showed that $\Delta S°$ favors the E conformation for most of the compounds, including methyl nitrite and the primary nitrites. The higher entropy of (E)-methyl nitrite ($\Delta S° = 1.6$ eu)[28] is related to the lower methyl top barrier of this conformation (29 cal/mol[29a] vs 1912 cal/mol[29b] for the Z isomer) and contributes −0.28 kcal/mol to $\Delta G°$ at −100 °C. The methyl rotational barrier of (Z)-methyl formate (1.19 kcal/mol)[30] is lower than the barrier for this conformation of methyl nitrite (1.91 kcal/mol),[29b] but the E conformation of methyl formate may also be stabilized by a favorable $\Delta S°$ term resulting from a lower methyl top barrier. The calculated barrier of 0.23 kcal/mol[11] for rotation of the phenyl group of (Z)-**2** through 90° is smaller than the corresponding barrier of 0.72 kcal/mol for the E isomer and suggests that the Z conformation may be favored by $\Delta S°$ in this case.

Conclusions

The large population (0.20) of the E isomer of phenyl formate is evidence for a significant contribution of "aromaticity" to the stabilization of the Z conformation of most esters. Because the Z conformation still predominates, other factors must also be important in determining the conformations of these compounds, as discussed above. "Aromaticity" is expected to have an important influence on the conformational equilibria in many related compounds, including secondary amides, alkyl nitrites,[31] and alkyl vinyl ethers.

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Registry No. Phenyl formate, 1864-94-4; 2,6-dimethylphenyl formate, 1865-00-5.

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A Comparative Study of the Kinetics of Selenol/Diselenide and Thiol/Disulfide Exchange Reactions

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Abstract: The kinetics of symmetrical selenol/diselenide and thiol/disulfide exchange reactions involving selenocysteamine/selenocystamine and cysteamine/cystamine have been studied in D_2O solution by NMR spectroscopy. The rate of selenol/diselenide exchange is so fast that resonances for the selenol and diselenide forms are coalesced in ^1H NMR spectra of millimolar selenocysteamine/selenocystamine mixtures at pD >2-3. In contrast, the rate of thiol/disulfide exchange is so slow that separate, sharp resonances are observed for both cysteamine and cystamine in mixtures at concentrations up to at least 0.2 M from pD <1 to >13. Rate constants for the selenol/diselenide exchange reaction were determined by line shape analysis of exchange-broadened resonances, while those for thiol/disulfide exchange were determined by an inversion-transfer method. The rate constants at 25 °C for exchange by reaction of $D_3N^+CH_2CH_2X^-$ with $D_3N^+CH_2CH_2XXCH_2CH_2ND_3^+$ are as follows: X = Se, $k = 1.65 \times 10^7$ L/mol·s; X = S, $k = 68.0$ L/mol·s. When the differences in the acidities of the selenol and thiol groups are accounted for, selenocysteamine/selenocystamine exchange is 1.2×10^7 times faster than cysteamine/cystamine exchange at physiological pH.

Selenium in the form of selenocysteine residues is an essential component of glycine reductase, certain formate dehydrogenases, and a hydrogenase of bacterial origin and of glutathione peroxidase in mammals and birds. Of these, glutathione peroxidase is the most studied.[1-3] Glutathione peroxidase catalyzes the reduction of hydroperoxides by glutathione by a mechanism thought to involve first the oxidation of the selenium and then its reduction, all by a sequence of nucleophilic displacement reactions.[2,3] In these reactions, the selenium, in its various oxidation states, is either the nucleophile, the central atom, or the leaving group, depending on the step in the catalytic cycle. Although the proposed mechanism is consistent with experimental results, it has not been proven. For example, it has not yet been possible to determine unequivocally the oxidation state of selenium in the various oxidized forms of glutathione peroxidase.[4]

As part of a project to characterize the chemistry of selenium in glutathione peroxidase, we have initiated a study of the kinetics and equilibria of nucleophilic displacement reactions involving model organoselenium compounds. In this paper, we report the results of a study of the kinetics of the selenol/diselenide exchange reaction

$$R^*SeH + RSeSeR \rightleftarrows R^*SeSeR + RSeH \quad (1)$$

where R is $H_3N^+CH_2CH_2^-$ and the asterisk serves to label otherwise identical R groups. We also report the results of a study of the thiol/disulfide exchange reaction of the analogous sulfur compounds for comparison. Rate constants were determined for

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the symmetrical reactions by dynamic NMR techniques: The rate constant for the selenol/diselenide reaction was determined by line shape analysis of exchange-broadened and averaged spectra while the rate constants for the thiol/disulfide reaction were determined by the inversion-transfer method.

Although the kinetics and mechanism of thiol/disulfide exchange reactions have been the subject of a number of studies,[5-8] the present paper, as far as we can determine, is the first report of a study of the kinetics of selenol/diselenide exchange reactions. The results indicate that selenol/diselenide exchange reactions are orders of magnitude faster than the analogous thiol/disulfide exchange reactions.

