MHz, $CDCl_3$) δ -4.1, -3.8, 11.2, 18.5, 19.4, 26.3, 32.2, 33.3, 34.9, 37.5, 38.3, 39.4, 55.1, 55.7, 67.7, 69.3, 71.3, 72.7, 73.3, 80.5, 94.9, 104.2, 114.2, 127.9, 128.7, 130.0, 130.4, 138.9, 159.6; $[\alpha]_D$ +25.2° (c 1.10, $CHCl_3$); HRMS calcd for $C_{32}H_{49}O_7Si$ (M - t-Bu) m/z 573.3248, found m/z 573.3232.

(2*S*,3*R*)-2-[(1*R*,2*R*)-4-(Benzyloxy)-2-[(*tert*-butyldimethylsilyl)oxy]-1-methylbutyl]-3-[(2*R*)-3-hydroxy-2-methylpropyl]-5-methoxy-1,2,3,4-tetrahydrofuran (48). To a solution of ether **47** (0.005 g, 0.01 mmol) in 0.5 mL of CH_2Cl_2 in a 2-mL round-bottomed flask at 0 °C was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.003 g, 0.01 mmol).[12] The resulting yellow-green solution was stirred 20 h at 0 °C and then was quenched with saturated $NaHCO_3$ solution and extracted with CH_2Cl_2. The combined extracts were dried over Na_2SO_4 and concentrated to give a yellow oil. Purification by silica gel flash column chromatography (hexanes–EtOAc, 8:2) gave recovered ether **47** (0.001 g, 20%) and tetrahydrofuran **48** (0.002 g, 42%) as a colorless liquid: ^1H NMR (500 MHz, $CDCl_3$) δ 0.01 (s, 3 H, $SiCH_3$), 0.03 (s, 3 H, $SiCH_3$), 0.85 (s, 9 H, $C(CH_3)_3$), 0.88 (d, 3 H, J = 6.8 Hz, $CHCH_3$), 0.94 (d, 3 H, J = 6.7 Hz, $CHCH_3$), 1.23 (s, 1 H, OH), 1.4–2.0 (m, 8 H, CH_2 and CH), 2.25 (m, 1 H, CH), 3.32 (s, 3 H, OCH_3), 3.35 (m, 2 H, $BnOCH_2$), 3.47 (dd, 2 H, J = 6.2, 6.1 Hz, $HOCH_2$), 3.83 (ddd, 1 H, J = 6.6, 6.4, 2.0 Hz, OCH), 4.00 (dd, 1 H, J = 7.8, 5.4 Hz, OCH), 4.47 (s, 2 H, OCH_2Ar), 4.97 (dd, 1 H, J = 5.3, 3.5 Hz, $CHOCH_3$), 7.31 (m, 5 H, phenyl); IR (neat) ν 3479 (m), 2930 (s), 2857 (s), 1462 (m), 1361 (m), 1252 (m), 1098 (s), 1040 (s), 985 (m), 869 (w), 836 (m), 774 (m), 698 (w) cm^{-1}; $[\alpha]_D$ +38.0° (c 0.20, $CHCl_3$); HRMS calcd for $C_{26}H_{45}O_4Si$ (M - OCH_3) m/z 449.3087, found m/z 449.3072. The infrared spectrum, rotation, and ^1H NMR spectrum were virtually identical to those of material prepared by K. C. Nicolaou.

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Supplementary Material Available: Experimental procedures for **27–37** and selected ^1H NMR spectra (49 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Kinetics and Equilibria of Thiol/Disulfide Interchange Reactions of Selected Biological Thiols and Related Molecules with Oxidized Glutathione

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Rate constants for reaction of coenzyme A and cysteine with oxidized glutathione (GSSG) and equilibrium constants for the reaction of coenzyme A; cysteine, homocysteine, cysteamine, and related thiols with GSSG by thiol/disulfide interchange were determined over a range of pD values by NMR spectroscopy. The rate constants for reaction of the thiolate anion forms of coenzyme A and cysteine with GSSG suggest that reduction of GSSG by coenzyme A and cysteine is a mechanistically uncomplicated S_N2 reaction. Equilibrium constants for the thiol/disulfide interchange reactions show a strong dependence on the Bronsted basicity of the thiolate anion. In a similar way, $\Delta E^{\circ\prime}$, the difference between the half-cell potentials for the RSSR/RSH and GSSG/GSH redox couples, is linearly dependent on the difference between the pK_A values of RSH and glutathione: $\Delta E^{\circ\prime} = 64\Delta pK_A - 7.7$ where $\Delta E^{\circ\prime}$ is in units of mV. The reducing strength at a given pH is also determined by the fraction of the thiol present in the reactive thiolate form. At pD 7, the half-cell potentials for coenzyme A, cysteine, homocysteine, and cysteamine are close to that of glutathione, the major intracellular thiol redox system, which suggests that small changes in the intracellular redox potential can cause significant changes in the intracellular distribution of these biological thiols between their reduced and oxidized forms.

