction was initiated by rapidly injecting and mixing in 0.200 mL of the solution of mercaptoethanol. The return of the absorbance due to the *p*-nitrothiophenol anion chromophore was monitored at 412 nm and these curves were translated into rate constants for the reactions in a manner previously described.^{2,4}

During the course of the observed reaction, some of the $HOCH_2CH_2SH$ could also react with $({}^+H_3NCH_2CH_2S)_2$. Using the previously determined structure-reactivity correlation, it was calculated that the rate for this process is much less than 1% of the rate for the observed reaction with ${}^+H_3NCH_2CH_2SSC_6H_4NO_2$. ($\beta_{LG} \times \Delta pK_a = -0.5 \times -3.63 = 1.8$ plus a factor of six for an aromatic leaving thiol gives $10^{1.8} \times 6 = 400$ -fold difference in rate favoring the observed reaction.) Thus, during one half-life of the observed reaction much less than 1% of the attacking thiol, present in excess, is depleted by this route. Linear slopes from semilog plots of the kinetic data obtained confirm that no significant competing process interferes with the rate measurement.

The thiols and disulfides were purified before use by distillation or recrystallization. The pK_a measurements for cysteamine, 3-mercaptopropionic acid, and 4-mercaptopbutanoic acid were measured spectrophotometrically in the presence of 2.5×10^{-4} M phosphate buffer at an ionic strength of 1.0 M maintained with KCl. The pK_a values were determined on the basis of 20 or more absorbance readings. Other pK_a values used in this study were determined under identical conditions.^{2,9}

Results

The pK_a values of ${}^+H_3NCH_2CH_2SH$ (8.23), ${}^-O_2CCH_2CH_2SH$ (10.05), and ${}^-O_2CCH_2CH_2CH_2SH$ (10.06) were measured spectrophotometrically at $\mu = 1.0$ M. The value for cysteamine^{1,10} was identical with that reported previously but those for the mercapto acids are lower than previously reported values.¹¹

The rate constants measured for the reappearance of *p*-nitrothiophenol from mixed disulfides upon addition of thiol are shown in Table I. Each rate constant was obtained by plotting the value of k_{obs} vs. the computed concentration of thiol anion as shown in Figure 2. These concentrations were calculated by knowing the pK_a of the thiol, the total thiol concentration, and the pH of the individual run.

The logarithms of the rate constants are plotted vs. their pK_a values in Figure 3. Also shown in this figure are the Brønsted slopes predicted for these reactions, using values of $\beta_{nuc} = 0.5$ for the attacking thiol anion² and $\beta_{CT} = -0.4$ for the central thiol.⁴ These are the values which have been determined for reactions with uncharged R groups on the attacking and central thiols. The point for the attack of the anion of mercaptoethanol on the mixed disulfide of

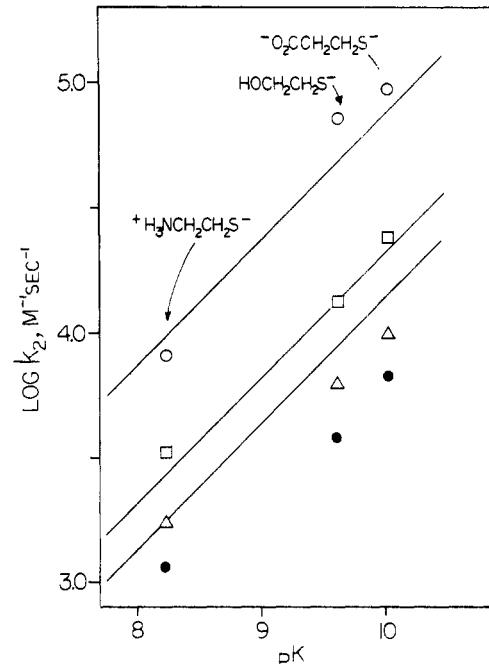


Figure 3. Brønsted-type plot for $\log k$ vs. pK_a of attacking nucleophile with the four mixed disulfides studied. The solid lines are those calculated with $\beta_{nuc} = 0.50$ and $\beta_{CT} = -0.40$, as described in the text. Data shown are for the reactions with ${}^+H_3NCH_2CH_2SSC_6H_4NO_2$ (○), $HOCH_2CH_2SSC_6H_4NO_2$ (□), ${}^-O_2CCH_2CH_2CH_2SSC_6H_4NO_2$ (△), and ${}^-O_2CCH_2CH_2SSC_6H_4NO_2$ (●). The reactions of these last two disulfides have coincident calculated lines because of the nearly identical pK_a values of the central thiols.

mercaptoethanol and *p*-nitrothiophenol was chosen as the starting value for drawing the lines, since all of the R groups are uncharged in this reaction. The lines for the cases where the mixed disulfide contains either 4-mercaptopbutanoate or 3-mercaptopropionate coincide because the pK_a values for these two thiols are very similar. The points for the cases in which the attacking or central thiol R group is charged deviate from these lines. In Figure 4 is shown a plot of the measured minus the calculated values of $\log k$ for each of the reactions studied vs. the charge on the R group of the attacking thiol anion.