Experimental Section

Chemicals and Solution Preparation. Selenocystamine dihydrochloride was used as received from Trans World Chemicals. Selenocysteamine solutions were prepared by reduction of selenocystamine in D_2O solution containing 1 mM EDTA and 0.2 M KCl at pD 7.4 over a Hg pool cathode set to −1.0 V vs the saturated calomel electrode.[9] The reductions were carried out in an H-cell with the Hg pool cathode (2.3 cm^2): the reference electrode and the RSeSeR solution in one compartment and saturated KCl solution and a platinum counter electrode in the second compartment. The two compartments were separated by a glass frit. A Princeton Applied Research Model 174 polarographic analyzer was used as the voltage source. Solutions were continuously bubbled with N_2 or Ar before, during, and after electrolysis. Progress of the reduction was monitored by ^1H NMR; as discussed below, exchange between the selenolate and diselenide forms is fast on the ^1H NMR time scale at pD 7.4 and thus the exchange-averaged chemical shift δ_{obs} provides a direct measure of the extent of reduction[10]

$$\delta_{obs} = f_{RSeH}\delta_{RSeH} + f_{RSeSeR}\delta_{RSeSeR} \quad (2)$$

where δ_{RSeH} and δ_{RSeSeR} are the chemical shifts and f_{RSeH} and f_{RSeSeR} the fractional concentrations of the selenol and diselenide forms. f_{RSeH} = [RSeH]/([RSeH] + 2[RSeSeR]) and f_{RSeSeR} = 2[RSeSeR]/([RSeH] + 2[RSeSeR]). When solutions for kinetic experiments were prepared, reduction was stopped when 40–60% of the diselenide was reduced, typically after 45–75 min of electrolysis. The partially reduced solutions were kept under an atmosphere of N_2 or Ar, and the pD was decreased from 7 to 2 with 4 M HCl. The pD was measured, and a 750-μL aliquot was withdrawn for ^1H NMR measurements. The pD was then increased with 5 M NaOH, and samples were withdrawn for NMR measurements every 0.5 pD unit. The HCl and NaOH solutions were stored in polyethylene bottles and were added to the selenocysteamine/selenocystamine solutions with use of a Mettler DV11 autotitrator. All NMR tubes were flushed with N_2 or Ar prior to the addition of sample solution.

Cysteamine hydrochloride (Sigma Chemical Co.) and cystamine dihydrochloride (Aldrich Chemical Co.) were used as received. Solutions containing cysteamine, cystamine, or a mixture of the two were prepared in D_2O that had been degassed by bubbling with N_2. Solutions were bubbled with N_2 as the pD was adjusted with HCl or NaOH in D_2O, and samples were withdrawn into NMR tubes that had been flushed with N_2 or Ar.

pH measurements were made at 25 °C with Orion Model 701A and EA920 pH meters equipped with Fisher Scientific Accu-pHast double-junction pH electrodes. The outer reference electrode compartment was filled with saturated KCl solution. The pH meters were calibrated with Fisher Scientific certified pH 4.00, 7.00, and 10.00 buffer solutions. pH meter readings for D_2O solutions were corrected for deuterium isotope effects with the relation pD = pH meter reading + 0.40.[11]

NMR Measurements. ^1H and ^{13}C NMR spectra were obtained at 500 and 125 MHz, respectively, with a Varian VXR-500 spectrometer. The probe temperature was 25 °C for ^1H measurements and 26.5 °C for ^{13}C measurements. ^1H chemical shifts were measured relative to the methyl resonance of *tert*-butyl alcohol (TBA) and are reported relative to 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). ^{13}C chemical shifts were measured relative to internal dioxane and are reported relative to tetramethylsilane. ^{13}C spectra were measured with Waltz decoupling to minimize sample heating. Rate constants for the cysteamine/cystamine exchange reaction were determined by both ^1H and ^{13}C NMR using the inversion-transfer method.[12,13] In this method, the resonance of one chemical species, e.g. B, of

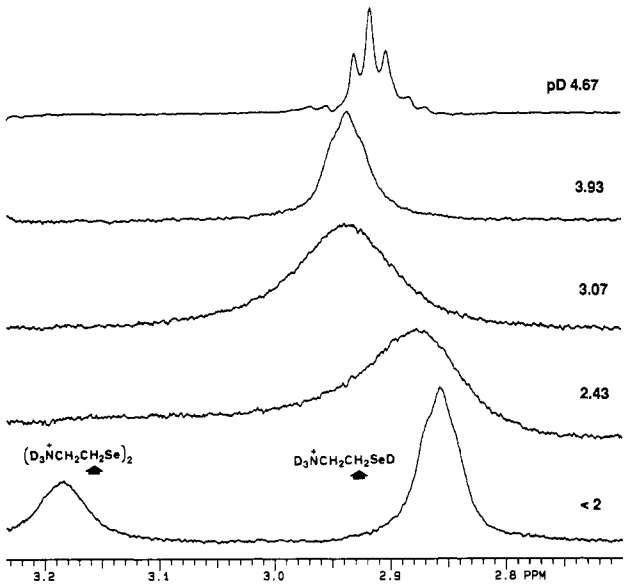

Figure 1. ^1H NMR spectra of a solution containing 2.9 mM selenocystamine and 14.2 mM selenocysteamine as a function of pD. Only the resonances for the methylene protons adjacent to the selenium are shown.

