Acknowledgment. This work was supported by NIH grants ES02611 and NS01208. We acknowledge the use of Duke Magnetic Resonance Center supported in part by NSF grant DMB8501010, NIH grant RR02780, and NC biotechnology grant 86U02151. We thank Mr. M. Anthony Moody for his help during the initial stages of this work. W. M. Valentine is an RJReynolds-Leon Golberg Memorial Fellow in Toxicology.

Supplementary Material Available: The ^1H and ^{13}C NMR spectra of pyrroles **16a,b,f-h** which are very rapidly oxidized (10 pages). Ordering information is given on any current masthead page.

Notes

Selenols Catalyze the Interchange Reactions of Dithiols and Disulfides in Water

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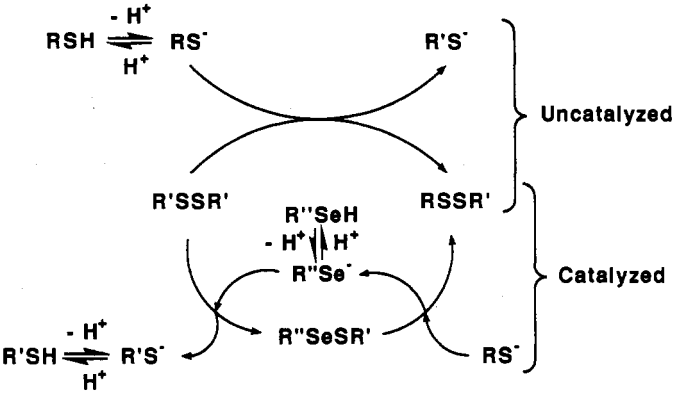
The mechanism of thiol–disulfide interchange reaction involves the nucleophilic attack of thiolate along the S–S bond axis of the disulfide.[1] The reaction is kinetically second order: first order in thiolate and in disulfide.[2-4] At pH 7, only 0.1–1% of typical thiol groups is present as thiolate in water, and the apparent rate constant of the thiol–disulfide interchange reaction is small ($k^{obsd} \approx 0.1$ M^{-1} s^{-1}).[2,5] Protein disulfide isomerase (EC 5.3.4.1) has been suggested to act as a catalyst for thiol-disulfide interchange in vivo, but its lack of specificity and the fact that it induces only moderate rate enhancements make its biological role uncertain.[6-9]

As part of a program examining thiolate–disulfide interchange, we surveyed a number of types of compounds (aromatic thiols, nonthiol nucleophiles, and cations) as potential catalysts for this reaction. The only significant rate enhancement was obtained with phenylselenol.[5] Selenolate is a strong nucleophile toward diselenides and a good leaving group in selenolate–diselenide interchange.[10] The pK_a of selenols is ≈ 7,[11] and at pH 7 the attack of selenol on diselenide is rapid ($k^{obsd} = 1.65 \times 10^7$ M^{-1} s^{-1}).[10] We expected selenolate to be a strong nucleophile toward disulfide and to be a good leaving group in the attack of thiolate on selenosulfide (RS–SeR). Scheme I shows the steps involved in the catalysis of a thiol–disulfide interchange reaction by selenol.