Introduction

Thiol/disulfide interchange reactions are important in maintaining the intracellular distribution of glutathione, coenzyme A, cysteine, and other thiols among their reduced and oxidized forms.[1] Glutathione is the most abundant nonprotein thiol in most cells, and the glutathione/oxidized glutathione (GSH/GSSG) pair forms the major intracellular thiol redox system.[2] The concentration of GSH in cells ranges between 0.5 and 10 mM,[3] and in most cells it is maintained largely in the form of free GSH by the activity of GSSG reductase (E.C. 1.6.4.2).[2,4] Glutathione participates in maintenance of the thiol/disulfide distribution of other thiol-containing molecules by reduction of symmetrical and mixed disulfides by GSH and oxidation of thiols by GSSG via thiol/disulfide interchange.

Thiol/disulfide interchange (eqs 1 and 2) takes place spontaneously and may also be catalyzed by thiol transferase (E.C. 2.5.1.18).[2] The distribution of intracellular

$$RSH + R'SSR' \rightleftharpoons RSSR' + R'SH \quad (1)$$

$$RSH + RSSR' \rightleftharpoons RSSR + R'SH \quad (2)$$

thiols among their thiol, disulfide, and mixed disulfide forms will depend on their half-cell potentials at the intracellular pH, the dynamics of their thiol/disulfide interchange reactions with GSH and GSSG, and the intra-

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Table I. Conditional Rate Constants for Thiol/Disulfide Exchange Reactions

RSH	R'SSR'	pD	k_{1c} (L/mol-s)	k_{-1c}[a] (L/mol-s)	k_{2c} (L/mol-s)	k_{-2c}[b] (L/mol-s)
CoASH	GSSG	6.40	0.011 ± 0.004	6.0 (±1.9) × 10^{-3}		
CoASH	CoASSG	6.55			3.0 (±0.7) × 10^{-3}	8.0 (±2.2) × 10^{-3}
CoASH	GSSG	6.66	0.022 ± 0.008	0.012 ± 0.004		
CoASH	CoASSG	6.90			4.9 (±2.1) × 10^{-3}	0.013 ± 0.006
CoASH	GSSG	7.07	0.062 ± 0.007	0.034 ± 0.005		
CoASH	GSSG	7.14	0.077 ± 0.022	0.042 ± 0.012		
CoASH	CoASSG	7.28			9.9 (±1.9) × 10^{-3}	0.026 ± 0.006
CoASH	CoASSG	7.41			0.013 ± 0.003	0.034 ± 0.009
CySH	GSSG	4.58	5.2 × 10^{-3}	5.7 × 10^{-3}		
CySH	GSSG	5.07	7.1 × 10^{-3}	7.8 × 10^{-3}		
CySH	GSSG	5.10	7.5 × 10^{-3}	8.3 × 10^{-3}		
CySH	GSSG	5.53	2.5 × 10^{-2}	7.2 × 10^{-2}		
CySH	GSSG	6.83	2.7 × 10^{-1}	3.0 × 10^{-1}		
CySH	GSSG	7.48	8.0 × 10^{-1}	8.8 × 10^{-1}		

[a] Calculated from k_{1c} using the relationship $K_{1c} = k_{1c}/k_{-1c}$. For the CoASH/GSSG system, K_{1c} = 1.84 ± 0.11. For the CySH/GSSG system, K_{1c} = 1.1. [b] Calculated using the relationship $K_{2c} = k_{2c}/k_{-2c}$ and K_{2c} = 0.374 ± 0.049.

cellular redox potential. Half-cell potentials have been reported for only a limited number of thiol-containing molecules of biological importance,[5-14] and these have been measured by indirect methods; their direct voltammetric measurement is not feasible due to the formation of stable metal–thiolate complexes at electrode surfaces[15] and the concomitant electrochemical irreversibility. Also, in spite of the importance of thiol/disulfide interchange reactions in biological systems, the dynamics of such reactions have been characterized for only a few biological molecules.[12-14,16] Szajewski and Whitesides reported rate and equilibrium constants for thiol/disulfide interchange reactions involving oxidized glutathione.[13a] However, because of interfering reactions between amino groups of amino thiols such as cysteine and the analytical reagents used to monitor the reactions, their study was restricted to small mono- and dithio alcohols and thio carboxylic acids.