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B \quad (3)$$

is selectively inverted, and transfer of inversion to the resonance of another chemical species with which B is in equilibrium is monitored. The following pulse sequence was used[14,15]

$$\pi/2(x)-\tau_1-\pi/2(-x)-\tau_2-\pi/2(x,y,-x,-y)\text{–acquisition}$$

where τ_1 is a fixed delay equal to $1/(2\Delta\nu)$ where $\Delta\nu = |\nu_A - \nu_B|$. τ_2 is a variable delay time during which transfer of inversion occurs; τ_2 values ranging from 0.0001 s to 5 times the longest T_1 of the two resonances were used. Spectra were measured at 17 or 18 τ_2 values in each experiment. When the resonance for B is to be inverted, the carrier is set on the resonance for A.

The exchange rate constant was extracted from the inversion-transfer data by equations relating the dependence of the intensity of the resonance for A on τ_2 to pseudo-first-order rate constants for the exchange reaction at equilibrium.[14] Data were fit to eq 4 of ref 14 by nonlinear least-squares regression analysis. Regression analysis, done on an Apple II Plus computer using the program NLLSQ,[16] gave values for the set of parameters ϕ_1, ϕ_2, λ_1, and λ_2 (defined in ref 14). The pseudo-first-order rate constant k_{-1}, defined by eq 3, was then calculated from λ_1 and λ_2 with eq 4, where T_1^A and T_1^B are spin-lattice relaxation times measured

$$k_{-1} = \frac{\lambda_1 + \lambda_2 - 1/T_1^A - 1/T_1^B}{1 + K_e} \quad (4)$$

at the pH and concentrations used in the inversion-transfer experiment and K_e = [B]/[A]. Thiol/disulfide exchange is a second-order reaction

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as discussed in the next section. The second-order exchange rate constant k was calculated from k_{-1} by the relationship $2k_{-1} = k$[RSH] or $k_{-1} = k$[RSSR], depending on whether the resonance for RSSR or RSH was inverted.

^{1}H spin-lattice relaxation times were determined at 25.0 °C for separate solutions containing 0.10 M RSH or 0.05 M RSSR in D_2O at the same pD values as used in the inversion-transfer experiments. The standard inversion recovery pulse sequence[17] was used with 90° and 180° pulse widths ranging from 15 to 18 and 30 to 36 μs, respectively. ^{13}C spin-lattice relaxation times were measured at 27.0 °C for separate solutions containing 0.20 M RSH or 0.10 M RSSR at pD 11.2.

Determination of pK_A Values. The acid dissociation constants for cysteamine (0.10 M) and cystamine (0.05 M) in D_2O were determined by ^{1}H NMR. Chemical shifts of the two triplets for each compound were measured vs pD over the pD range 6–13. pK_A values were obtained by fitting the chemical shift data for both compounds to a diprotic acid model[18] using the nonlinear least-squares program KINET.[19] The values obtained for cysteamine in D_2O are pK_{A1}(SD) = 8.67 ± 0.01 and pK_{A2}(ND_3^+) = 11.43 ± 0.01; for cystamine in D_2O, pK_{A1} = 9.39 ± 0.01 and pK_{A2} = 10.22 ± 0.01. pK_A values have been reported previously for cysteamine[20] and cystamine[21] in H_2O solution; however, no values are available for comparison with the values reported here for D_2O solution.

Results

Selenol/Diselenide Exchange Kinetics. ^{1}H NMR spectra of mixtures of RSeH and RSeSeR consist of two separate multiplets for the two methylene groups of each compound at pD <2. As the pD is increased above 2, the resonances coalesce and then the coalesced resonances sharpen up as the pD is increased further. To illustrate, spectra are shown in Figure 1 for the methylene groups adjacent to the selenium of RSeH and RSeSeR. At pD <2, separate resonances are observed; however, even at this low pD, they are exchange broadened. At higher pD, the two resonances broaden further (pD 2.43), coalesce, and eventually give a single, exchange-averaged triplet. Also, as the pD is increased, the chemical shift of the exchange-averaged resonance changes because the resonance for the selenol forms shifts due to titration of the selenol group; for selenocysteamine, pK_A(SeD) = 5.50 ± 0.02 and pK_A(ND_3^+) = 11.45 ± 0.02.[9]

The spectra in Figure 1 indicate that the rate of selenol/diselenide exchange increases as the pD is increased, which is consistent with the deprotonated selenolate, $D_3N^+CH_2CH_2Se^-$, being the reactive form in the exchange reaction.

$$R^*Se^- + RSeSeR \underset{}{\overset{k}{\rightleftharpoons}} R^*SeSeR + RSe^- \qquad (5)$$

In the analogous thiol/disulfide exchange reactions, it is well established that the deprotonated thiolate, RS^-, is the reactive thiol species.[5b,7a,22-25]