Discussion

Previous studies of the thiol-disulfide interchange reaction done under conditions identical with those used in this work indicate a value of $\beta_{nuc} = 0.5$ for the attacking thiol and $\beta_{CT} = -0.4$ for the central thiol when the substituent groups are uncharged.^{2,4} There is an obvious ambiguity that arises with mixed disulfides since either of two

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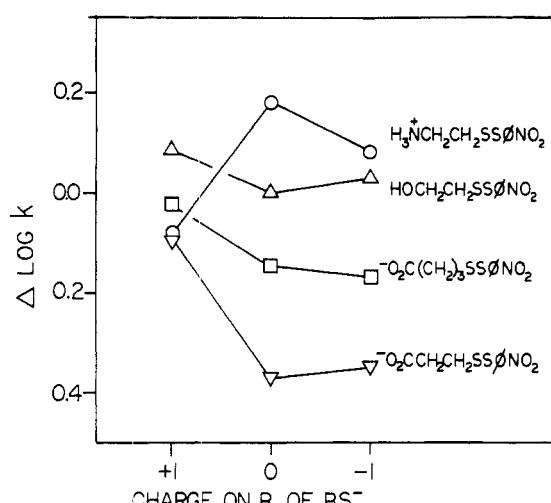
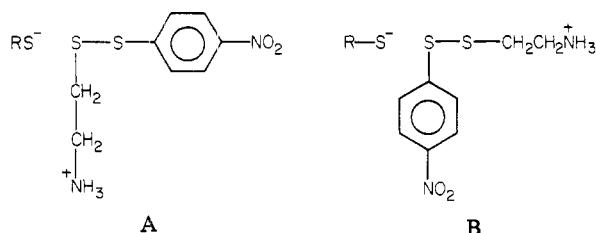


Figure 4. Plot of the calculated $\log k$ value minus the measured $\log k$ value vs. the charge on the R group of the attacking RS^- .

sulfur atoms may be attacked by the nucleophilic thiol anion as shown in structures A and B. By use of the value



of β_{CT} and that for the leaving group ($\beta_{LG} = -\beta_{nuc} = -0.5$) and by use of the fact that aryl thiol anions leave sixfold faster than alkyl thiol anions,² a calculation of the relative rates of attack at the two sites may be made. The ratio of rates of the desired reaction (A) to the undesired reaction (B) is calculated to be about 15 in the worst case. This small percentage of attack on the wrong sulfur has no effect on the rate if the entering and leaving thiol are identical or similar in pK_a . In the few cases where the small amount of new mixed disulfide would have differed substantially in rate from the starting mixed disulfide, semilog plots of the absorbance data were made. The linear plots found ensured that there was very little, if any, error in the measured rate due to initial attack at the wrong sulfur.

The points for the variously charged disulfides and thiols exhibit small but significant deviations from the Brønsted type plots found for the uncharged systems. The deviations are plotted in Figure 4 and show a pattern that indicates that pK_a values alone provide a good but not perfect index of how the charge will affect the stability of a transition state. The negative charges on R groups destabilize the calibrating ionization reaction of the thiols and the positive charges stabilize the same reaction. The fact that there are negative deviations for negative groups and positive deviations for positively charged groups implies that charge has a proportionally greater effect on transition-state energy than inductive effects when compared to the calibrating ionization reaction.

One interesting feature of Figure 4 is that the deviation from uncharged values is greatest when the charge is on the central group rather than on the attacking group and that increasing the distance from the reacting site diminishes the effect of the charge. Coulombic interactions are apparently also evident between attacking and central thiol R groups in the relatively low rates found when both are positively charged.

We conclude that predictions of rate constants for the thiol-disulfide interchange reaction in aqueous solution may be in error by as much as a factor of 2.5 when structure-reactivity correlations measured with uncharged substituents are used to predict the rates of those with charged substituents. The effect of these deviations can have a profound effect on the β value measured for a reaction. If a series of reactants is used in which the highest pK_a reactants are negatively charged and the lowest positively charged, there will be a tendency to tilt the slope of the plot. A total set of deviations of 0.6 $\log k$ unit over a range of 3 pK_a units would give a β value in error by 0.2 and thus give a much different estimate of the degree of bond formation than would be obtained with neutral substituent groups.

Acknowledgment. This work was supported by the National Science Foundation, Grant CHE-78-08723.

Registry No. $O_2NC_6H_4SH$, 1849-36-1; $(O_2CCH_2CH_2S)_2$, 58823-20-4; $(^+H_3NCH_2CH_2S)_2$, 58823-21-5; $(HOCH_2CH_2S)_2$, 111-48-8; $(O_2C(CH_2)_3S)_2$, 73873-03-7; $(^+H_3NCH_2CH_2SSC_6H_4NO_2)_2$, 73873-04-8; $HOCH_2CH_2SSC_6H_4NO_2$, 53595-97-4; $(O_2CCH_2CH_2SSC_6H_4NO_2)_2$, 73891-30-2; $(O_2C(CH_2)_3SSC_6H_4NO_2)_2$, 73891-31-3; $(^+H_3NCH_2CH_2SH)_2$, 40302-08-7; $HOCH_2CH_2SH$, 60-24-2; $(O_2CCH_2CH_2SH)_2$, 2365-49-3.

Structure-Reactivity Relationships in Nucleophilic Reactions on Cinnamoyl Azide and Phenyl Cinnamates. Kinetic Stability of the Acyl Azide and Relative Leaving Ability of Nitrogen and Oxygen

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The alkaline hydrolysis and aminolysis of cinnamoyl azide and aryl-substituted phenyl cinnamates and the azidolysis of the phenyl cinnamates have been studied. The positions of the rate-determining steps in the reactions are identified by the structure-reactivity relationships. The enhanced affinity of azide ion for an acyl carbon compared with its basicity is proposed to account for the observed kinetic stability of cinnamoyl azide toward alkaline hydrolysis and aminolysis. Azide ion resists expulsion from the tetrahedral intermediate more than quinuclidine derivatives but less than phenolates. This is explained in terms of the electron withdrawal as well as electron donation by resonance.

Nucleophilic reactions on acyl derivatives, especially esters and amides, have been the subject of extensive

studies. The existence of a tetrahedral intermediate with a finite lifetime, the nature of the rate-determining steps,