As part of a program to study the biological chemistry of sulfur, we have characterized the thiol/disulfide interchange reactions of coenzyme A, homocysteine, cysteamine, *N,N*-dimethylcysteamine, cysteine, and 2-amino-2-methyl-1-propanethiol (AMPT) with glutathione and of AMPT with 2-hydroxyethyl disulfide and 3,3'-dithiodipropionic acid. These systems were chosen for study because of their importance in biology and because their thiolate groups cover a range of Bronsted basicity. Rate constants were measured for selected reactions, and equilibrium constants were determined for all the thiol/disulfide interchange reactions by 500-MHz ^{1}H or 125-MHz ^{13}C NMR spectroscopy. Half-cell potentials were calculated for the RSSR/RSH redox systems from the equilibrium constants.

Results

Thiol/Disulfide Interchange Kinetics. Rate constants were determined by ^{1}H NMR for both steps in the overall reaction of coenzyme A with GSSG over the pD range 6.4 to 7.4 by measuring initial rates of the reaction of CoASH with GSSG and of CoASH with CoASSG, respectively. Typical concentrations were 4–6 mM CoASH,

$$\text{CoASH} + \text{GSSG} \underset{k_{-1c}}{\overset{k_{1c}}{\rightleftharpoons}} \text{CoASSG} + \text{GSH} \quad (3)$$

$$\text{CoASH} + \text{CoASSG} \underset{k_{-2c}}{\overset{k_{2c}}{\rightleftharpoons}} \text{CoASSCoA} + \text{GSH} \quad (4)$$

3 mM GSSG, and 3 mM CoASSG. The results are presented in Table I. Rate constants for the reaction of GSH with CoASSG and CoASSCoA, k_{-1c} and k_{-2c}, respectively, were calculated from k_{1c} and k_{2c} and the equilibrium constants reported in the next section using the relationships $K_{1C} = k_{1C}/k_{-1C}$ and $K_{2C} = k_{2C}/k_{-2C}$.

The rate constant for reaction of cysteine with GSSG was determined by ^{1}H NMR by measuring the initial rate of the reaction over the pD range 4.18–7.08. The CySH and GSSG concentrations were 20 and 10 mM, respectively. The results are presented in Table I.

$$\text{CySH} + \text{GSSG} \underset{k_{-1c}}{\overset{k_{1c}}{\rightleftharpoons}} \text{CySSG+} + \text{GSH} \quad (5)$$

Thiol/disulfide interchange reactions proceed via the thiolate anion.[9,12,13,14a,17] For the systems studied in this work, thiol/disulfide interchange can be described by the reaction sequence

$$\text{RSH} \rightleftharpoons \text{RS}^- + \text{H}^+ \quad (6)$$

$$\text{RS}^- + \text{GSSG}^{2-} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{RSSG}^- + \text{GS}^{2-} \quad (7)$$

$$\text{RS}^- + \text{RSSG}^- \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{RSSR} + \text{GS}^{2-} \quad (8)$$

$$\text{GSH}^- \rightleftharpoons \text{GS}^{2-} + \text{H}^+ \quad (9)$$

where k_1, k_{-1}, k_2, and k_{-2} are pD-independent rate constants.

pD-independent rate constants were calculated from the pD-dependent (conditional) rate constants in Table I. Over the pD regions used, GSH and GSSG are present predominantly in the GSH^- (CO_2^-, ND_3^+, SD) and $GSSG^{2-}$

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Table II. Thiol pK_A Values[a]

thiol	pK_A	thiol	pK_A
glutathione	9.33	3-mercaptopropionic acid	10.83[d]
coenzyme A	9.83	2-amino-2-methyl-1-propanethiol	8.78
cysteamine	8.67[b]	captopril	10.31[e]
N,N-dimethyl-cysteamine	8.38	penicillamine	8.60[f]
		cysteine	8.91[g]
homocysteine	9.65[c]		
mercapto-ethanol	10.14[d]		

[a] In D_2O solution.[18] [b] From ref 16a. [c] pK_A for the thiol group of the (ND_3^+, SD, CO_2^-) form of homocysteine. Calculated from pK_2 = 9.39 and $k(SD)/k(ND_3^+)$ = 1.74, as determined by spectrophotometry. [d] Determined by pH titration. [e] From ref 14d. [f] pK_A for the thiol group of the (ND_3^+, SD, CO_2^-) form of penicillamine. Calculated from pK_2 = 8.49 (Kadima, W. Ph.D. Thesis, University of Alberta) and $k(SD)/k(ND_3^+)$ = 3.39, as determined by spectrophotometry. [g] pK_A value for the thiol group of the (ND_3^+, SD, CO_2^-) form of cysteine. Calculated from pK_2 = 8.61 (Kadima, W. Ph.D. Thesis, University of Alberta) and pK(SD) = pK(ND_3^+) as determined by spectrophotometry.