The rate constant for the reaction in eq 5 was determined by analysis of exchange-broadened spectra measured as a function of pD for solutions containing initially 2, 5, and 10 mM diselenide. The procedure involved matching experimental and computer-simulated spectra to determine preexchange lifetimes for RSeSeR and RSeH, from which the rate constant was calculated. Exchange-broadened spectra were simulated by Bloch equations modified for chemical exchange.[26] The resonances for the methylene groups adjacent to selenium were used. In the absence of chemical exchange, the chemical shift of the resonance for these protons of RSeSeR is constant over the pD range used (pD 2–7; pK_{A1}(ND_3^+) = 9.65 ± 0.03 and pK_{A2}(ND_3^+) = 10.77 ± 0.11[10]),

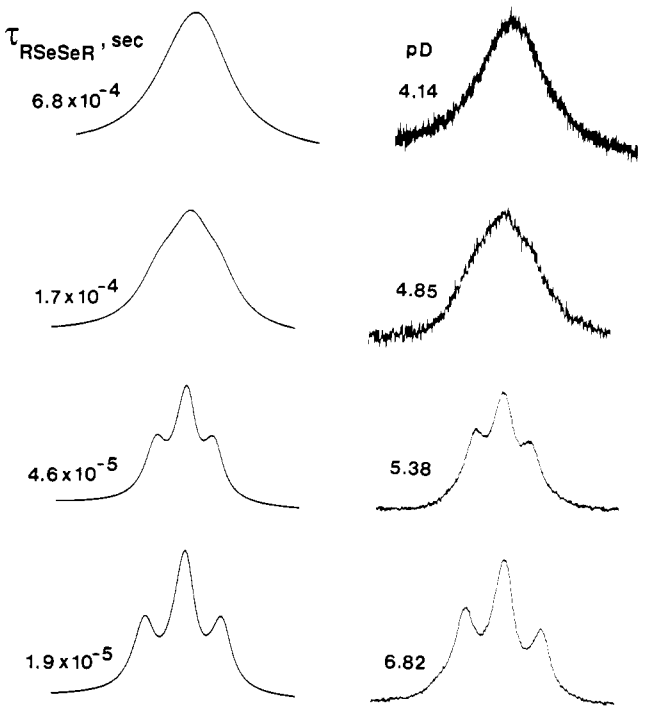

Figure 2. Experimental and simulated ^{1}H NMR spectra for the methylene protons adjacent to the selenium in solutions containing 1.92 mM selenocysteamine and 1.04 mM selenocystamine. Averaged resonances are observed for these protons due to fast selenol/diselenide exchange.

while the chemical shift of RSeH is a function of pD, shifting from 2.854 ppm at pD <2 to 2.575 ppm at pD 7.4 due to titration of the selenol group. The chemical shift of RSeH was obtained from the slow-exchange spectrum of a mixture of selenol and diselenide, while the chemical shift of RSe^- was determined from the spectrum obtained after reduction of 10 mM RSeSeR for 22 h at pD 7.4. This chemical shift was confirmed by measuring the spectrum for selenol produced by reduction of the diselenide with $NaBH_4$. The chemical shift used for the selenol in simulating spectra at a particular pD was calculated from the chemical shifts of the RSeH and RSe^- forms and pK_A(SeD) using eq 2. The chemical shift difference, $\Delta\nu$, between the centers of the triplets for the selenol and diselenide forms varies from 155.0 to 293.8 Hz over the pD range (3.07–7.57) where spectra were simulated. Coupling constants of 7.2 and 7.0 Hz were used for the selenol and diselenide triplets in the spectral simulations.

A typical series of matched experimental and computer-simulated spectra are shown in Figure 2. The solution used in these experiments had an initial RSeSeR concentration of 2mM. The solution was reduced until the chemical shift of the exchange-averaged resonance indicated 58% of the RSeSeR was converted to selenol, and then spectra were taken as the pD was adjusted.

RSeSeR lifetimes were obtained by matching simulated and experimental spectra measured over a wide pD range; for initial RSeSeR concentrations of 2, 5, and 10 mM, the pD ranges used were 4.14–7.57, 4.04–4.88, and 3.07–4.67, respectively. Because the reaction is second order, the maximum pH at which lifetimes could be determined from exchange-averaged spectra decreased as the concentration increased.

A value was calculated for the rate constant for the exchange reaction defined by eq 5 from each lifetime measured for RSeSeR, τ_{RSeSeR}, using the relation in eq 6.[27] The concentration of RSe^-

$$k = 1/\tau_{RSeSeR}[RSe^-] \qquad (6)$$

was calculated from the total concentration of the selenol form with pK_A(SeD). The average rate constants obtained from experiments done at the three different initial concentrations of

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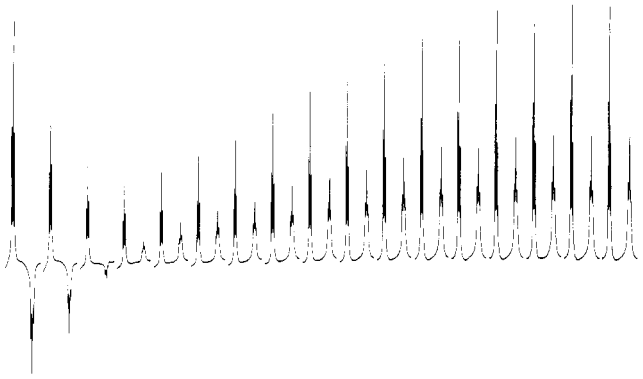

Figure 3. ^{1}H NMR spectra measured by the inversion-transfer pulse sequence for a pD 10.87 solution containing a 0.20 M cysteamine and 0.10 M cystamine. The resonances shown are for the methylene protons adjacent to sulfur; the resonance for cysteamine was inverted. In the pulse sequence, τ_1 was set to 0.005 35 s and τ_2 had values of (from left to right in the figure) 0.0001, 0.1, 0.3, 0.6, 0.9, 1.2, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 15, and 20 s.