Scheme I. Catalysis of Thiol–Disulfide Interchange by Selenol



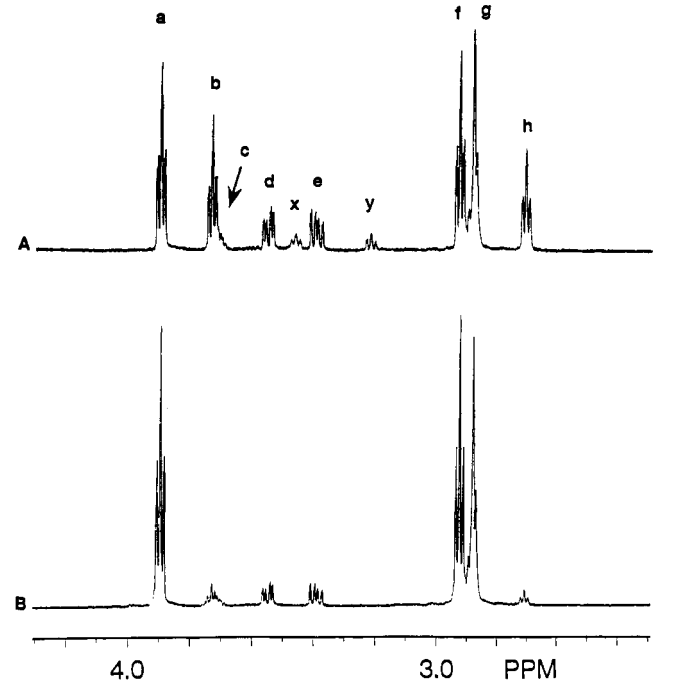


Figure 1. ^1H NMR spectra (500 MHz) of reaction mixtures initially containing dihydroasparagusic acid (DHA, 5 mM) and bis(2-hydroxyethyl) disulfide (ME^{ox}, 5 mM) in phosphate-buffered (50 mM) D_2O at pD 7 under argon: (A) in the presence of 10 mol % 2-aminoethaneselenol (0.5 mM), quenched with DCl after 1.5 min; (B) in the absence of selenol, quenched with DCl after 2.2 min. The peak assignments are a = CH_2OH (ME^{ox}), b = CH_2OH (ME), c = CH (DHA^{ox}), d,e = CH_2 (DHA^{ox}), f = CH_2S (ME^{ox}), g = CH, CH_2 (DHA), h = CH_2S (ME), x = $CH_2NH_3^+$, y = CH_2Se.

In this study we have surveyed in greater detail the ability of several alkaneselenols to catalyze thiol–disulfide interchange reactions. We have examined thiol–disulfide

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Table I. Catalysis of Thiol–Disulfide Interchange by Selenols in Water at pH 7[a]

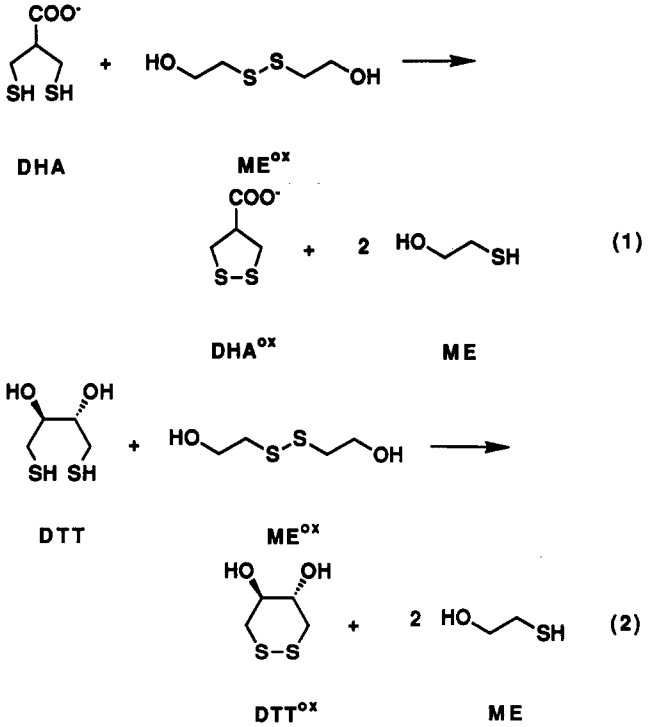
reactants[b]	catalyst	equiv[c]	k^{obsd} (M^{-1} min^{-1}) k_{cat}^{obsd}	k_{uncat}^{obsd}	$k_{cat}^{obsd}/k_{uncat}^{obsd}$	$k_{cat}^{obsd}/[RSeH]k_{uncat}^{obsd}$ (mM^{-1})	method
DHA + ME^{ox}	$^+H_3NCH_2CH_2SeH$	0.05	70	2.3	30	150	UV
		0.10	110	2.3	48	120	UV
	$(^+H_3NCH_2CH_2Se)_2$	0.05[d]	35	2.6	13	65	UV
		0.025[d]	18	2.6	7	70	UV
	$^+H_3NCH_2CH_2SeH$	0.10	70	4	18	36	NMR
DTT + ME^{ox}	$(^+H_3NCH_2CH_2Se)_2$	0.05[d]	130	9	15	75	UV
		0.025[d]	65	9	7	70	UV
	$^+H_3NCH_2CH_2SeH$	0.10	420	11	38	76	NMR
	$HOCH_2CH_2SeCN$	0.05	170	11[e]	16	64	NMR
L-Cys + ME^{ox}	$^+H_3NCH_2CH_2SeH$	0.05	30	27	1		NMR
N-AcCys + ME^{ox}	$^+H_3NCH_2CH_2SeH$	0.05	9	6	1		NMR
	$HOCH_2CH_2SeCN$ + DTT	0.05	8	6[e]	1		NMR