(CO_2^-, ND_3^+) forms. The thiol groups of CoASH and CySH are predominantly in the thiol form. Therefore, the concentration of the thiolate species, and thus the conditional rate constants, will increase as the pD increases. For the CoASH/GSSG system, $k_1 = k_{1c}/\alpha_3$ and $k_2 = k_{2c}/\alpha_3$ where α_3, the fraction of CoASH in the thiolate form, is given by $K_A/(K_A + [D^+])$. Using the K_A value in Table II[18] for the thiol group of CoASH and the rate constants in Table I, $k_1 = 34 \pm 4$ L/mol-s and $k_2 = 4.2 \pm 1.1$ L/mol-s. From these values and the pD-independent equilibrium constants in Table IV, $k_{-1} = k_1/K_1 = 5.9 \pm 0.6$ L/mol-s and $k_{-2} = k_2/K_2 = 3.6 \pm 0.9$ L/mol-s. For the CySH/GSSG system, $k_1 = k_{1c}/\alpha_3$, where α_3 is the fraction of cysteine in the CyS^- (CO_2^-, ND_3^+, S^-) form. Using the K_A values for cysteine in Table II and the conditional rate constants in Table I, $k_1 = 42 \pm 15$ L/mol-s. From this value and the value for K_1 in Table IV, k_{-1} is calculated to be 7.5 ± 2.6 L/mol-s.

Thiol/Disulfide Interchange Equilibrium Constants. Conditional equilibrium constants were determined for the two steps in the reduction of GSSG by RSH (eqs 10 and 11) and for the overall reaction (eq 12) from the intensities of resonances in ^1H or ^{13}C NMR spectra of reaction mixtures which had reached equilibrium.

$$RSH + GSSG \overset{K_{1c}}{\rightleftharpoons} RSSG + GSH \quad (10)$$

$$RSH + RSSG \overset{K_{2c}}{\rightleftharpoons} RSSR + GSH \quad (11)$$

$$2RSH + GSSG \overset{K_{3c}}{\rightleftharpoons} RSSR + 2GSH \quad (12)$$

The conditional equilibrium constants are defined in eqs 13–15

$$K_{1c} = \frac{[RSSG]_{total}[GSH]_{total}}{[RSH]_{total}[GSSG]_{total}} \quad (13)$$

$$K_{2c} = \frac{[RSSR]_{total}[GSH]_{total}}{[RSH]_{total}[RSSG]_{total}} \quad (14)$$

$$K_{3c} = K_{1c}K_{2c} = \frac{[RSSR]_{total}[GSH]^2_{total}}{[RSH]^2_{total}[GSSG]_{total}} \quad (15)$$

(18) The pK_A values in Table II are for D_2O solution. Comparison with values measured in H_2O suggests that thiol pK_A values are ~0.5 pK units larger in D_2O. For example, the pK values for the thiol groups of mercaptoethanol and the (SH, NH_3^+, CO_2^-) forms of penicillamine and cysteine in H_2O are 9.62, 8.03 and 8.38, respectively (Backs, S. J.; Rabenstein, D. L. *Inorg. Chem.* 1981, *20*, 410–415) while the pK of the thiol group of glutathione in H_2O is 8.93 (Rabenstein, D. L. *J. Am. Chem. Soc.* 1973, *95*, 2797–2803).