RSeSeR are as follows: 2 mM, $k = 1.80\ (\pm 0.48) \times 10^7$ L/mol·s ($n = 6$); 5 mM, $k = 1.55\ (\pm 0.20) \times 10^7$ L/mol·s ($n = 4$); 10 mM, $k = 1.81\ (\pm 0.32) \times 10^7$ L/mol·s ($n = 4$). The overall average is $1.65\ (\pm 0.16) \times 10^7$ L/mol·s.

Thiol/Disulfide Exchange Kinetics. In contrast to the dynamic effects observed in ^{1}H NMR spectra of mixtures of selenocysteamine and selenocystamine, ^{1}H NMR spectra of solutions containing cysteamine and cystamine consist of separate, sharp resonances for the two compounds, with no evidence of any exchange broadening from pD 1 to 13 at concentrations up to at least 0.2 M. Similar results have been obtained for other thiol/disulfide pairs.[5e,8,10,28,29] We have discovered, however, that the rates of thiol/disulfide exchange are fast enough for measurement by spin-transfer techniques.[30] In this study, we used the inversion-transfer method to measure the rate constant for cysteamine/cystamine exchange as a function of pD.

$$\mathrm{R^*SH + RSSR} \underset{}{\overset{k}{\rightleftharpoons}} \mathrm{R^*SSR + RSH} \qquad (7)$$

The procedure involved measuring a series of spectra as a function of τ_2 in the inversion-transfer pulse sequence, as described in the Experimental Section. The triplet resonance for one of the methylene groups of RSH or RSSR was selectively inverted, and transfer of inversion to the corresponding methylene protons of RSSR or RSH, respectively, was measured.

A typical series of inversion-transfer spectra are shown in Figure 3; in this experiment, the resonance for the CH_2S protons of cysteamine was inverted. At the pD (10.87) of the experiment shown in Figure 3, $\Delta\nu = 130.1$ Hz. The integrated intensity obtained for the cystamine resonance from another experiment is plotted vs τ_2 in Figure 4; the smooth curve through the points is the theoretical curve calculated with the parameters derived from the nonlinear least-squares regression analysis of the data. Rate constants, measured as a function of pD, together with the spin-lattice relaxation times measured at the same pD values by the inversion-transfer method, are presented in Table I.[31]

Since the resonances used for the inversion-transfer experiment are multiplet patterns as a result of homonuclear scalar coupling, several experiments were performed to determine whether the intensity of the resonance being observed was affected by nuclear Overhauser effects or multiple quantum effects.[32] In one set of

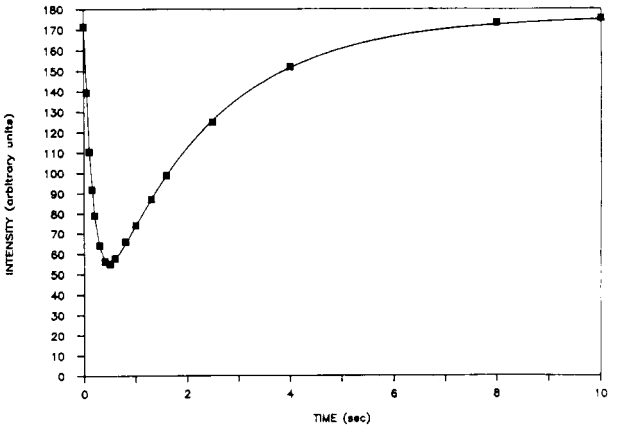

Figure 4. Integrated intensity of the resonance for the methylene protons adjacent to the sulfur of cystamine as a function of the delay time τ_2 in an inversion-transer experiment on a solution containing 0.10 M cysteamine and 0.05 M cystamine at pD 9.40. The smooth curve through the points is the theoretical curve calculated from the parameters obtained by nonlinear regression analysis.