[a] Reactions were carried out in buffered deoxygenated aqueous solution at room temperature (23–24 °C) under an atmosphere of argon. The error in the observed rate constants is ±10%. [b] The initial concentrations of thiols and disulfides were 4 mM each in the UV experiments, 10 mM each in the NMR experiments involving cysteine or *N*-acetylcysteine with $^+H_3NCH_2CH_2SeH$ as catalyst, and 5 mM each in all other NMR experiments. [c] The quantity of RSeH used is given in terms of equivalents of the reactants—thiols and disulfides. [d] The equivalents of RSeH reported are two times the initial molar equivalents of $(^+H_3NCH_2CH_2Se)_2$. [e] The values of the uncatalyzed rate constant, k_{uncat}^{obsd}, for the $HOCH_2CH_2SeCN$ experiments are assumed to be the same as determined by the NMR method for $^+H_3NCH_2CH_2SeH$ experiments.

interchange reactions involving both monothiols or dithiols and used selenols and precursors of selenols (diselenide, selenocyanate) as catalysts. Assays were based on ^1H NMR and UV spectroscopic analyses.

Results and Discussion

Table I shows the apparent rate constants (k^{obsd}) of thiol–disulfide interchange reactions in the presence or absence of selenols. The reactions involving strongly reducing dithiols (eqs 1 and 2; DHA is dihydroasparagusic



(1)

(2)

acid) are significantly catalyzed by the presence of alkyl selenols. Figure 1 illustrates the rate enhancements observed in these thiol–disulfide interchange reactions in the presence of catalytic amounts of selenol.

Table I reports observed rate enhancements ($k_{cat}^{obsd}/k_{uncat}^{obsd} = v_{cat}/v_{uncat}$) and ratios of observed rate constants adjusted for the initial concentration of selenol ($k_{cat}^{obsd}/[RSeH]k_{uncat}^{obsd}$) estimated from eqs 3 and 4. We emphasize that these are

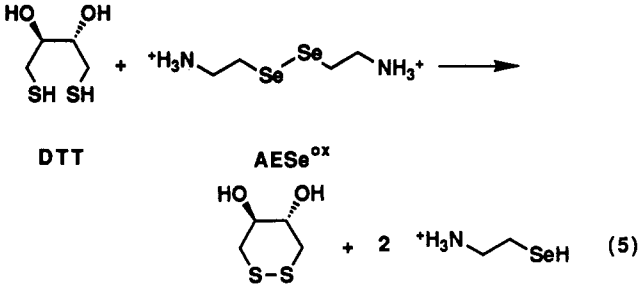
$$v_{uncat} = \tfrac{1}{2}d[ME]/dt = k_{uncat}^{obsd}[ME^{ox}][R(SH)_2] \quad (3)$$

$$v_{cat} = \tfrac{1}{2}d[ME]/dt = k_{cat}^{obsd}[ME^{ox}][R(SH)_2] \quad (4)$$

observed rate constants and do not contain corrections for the values of pK_a of the sulfur and selenium compounds. They cannot, therefore, be related directly to the reactions in Scheme I. The values of concentrations of selenols used for calculating the ratio of observed rate constants adjusted for the concentration of selenol ($k_{cat}^{obsd}/[RSeH]k_{uncat}^{obsd}$) are the initial concentrations of selenol (diselenide or selenocyanate) and are therefore approximate measures of the catalytic concentrations of the selenol. The values of rate constants obtained from UV experiments are more accurate than those from NMR experiments.

Most of the work has used 2-aminoethaneselenol (AESeH) as the selenium-containing component. The reasons for this choice are that its diselenide, bis(2-aminoethyl) diselenide ($AESe^{ox}$, selenocystamine), is commercially available, and both $AESe^{ox}$ and AESeH are water soluble.