Table III. Conditional Equilibrium Constants for the Reaction of Coenzyme A, Homocysteine, and 2-Amino-2-methyl-1-propanethiol with Oxidized Glutathione

thiol	pD	K_{1c}	K_{2c}	K_{3c}
coenzyme A[a]	5.92	1.80	0.459	0.819
coenzyme A	6.41	1.80	0.398	0.716
coenzyme A	6.92	1.68	0.333	0.560
coenzyme A	7.41	1.98	0.337	0.667
coenzyme A	7.92	1.95	0.341	0.667
coenzyme A	8.90	2.11	0.445	0.941
homocysteine[b]	6.47	3.06	0.305	0.933
homocysteine	6.95	2.54	0.241	0.612
homocysteine	7.48	2.34	0.227	0.531
homocysteine	7.95	2.27	0.244	0.554
homocysteine	8.44	2.31	0.238	0.550
AMPT[c]	5.50	0.469	0.0765	0.0359
AMPT	5.98	0.460	0.0757	0.0348
AMPT	6.42	0.466	0.0756	0.0352
AMPT	6.86	0.485	0.0809	0.0393
AMPT	7.32	0.553	0.0873	0.0485
AMPT	7.76	0.541	0.0830	0.0451
AMPT	8.32	0.716	0.0967	0.0703

[a] Values reported for the CoASH/GSSG system are the average of from 3–5 determinations. The average standard deviations for K_{1c}, K_{2c}, and K_{3c} were 3%, 5%, and 6%, respectively. [b] Values reported for the homocysteine/GSSG system are the average of three determinations. The average standard deviations for K_{1c}, K_{2c}, and K_{3c} were 8%, 14%, and 17%. [c] Values reported for the AMPT/GSSG system are the average of three determinations. The average standard deviations for K_{1c}, K_{2c}, and K_{3c} were 6%, 4%, and 10%.

where the concentrations are the total concentrations of all the protonated species for each thiol or disulfide and RSH represents coenzyme A, cysteine, homocysteine, cysteamine, *N,N*-dimethylcysteamine, and AMPT. As examples of the results, conditional equilibrium constants for the reaction of coenzyme A, homocysteine, and AMPT with GSSG are presented in Table III.

pD-independent equilibrium constants (eqs 16–18)

$$K_1 = \frac{[GS^{2-}][RSSG]}{[RS^-][GSSG^{2-}]} \quad (16)$$

$$K_2 = \frac{[GS^{2-}][RSSR]}{[RS^-][RSSG]} \quad (17)$$

$$K_3 = K_1K_2 = \frac{[GS^{2-}]^2[RSSR]}{[RS^-]^2[GSSG]} \quad (18)$$

in terms of the reactive thiolate anions were calculated from the conditional equilibrium constants, using the equations $K_1 = K_{1c}(\alpha_2/\alpha_3)$ and $K_2 = K_{2c}(\alpha_2/\alpha_3)$, where α_2 and α_3 are the fractions of GSH and RSH in the thiolate form. α_2 and α_3 are a function of pD and can be calculated as described above. The results are summarized in Table IV.

Conditional equilibrium constants were also determined for the reaction of AMPT with 2-hydroxyethyl disulfide and 3,3′-dithiodipropionic acid over the pD range 6–7.8. For the reaction with 2-hydroxyethyl disulfide, pD independent equilibrium constants of $K_1 = 4.1 (\pm 0.7) \times 10^{-2}$, $K_2 = 4.0 (\pm 0.3) \times 10^{-3}$ and $K_3 = 1.6 (\pm 0.4) \times 10^{-4}$ were obtained from conditional equilibrium constants. For the reaction with 3,3′-dithiodipropionic acid, values of $K_1 = 1.4 (\pm 0.3) \times 10^{-2}$, $K_2 = 3.2 (\pm 0.3) \times 10^{-4}$, and $K_3 = 4.6 (\pm 1.5) \times 10^{-6}$ were obtained. From these values for K_3 and the pD independent equilibrium constant for the reaction of AMPT with GSSG in Table IV, pD independent equilibrium constants were calculated for the reaction of mercaptoethanol and 3-mercaptopropionic acid with

Table IV. Equilibrium Constants for the Reaction of Thiols with GSSG and Half-Cell Potentials for Thiol/Disulfide Redox Systems