Table I. Rate Constants for Cysteamine/Cystamine Exchange and Spin-Lattice Relaxation Times for Cysteamine and Cystamine as a Function of pD[a]

pD	k,[b,c] L/mol·s	cysteamine T_1,[d,e] s		cystamine T_1,[e,f] s	
		NCH_2	CH_2S	NCH_2	CH_2S
7.43	3.6	6.36	6.54	2.09	1.92
7.83	7.8	6.11	6.25	2.14	1.97
8.20	16	6.16	6.27	2.17	1.98
8.61	30	6.09	6.06	2.19	1.98
9.02	46	5.34	5.23	2.20	2.05
9.40	52	3.86	3.64	2.23	2.14
9.83	44	3.49	3.30	2.35	2.24
10.09	38	3.40	3.22	2.46	2.32
10.40	30	3.22	3.21	2.52	2.27
10.74	20	3.26	3.41	2.41	2.34
11.04	16	3.14	3.30	2.57	2.53
11.65	17	2.96	3.13	2.34	2.50
12.58	22	2.80	2.98	2.71	2.66
13.63	24	2.74	2.96	2.76	2.66

[a] 25 °C. [b] 0.10 M cysteamine and 0.05 M cystamine. [c] Relative uncertainties are estimated to be ±3%. [d] 0.10 M cysteamine. [e] Relative uncertainties are estimated to be ±3%. [f] 0.05 M cystamine.

experiments, spectra were measured by the inversion-transfer pulse sequence for solutions containing only cysteamine or cystamine. The carrier was set on the CH_2S resonance, τ_1 was set to the value used to achieve inversion of the CH_2S resonance of the exchange partner in the identical experiment where both cysteamine and cystamine were present in solution, and τ_2 was set to the same values used to determine the rate constant in the inversion-transfer experiment. It was found that the integrated intensity of the observed resonance showed a small and apparently random variation; typically the relative standard deviation of the average integrated intensity was 1.4%. In another experiment, the rate constant for cysteamine/cystamine exchange was measured by the inversion-transfer method using ^{13}C NMR;[33] because of the low natural abundance of ^{13}C, multiple quantum transitions are negligible. The experiment was performed at pD 10.17 and 27.0 °C, with a cysteamine concentration of 0.183 M and a cystamine concentration of 0.108 M. The resonance for the methylene carbon

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(32) The pulse sequence used here for the inversion-transfer experiment is similar to that used in multiple quantum NMR: Mareci, T. H. In *Pulse Methods in 1D and 2D Liquid Phase NMR*; Brey, W. S., Ed.; Academic Press: New York, 1988; pp 259–342.
(33) ^{13}C resonances were assigned on the basis of literature data (Pretsch, E.; Seibl, J.; Simon, W.; Clerc, T. *Tables of Spectral Data for Structure Determination of Organic Compounds*; Springer-Verlag: New York, 1983) and the results of a ^{13}C 2-dimensional exchange spectroscopy (EXSY) experiment (Huang, V.; Macura, S.; Ernst, R. R. *J. Am. Chem. Soc.* **1981**, *103*, 5327).

bonded to the sulfur of cysteamine was inverted.[33] A value of 44 L/mol·s was obtained for the rate constant. Considering the difference in temperature, this rate constant is in good agreement with the results in Table I. The results of these two experiments indicate that nuclear Overhauser effects and multiple quantum transitions have a negligible effect on the rate constants determined by ^{1}H NMR for the cysteamine/cystamine exchange.

Discussion

The first observations that the kinetics of selenol/diselenide exchange reactions are faster than those of thiol/disulfide exchange reactions came from ^{1}H NMR spectra of solutions containing selenol/diselenide and thiol/disulfide mixtures.[10,34] For example, ^{1}H NMR spectra of solutions containing $HOCH_2CH_2SeH$ and $(HOCH_2CH_2Se)_2$ consist of separate, exchange-broadened resonances for both compounds at low pH (<4–5, depending on concentrations).[10] At higher pH, resonances from the two compounds coalesce into exchange-averaged resonances, which sharpen as the pH is increased. In contrast, ^{1}H NMR spectra of mixtures of the analogous sulfur compounds consist of sharp, separate resonances for both compounds over the entire pH range, with no evidence of line broadening due to thiol/disulfide exchange. Qualitatively, the results obtained in the present study are identical for $D_3N^+CH_2CH_2SeD/(D_3N^+CH_2CH_2Se)_2$ and the analogous sulfur compounds; however, we have now quantitatively characterized the much faster kinetics for selenol/diselenide exchange with these two systems.

The rate of RSeH/RSeSeR exchange is so fast that it can be measured by ^{1}H NMR only at pD <7.6, depending on concentration. At pD <7.6, the amino groups of both RSeH and RSeSeR are protonated. Thus, the rate constant determined in this work is for the reaction of $D_3N^+CH_2CH_2Se^-$ with $(D_3N^+CH_2CH_2Se)_2$. However, over the higher pD range used to measure the rate of the RSH/RSSR exchange reaction, the ammonium groups of RSH and RSSR are titrated. Thus, the rate constants in Table I are pD dependent due to changes in species present as the pD is increased from 7.43 to 13.63. Considering the species present, the possible reactions are $D_3N^+CH_2CH_2S^-$ reacting with $D_3N^+CH_2CH_2SSCH_2CH_2ND_3^+$, $D_3N^+CH_2CH_2SSCH_2CH_2ND_2$, and $D_2NCH_2CH_2SSCH_2CH_2ND_2$ (rate constants k_1–k_3, respectively) and $D_2NCH_2CH_2S^-$ reacting with the same disulfide species (rate constants k_4–k_6, respectively). The observed rate constants in Table I are a function of