Strongly reducing dithiols (e.g., dithiothreitol (DTT)) reduce diselenide to selenol. The value of the equilibrium constant (0.14 M, eqs 5 and 6) for the reduction of sele-



(5)

$$K_{eq} = \frac{[AESeH]^2[DTT^{ox}]}{[AESe^{ox}][DTT]} = 0.14\ M \quad (6)$$

nocystamine ($(^+H_3NCH_2CH_2Se)_2$, $AESe^{ox}$) by DTT is, however, significantly lower than that (9.4×10^4 M) for the analogous reduction of bis(2-hydroxyethyl) disulfide (ME^{ox}) by DTT.[12] Monothiols are more weakly reducing

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than dithiols such as DTT and cannot reduce diselenide significantly (eq 7). The rate of thiol–disulfide interchange

$$2ME + AESe^{ox} \rightleftharpoons ME^{ox} + 2AESeH \quad (7)$$

reaction of cysteine (or *N*-acetylcysteine) with bis(2-hydroxyethyl) disulfide (ME^{ox}) is not enhanced in presence of 2-aminoethaneselenol ($^+H_3NCH_2CH_2SeH$) or 2-hydroxyethaneselenol ($HOCH_2CH_2SeH$) (Table I). The thiol–disulfide reactions involving monothiols and disulfides are not catalyzed by selenol because selenol is oxidized to diselenide by reaction with disulfide.

In the reactions involving strongly reducing dithiols and disulfides, the catalytic selenol can be generated from diselenide (RSeSeR) or selenocyanate (RSeCN).[18] The addition of diselenide or selenocyanate to the reaction mixture is more convenient than the addition of selenols because selenols are easily oxidized and are thus difficult to manipulate.

In conclusion, selenols catalyze the thiol–disulfide interchange reactions involving dithiols significantly in water. They are the first nonbiological materials that have even marginal utility as catalysts for this reaction.[19] We hypothesize, in the absence of any firm, relevant experimental evidence that this catalytic activity is attributable to their acidity ($pK_a \approx 7$, a number that probably provides a near-optimal combination of conversion of selenol to negatively charged selenolate nucleophile at pH 7 and useful nucleophilicity of this species) and to the weak solvation and high polarizability (and hence high nucleophilicity) of the selenolate ion. Thiolate–disulfide interchange reactions involving monothiols are, however, not accelerated in the presence of selenols. Selenols are oxidized to diselenides by noncyclic dialkyl disulfides. Their ability to catalyze reactions of eqs 1 and 2 is due to the ability of the dithiols to reduce diselenides to selenols.

The selenol precursors—diselenide or selenocyanate—can also be conveniently used to catalyze the thiol–disulfide interchange reactions involving strongly reducing dithiols.

Experimental Section

General. Selenocystamine hydrochloride ($AESe^{ox}$), potassium selenocyanate, 2-bromoethanol, dithiothreitol (DTT), cysteine (L-Cys), *N*-acetylcysteine (*N*-AcCys), and bis(2-hydroxyethyl) disulfide (ME^{ox}) were all purchased from Aldrich. Dihydroasparagusic acid (DHA) was prepared as described.[5] 2-Hydroxyethyl selenocyanate ($HOCH_2CH_2SeCN$) was prepared by a literature procedure.[17]

All flasks, quartz cuvettes, and NMR tubes were stoppered with rubber septa and were flushed with argon before use. All solutions were deoxygenated by bubbling argon through them for ~45 min. All transfers were done using gas-tight syringes.