thiol	ΔpK_A-(SH)[a]	K_1[b]	K_2[b]	K_3[b]	$\Delta E^{\circ\prime}$, mV (pD independent)[c]	K_{1c}[d]	K_{2c}[d]	K_{3c}[d]	$E^{\circ\prime}$, V (pD = 7.0)[e]
N,N-dimethylcysteamine	−0.95	0.317	0.0839	0.027	−46.2 ± 4.0	2.72	0.72	1.96	−0.214 ± 0.003
penicillamine[f]	−0.73	0.56	0.0143	8.04×10^{-3}	−61.8	2.95	0.075	0.222	−0.186
cysteamine	−0.66	0.542	0.075	0.0409	−41.0 ± 2.1	2.44	0.337	0.823	−0.203 ± 0.001
2-amino-2-methyl-1-propanethiol	−0.55	0.143	0.0229	0.003 29	−73.2 ± 2.1	0.50	0.80	0.040	−0.164 ± 0.002
cysteine	−0.42	0.427	0.0914	0.039	−41.6 ± 1.6	1.11	0.240	0.265	−0.188 ± 0.001
glutathione	0	1.0	1.0	1.0	0	1.0	1.0	1.0	−0.205[g]
homocysteine	0.32	4.47	0.448	2.03	9.1 ± 3.3	2.46	0.247	0.607	−0.199 ± 0.05
coenzyme A	0.50	5.73	1.08	6.17	23.3 ± 1.6	1.81	0.343	0.622	−0.199 ± 0.002
mercaptoethanol	0.81			20.1	38.4 ± 2.8			0.486	−0.196 ± 0.003
captopril[h]	0.98	31.5	19.5	210	68.5 ± 1.4	3.31	2.05	6.79	−0.230 ± 0.001
3-mercaptopropionic acid	1.50			720	84.3 ± 3.7			0.727	−0.20 ± 0.004

[a] $pK_A(RSH) - pK_A(GSH)$. [b] pD independent equilibrium constants for the reaction of RSH with GSSG. The average uncertainties in K_1, K_2, and K_3 were ±8, ±17, and ±24%, respectively. [c] $\Delta E^{\circ\prime} = E^{\circ\prime}_{GSH} - E^{\circ\prime}_{RSH}$; calculated using the pD-independent value for K_3. [d] Calculated for pD = 7.0 using the pD-independent equilibrium constants and the pK_A values in Table I. [e] Calculated using the pD 7.0 value for K_{3c}. [f] Reference 14a. [g] Reference 13a. [h] Reference 14d.

GSSG. The results are reported in Table IV.

Discussion

The thiolate anion is the reactive species in thiol/disulfide interchange reactions.[9,12,13,14a,17] Over the pD ranges used in this work, the molecules are predominantly in the thiol form. Thus, the rate constants in Table I for the reaction of CoASH and CySH with GSSG increase as the pD and the fraction of CoASH and CySH in the reactive thiolate form increases. In contrast, the equilibrium constants in Table III are essentially pD independent up to pD values where the thiol groups are titrated. Using the above equations which relate the conditional and pD independent equilibrium constants, it can be shown that, when K_A^{RSH} and $K_A^{GSH} \ll [D^+]$, $K_{1C} = K_1(K_A^{RSH}/K_A^{GSH})$, $K_{2C} = K_2(K_A^{RSH}/K_A^{GSH})$ and $K_{3C} = K_3(K_A^{RSH}/K_A^{GSH})^2$, and thus the equilibrium constants are predicted not to vary with pD. When $[D^+]$ is similar in magnitude to K_A, the equilibrium constants will vary with pD if $K_A^{RSH} \neq K_A^{GSH}$. At higher pD where $[D^+] \ll K_A^{RSH}$ and K_A^{GSH}, the conditional equilibrium constants will equal the pD independent equilibrium constants defined in eqs 16–18.

The pD independent rate constant, k_1, for the reaction of the thiolate forms of CoASH and CySH with GSSG are similar in magnitude. Szajewski and Whitesides found that the pH independent rate constants for the reduction of GSSG by a series of thio alcohols and thio carboxylic acids increase as the thiol pK_As increase according to the relationship $\log k_1 = -1.29 + 0.5pK_A$, where the units of k_1 are L/mol-min.[13a] This equation predicts a value of $k_1 = 70$ L/mol-s for the reaction of CoASH with GSSG, as compared to the experimental value of 34 ± 4 L/mol-s, and $k_1 = 24$ L/mol-s for the reaction of CySH with GSSG, as compared to the experimental value of 42 ± 15 L/mol-s. Considering that the above equation was derived from rate constants measured for the reaction of thio alcohols and thiocarboxylic acids with GSSG under solution conditions different from those used in this work, the agreement between the predicted and experimental rate constants is quite good and suggests that reaction of CoASH and CySH with GSSG is mechanistically uncomplicated and proceeds through a S_N2 transition state, as is considered to be the case for other thiol/disulfide interchange reactions.[13a,17]