$$k_{obs} = k_1\alpha_1\alpha_0' + k_2\alpha_1\alpha_1' + k_3\alpha_1\alpha_2' + k_4\alpha_2\alpha_0' + k_5\alpha_2\alpha_1' + k_6\alpha_2\alpha_2' \quad (8)$$

where α_1 and α_2 are the mole fractions of cysteamine present as $D_3N^+CH_2CH_2S^-$ and $D_2NCH_2CH_2S^-$, respectively, and α_0', α_1', and α_2' are the mole fractions of cystamine present in the di-, mono-, and nonprotonated forms. Individual rate constants were calculated from the observed rate constants in Table I by least-squares regression analysis. The values used for α_1, α_2, α_0', α_1', and α_2' were calculated from the acid dissociation constants determined for cysteamine and cystamine in D_2O. Because rate constants k_2 and k_4 and rate constants k_3 and k_5 are related by the principle of microscopic reversibility and thus highly correlated, it was not possible to determine all six rate constants. When the terms involving rate constants k_3 and k_4 are dropped from eq 8, the results obtained by regression analysis are $k_1 = 68 \pm 2$ L/mol·s; $k_2 = 61 \pm 2$ L/mol·s; $k_5 = 122 \pm 24$ L/mol·s; and $k_6 = 23 \pm 1$ L/mol·s. The observed rate constants are plotted vs pD in Figure 5; the solid curve through the points is the theoretical curve predicted with these values for k_1, k_2, k_5, and k_6. Rate constants k_1 and k_6 were also calculated independently from the observed rate constants at the low- and high-pH ends, respectively, since the α values for cysteamine and cystamine indicate that interchange in these pH regions is completely by the k_1 and k_6 processes. The values obtained are $k_1 = 67$ L/mol·s and 64 L/mol·s at pD 7.43 and 7.83, respectively, and $k_6 = 24$ L/mol·s at both pD 12.58 and 13.63, in good agreement with the values obtained by regression analysis on the entire set of observed rate constants.

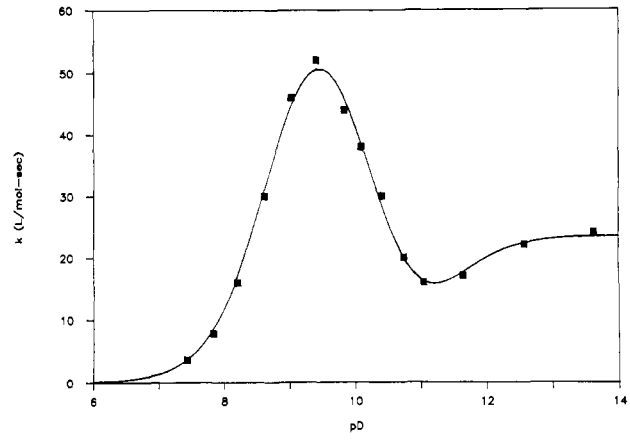

Figure 5. Observed rate constant for cysteamine/cystamine exchange as a function of pD. The smooth curve through the experimental values is the theoretical curve predicted by eq 9 and the values given in the text for k_1, k_2, k_5, and k_6.

The values obtained for rate constants k_2 and k_5 from the regression analysis are upper limits, since they are too large if the k_4 and k_3 processes, respectively; contribute significantly to interchange. Applying the principle of microscopic reversibility, the term $k_4[RS^-][R^+SSR^+]$ equals that portion of the term $k_2[R^+S^-][R^+SSR]$ in which R^+S^- displaces RS^- from R^+SSR. If this is the dominant pathway in the reaction of R^+S^- with R^+SSR, the calculated value for k_2 is too large by a factor of 2 and $k_4 = k_2K_{A1}^{RSSR}/K_{A2}^{RSH}$. If, however, the displacement of R^+S^- from R^+SSR by R^+S^- is the dominant pathway, the calculated value for k_2 is true value. If the k_2 reaction proceeds by both pathways, the actual value for k_2 will lie somewhere between the two limits. Similarly, the term $k_3[R^+S^-][RSSR]$ equals that portion of the term $k_5[RS^-][R^+SSR]$ in which RS^- displaces the leaving group R^+S^- from R^+SSR. Depending on the fraction of the k_5 process that proceeds by this pathway, k_5 will be either too large by a factor of 2 and $k_3 = k_5K_{A2}^{RSH}/K_{A2}^{RSSR}$, the value calculated above for k_5 is the true value, or the actual value for k_5 is somewhere between those two limits.

Rate constant k_1 is for the cysteamine/cystamine exchange reaction involving species with the same protonation states as in the selenocysteamine/selenocystamine reaction. The two rate constants indicate that the selenol/diselenide exchange reaction is 2.4×10^5 times faster.