Preparation of 2-Aminoethaneselenol ($^+H_3NCH_2CH_2SeH$). To a solution of selenocystamine hydrochloride (0.0028 g, 8.8 μmol) in deoxygenated ethanol (0.5 mL) was added sodium borohydride (0.0020 g, 53 μmol). The resulting solution was kept at room temperature under argon for 20 min until the solution became clear and no effervescence was seen. The solution was cooled in an ice bath and was quenched with glacial acetic acid (18 μL, 310 μmol). The concentration of 2-aminoethaneselenol was 40 mM by Ellman's assay.[20]

UV Assay for Catalysis of Thiol–Disulfide Interchange Involving DHA and ME^{ox} by 2-Aminoethaneselenol ($^+H_3NCH_2CH_2SeH$). Stock solutions of DHA and ME^{ox} (both 8 mM) were prepared from DHA (0.0131 g) in 10.8 mL of deoxygenated phosphate buffer (0.1 M in phosphate, pH 7.0, 2 mM in EDTA), and from ME^{ox} (0.0129 g) in 10.5 mL of deoxygenated phosphate buffer. In a quartz cuvette containing 1.5 mL of DHA stock solution and 30 μL of 2-aminoethaneselenol stock solution (40 mM) was added 1.5 mL of ME^{ox} stock solution, and the increase in absorbance at 330 nm was recorded. The initial concentrations in the cuvette were $[DHA] = [ME^{ox}] = c_{initial} = 4.0$ mM, $[^+H_3NCH_2CH_2SeH] = 0.4$ mM. The apparent rate constant (k^{obsd}) was calculated using the integrated form of the rate equation $d[DHA^{ox}]/dt = k^{obsd}[DHA][ME^{ox}]$: $k^{obsd} = (1/c_{final} - 1/c_{initial})/t$. For monitoring the reactions of DTT and ME^{ox}, the increase in absorbance at 310 nm was recorded.[2]

^1H NMR Assay for Catalysis of Thiol–Disulfide Interchange Involving DTT and ME^{ox} by 2-Aminoethaneselenol ($^+H_3NCH_2CH_2SeH$). Solutions of DTT (10 mM, 0.0031 g in 2 mL of deoxygenated D_2O buffer (50 mM in phosphate, pD 7.0)) and ME^{ox} (10 mM, 0.0046 g in 3 mL of deoxygenated D_2O buffer) were prepared. To an NMR tube was added $^+H_3NCH_2CH_2SeH$ (21 μL of a 11.8 mM solution in ethanol), and the solvent was removed in vacuo; to the NMR tube were added 0.25 mL of the DTT solution and 0.25 mL of the ME^{ox} solution, and the reaction was quenched after 1.5 min by addition of DCl (10 μL of a 37 wt % solution in D_2O). The initial concentrations in the NMR tube were $[DTT] = [ME^{ox}] = 5$ mM, $[^+H_3NCH_2CH_2SeH] = 0.5$ mM. The final concentrations of ME^{ox} and ME were determined by integration of the NMR peak areas. For the uncatalyzed reaction, 0.25 mL of DTT solution and 0.25 mL of ME^{ox} solution were mixed in an NMR tube, and the reaction was quenched after 5 min by addition of DCl (10 μL of a 37 wt % solution in D_2O).

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Registry No. DHA, 136202-27-2; DTT, 3483-12-3; L-Cys, 52-90-4; *N*-AcCys, 616-91-1; ME^{ox}, 1892-29-1; $^+H_3NCH_2CH_2SeH$, 116303-19-6; $(^+H_3NCH_2CH_2Se)_2$, 84250-77-1; $HOCH_2CH_2SeCN$, 115423-26-2.

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(18) Selenols can be conveniently prepared by reduction of diselenide (RSeSeR) or selenocyanate (RSeCN) with sodium borohydride,[13,14] or with dithiothreitol (DTT).[15,16] Selenocyanates are conveniently prepared from alkyl bromide by reaction with potassium selenocyanate (KSeCN).[17] Selenocyanates are stable to chromatography on silica gel and are therefore useful intermediates in the synthesis of selenols.

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Sigmatropic Rearrangements of Ammonium Benzylides: New Preparative and Mechanistic Aspects[1]

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Benzylammonium salts, when treated with a base, generate ylides, which undergo the Stevens [1,2] and/or Sommelet–Hauser [2,3] rearrangements.[2] The latter is an attractive method for the synthesis of aromatic compounds with ortho-located substituents.

We report that the Sommelet–Hauser rearrangement of benzylides, generated from suitably substituted benzylammonium salts, is a new and convenient synthetic route leading to *o*-cyanomethylated derivatives of aromatic aldehydes. Furthermore, this reaction applied to ring-substituted benzylammonium salts allowed us to present a new mechanistic pathway.[3]

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