A major objective of this research has been to quantitatively characterize the oxidation–reduction chemistry of thiol groups in molecules of biological importance. Examination of the pD independent equilibrium constants in Table IV for oxidation of thiols by GSSG reveals a correlation between the reducing strength of the thiolate form and its Bronsted basicity (Table II), with the reducing strength increasing as the Bronsted basicity increases. A plot of $\log K_3$ vs ΔpK_A, where $\Delta pK_A = pK_A(RSH) - pK_A(GSH)$, is linear, and a least-squares fit of the data yields the relationship $\log K_3 = 2.17\Delta pK_A - 0.26$, with standard errors for the estimated slope and intercept of ±0.19 and ±0.15, respectively, and a correlation coefficient of 0.97.[19,20] The pD-independent half-cell potentials of the RSSR/RSH and GSSG/GSH redox couples are related to K_3:

$$E^{\circ\prime}_{RSSR/RSH} = E^{\circ\prime}_{GSSG/GSH} - (RT/nF) \ln K_3 \quad (19)$$

If the pD-independent half-cell potential for the GSSG/GSH couple were known, pD-independent half-cell potentials for the RSSR/RSH couples could be calculated from the values for K_3 in Table IV. However, a pD-independent half-cell potential has not been reported, and thus we are limited to calculating $\Delta E^{\circ\prime}$, the difference between the pD-independent half-cell potentials of the GSSG/GSH and RSSR/RSH couples. The results are listed in Table IV. A plot of $\Delta E^{\circ\prime}$ vs ΔpK_A is linear, and a least-squares fit to the data yields the relationship $\Delta E^{\circ\prime} = 64\Delta pK_A - 7.7$, with standard errors of the estimated slope and intercept of ±5.7 and ±4.4 and a correlation coefficient of 0.97.[19]

The conditional equilibrium constants and half-cell potentials at physiological pH will be different from the pD independent values listed in Table IV if the pK_As of the thiol groups of RSH and GSH are different. Conditional equilibrium constants for pD = 7 were calculated using the pD independent equilibrium constants in Table IV and the pK_A values in Table II. The results are listed in Table IV. Also listed in Table IV are half-cell potentials for the various RSSR/RSH couples at pD = 7, calculated using the listed values for K_{3C} and a value of −0.205 V for the half-cell potential for the GSSG/GSH couple at pH = 7.[13a,21] The conditional equilibrium constants and half-cell potentials at pD 7 cover a much smaller range

(19) The dimethyl-containing thiols *N,N*-dimethylcysteamine, AMPT, and penicillamine deviate most from the linear relationship. When they are removed from the data sets, the linear equations obtained from the least-squares fits are: $\log K_3 = 2.12(\pm 0.14)\Delta pK_A - 0.18(\pm 0.11)$ with a correlation coefficient of 0.99 and $\Delta E^{\circ\prime} = 62.5(\pm 4.2)\Delta pK_A - 5.5(\pm 3.2)$ with a correlation coefficient of 0.99.

(20) Szajewski and Whitesides[13a] report a similar linear dependence of $\log K$ on ΔpK_A ($\log K = 2.42\ \Delta pK_A$) derived from literature equilibrium constants for thiol/disulfide interchange reactions of a variety of thiols with several disulfides.

(21) The difference of 0.017 V between the $\Delta E^{\circ\prime}$ values for cysteine and glutathione at pD 7 is in excellent agreement with the value of 0.018 V reported by Jocelyn.[10]

than the pD-independent constants and, with the exception of AMPT and captopril, the values of K_{3C} are close to 1 and the half-cell potentials are close to that of the GSSG/GSH couple. For example, even though the thiolate form of CoASH has an intrinsic reducing strength 23.3 mV more negative than that of GSH, CoASH is actually 6 mV less reducing than GSH at pD 7 because the fraction of CoASH in the reactive thiolate anion form is much less at pD 7. Thus, the conditional half-cell potentials of these biological thiols at pD 7 are sufficiently close to that of GSH, the major intracellular thiol redox system, that their distribution between thiol and disulfide forms will be sensitive to small changes in intracellular conditions. For example, the similarity of the reduction potentials of CoASH and GSH at pD 7 suggests that small changes in the intracellular reduction potential and thus in the distribution of glutathione between its GSH and GSSG forms will cause substantial changes in the position of the CoASH/CoASSG equilibrium. The CoASSG mixed disulfide has been identified in a number of organisms,[22-25] and its level in Escherichia coli has been found to increase substantially in response to oxygen starvation and glucose starvation.[25] Although no conclusions have been drawn concerning its role in vivo, it has been suggested that CoASSG might be a storage form of CoASH during certain stages of growth.[26]

As noted in the introduction, half-cell potentials have been reported for only a limited number of thiol-containing molecules of biological importance because of problems associated with their measurement by electrochemical methods. The linear dependence of log K_3 and $\Delta E^{\circ\prime}$ on ΔpK_A for the RSH and GSH thiol groups suggests that the relationships derived in this study can be used to predict the redox properties of other biological thiols. For example, if the pK_A of a thiol group is known, the above equations can be used to calculate the pD-independent equilibrium constant K_3 and $\Delta E^{\circ\prime}$. The conditional equilibrium constant and the half-cell potential at pD 7 can then be calculated from this value for K_3 and the thiol pK_A value.