Thiol/disulfide exchange is a mechanistically simple S_N2 displacement reaction, with nucleophilic attack occurring along the sulfur–sulfur bond axis of the disulfide group.[5,35,36] Presumably selenol/diselenide exchange is by a similar mechanism. Thus, it is to be expected that selenol/diselenide exchange will be faster than thiol/disulfide exchange; because of their higher polarizability, selenolates are both better nucleophiles and better leaving groups than the corresponding thiolates.[37,38] Also, the energy of the Se–Se bond (46 kcal/mol) is considerably less than that of the S–S bond (64 kcal/mol).[39] Although it is not possible to determine the relative contributions of these factors to the faster selenol/diselenide exchange, the results indicate that alkane selenolates are much better nucleophiles and leaving groups than the corresponding alkane thiolates.

It is of interest to consider these results with reference to the catalytic activity of glutathione peroxidase. The rate constants for selenol/diselenide and thiol/disulfide exchange involving similarly protonated species differ by a factor of 2.4×10^5.

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However, the difference in effective rate constants at pD 7 is even larger due to the greater basicity of the thiolate anion. Thus, at pD 7, thiol groups are predominantly present in the RSH form, whereas selenol groups are essentially completely ionized to RSe^-. When this is accounted for, the conditional rate constant for cysteamine/cystamine exchange at pD 7 is 1.4 L/mol·s as compared to 1.65×10^7 L/mol·s for selenocysteamine/selenocystamine exchange. Thus, at physiological pH, the rates differ by a factor of 1.2×10^7. Although, the catalytic activity of glutathione peroxidase does not involve selenol/diselenide exchange, these results indicate that, because of the combined effects of selenolate being both a better nucleophile and a better leaving group and RSeH being completely deprotonated at physiological pH, the nucleophilic displacement reactions that do occur are much faster than if sulfur were at the active site.

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Fluoride Ion Coordination Chemistry of Iron(II) Porphyrins: Unique Spectral Properties and Associated Dioxygen Activation Chemistry of the Fluoroiron(II) Complex

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Abstract: Fluoroiron(II) porphyrin anionic complexes are generated in a variety of organic solvents by addition of tetrabutylammonium fluoride to the respective square-planar iron(II) porphyrin. The paramagnetic fluoroiron(II) tetraphenylporphyrin pyrrole proton NMR chemical shift value of 30.3 ppm resembles that for the corresponding hydroxide ion complex and is consistent with a high-spin iron(II) configuration. This even-spin complex surprisingly exhibits a broad 750 G X-band EPR signal at liquid helium temperature. Such a signal is expected only for a complex with a low-symmetry ligand field component. A very large Mössbauer quadrupole splitting parameter of 4.06 mm/s (78 K) serves to demonstrate further the strong ligand field perturbation by an axial fluoride ligand. The five-coordinate fluoroiron(II) porphyrin complex also exhibits unique reactivity with dioxygen, in that the detectable products at ambient temperature are the peroxoiron(III) porphyrin and the difluoroiron(III) porphyrin anionic complexes. This observation is in contrast to the usual dioxygen conversion of simple iron(II) porphyrins to the dinuclear oxo-bridged iron(III) porphyrin.

A number of spectroscopic studies have provided evidence for anion coordination to iron(II) porphyrins contained in nonaqueous solution.[1] In this regard, Kadish and Rhodes were the first to detect fluoride ion binding to square-planar iron(II) porphyrins through electrochemical measurements.[2] Fluoride ion coordination serves to modulate iron porphyrin redox potentials[2] and to dictate formation of novel high-valent metalloporphyrin complexes.[3] This report details in part the unique spectroscopic properties of fluoroiron(II) tetraphenylporphyrin, (TPP)FeF^-, and fluoroiron(II) octaethylporphyrin, (OEP)FeF^-, complexes in terms of an anomalously large iron-57 Mössbauer quadrupole splitting parameter and an EPR detectable signal for the even-spin iron(II) compound.

The novel dioxygen reaction chemistry of the fluoroiron(II) porphyrin anion is also described here. The generally low-temperature reversible reaction of dioxygen with iron(II) porphyrins has been intensively studied in order to model the dioxygen binding properties of hemoglobin and myoglobin.[4] The competing autoxidation chemistry of simple iron(II) porphyrins has also been the subject of mechanistic studies.[5] In nonaqueous solution at ambient temperature simple iron(II) porphyrins react with dioxygen to yield ultimately the dinuclear μ-oxoiron(III) porphyrin, PFe–O–FeP. A likely pathway for μ-oxo formation involves initial appearance of a μ-peroxoiron(III) dimer with subsequent homolytic scission of the O–O bond. The intermediate μ-peroxo

$$PFe^{II} + O_2 \rightleftharpoons PFe^{III}O_2 \xrightarrow{PFe^{II}} PFe^{III}\text{-}O\text{-}O\text{-}Fe^{III}P \longrightarrow$$

$$2PFe^{IV}(O) \xrightarrow{2PFe^{II}} 2PFe^{III}\text{-}O\text{-}Fe^{III}P$$

species, PFe^{III}–O–O–Fe^{III}P, have been detected and characterized by ^{1}H NMR and electronic spectroscopic methods in the temperature range −80 to −20 °C.[6] Increasing temperature or addition of amines causes homolytic cleavage of the μ-peroxo bridge to yield monomeric oxoiron(IV) porphyrin derivatives detectable at low temperature.[7]

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