Experimental Section

Materials and Methods. The free acid form of glutathione, the sodium salt forms of glutathione disulfide, coenzyme A (CoASH), and coenzyme A–glutathione mixed disulfide (CoASSG) and the hydrochloride forms of cysteine (CySH), cystine (CyS-SCy), homocysteine (HCSH), and homocystine (HCSSCH) were obtained from Sigma Chemical Co. Cysteamine hydrochloride (CSH), cystamine dihydrochloride (CSSC), 2-hydroxyethyl disulfide, and 3,3'-dithiodipropionic acid were obtained from Aldrich Chemical Co. *N,N*-Dimethylcysteamine hydrochloride (NCySH) was supplied by Fluka Chemical Co. AMPT was synthesized by a literature procedure.[27]

Solutions were prepared in degassed D_2O containing 0.15 M NaCl. Initial thiol concentrations were generally twice the disulfide concentration. 2-Methyl-2-propanol and dioxane was added for ^1H and ^{13}C chemical shift references. For the equilibrium studies, thiol and disulfide solutions were prepared separately and then mixed in a temperature-controlled cell under nitrogen or argon to minimize oxidation of the thiol. The pD was adjusted with DCl or NaOD, three aliquots were placed in NMR tubes which were flushed with nitrogen, capped, and sealed with Parafilm, and then the pD was adjusted and the process repeated. The NMR tubes were placed in a glovebox under nitrogen for at least 24 h before measurement of NMR spectra. Spectra were measured each day for several days to ensure that equilibrium was reached. In general, the lower the pD, the longer the time required to reach equilibrium; at the lowest pD values used (pD ~5), equilibrium was reached in 48 h or less.

For kinetics experiments, spectra were measured as a function of reaction time. At acidic pD values, the reactions are slow enough to follow directly by ^1H NMR. Solutions of the thiol and disulfide were prepared in D_2O and their pDs adjusted to the appropriate value. Aliquots of each solution were added to NMR tubes and ^1H NMR spectra measured at intervals of 10–30 min. After measurement of time-course spectra, the pD of the solution was measured. At pD values greater than 7, reactants were mixed in a cell thermostatted at 25 °C, and aliquots were removed at 30 s–1 min intervals, quenched by addition of DCl to pD <2, and then analyzed by NMR. All kinetics experiments were carried out in degassed D_2O containing 0.15 M NaCl.

NMR Measurements. ^1H and ^{13}C NMR spectra were measured with a Varian VXR-500S spectrometer at a probe temperature of 25 °C using a repetition time of at least five times the longest spin-lattice relaxation time of the resonances of interest to obtain quantitative NMR spectra. Spin-lattice relaxation times were measured by the inversion–recovery pulse sequence. Quantitative ^{13}C NMR spectra were measured with WALTZ decoupling gated on only during acquisition of the free induction decay. NMR peak areas were obtained by integration using Varian software when resonances were sufficiently separated so that an integration range of 32 times the width at half height to either side of the resonance of interest could be used. When resonances were more closely spaced, peak areas were determined using Varian software which fits Lorentzian lineshapes to the peaks.

pH measurements in D_2O solutions were made using combination microelectrodes which were calibrated with pH 4.00, 7.00, and 10.00 aqueous buffers (Fisher Scientific Co.). The exact pH values of the buffers were determined by comparison with primary standard buffers prepared according to NBS procedures. pH meter readings were converted to pD values for D_2O solutions with the equation pD = pH meter reading +0.40.[28]

Determination of Thiol pK_A Values. The thiol pK_A values in Table II were determined by pH titration or by NMR for the conditions used in these studies. Exchange of protons between thiol groups and solvent molecules is fast on the NMR time scale, and pK_A values were determined from exchange-averaged chemical shift data by methods described previously.[29]

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Registry No. GSSG, 27025-41-8; GSH, 70-18-8; NCySH, 108-02-1; CSH, 60-23-1; CySH, 52-90-4; HCSH, 6027-13-0; CoASH, 85-61-0; 3-mercaptopropionic acid, 107-96-0; 2-amino-2-methyl-1-propanethiol, 13893-24-8; mercaptoethanol, 60-24-2.

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