Contribution from the Department of Chemistry,
Texas Tech University, Lubbock, Texas 79409

Stability of the Cu(II)-S Bond in Mercapto Amino Acid Complexes of [2,2',2''-Tris(dimethylamino)triethylamine]copper(II) and [Tris(2-pyridylmethyl)amine]copper(II)

HAESUN KANG BAEK, ROBIN L. COOPER, and ROBERT A. HOLWERDA*

Received September 24, 1984

Comparative kinetic studies (pH 5.6-11.1) are reported for the oxidations of cysteine (cys-SH), cysteine methyl ester (cme-SH), penicillamine (pen-SH), and glutathione (glu-SH) coordinated to [2,2',2''-tris(dimethylamino)triethylamine]copper(II) (Cu(Me_6tren)$^{2+}$) and [tris(2-pyridylmethyl)amine]copper(II) (Cu(tmpa)$^{2+}$). Relative stabilizations of (tmpa)Cu^{II}-SR species by factors of ca. 10^4 and 10 were found at low- and high-pH limits, respectively, suggesting that delocalization of thiolate sulfur negative charge over the π systems of aromatic nitrogen donor ligands contributes significantly to Cu(II)-S bond stability. Excluding the glutathione complexes, all of the Cu(tmpa)$^{2+}$ and Cu(Me_6tren)$^{2+}$ adducts exhibit reactivity decreases from intermediate to high pH, where S,N chelation by the amino acids is favored. A mechanism involving reductive elimination of sulfur to give Cu(I) and a thiyl radical is supported by this and other results. Oxidation rate constants of S,N-bonded cysteine and its methyl ester are not greatly different in complexes with both (tmpa)Cu^{II} and (Me_6tren)Cu^{II}, but the reactivity of penicillamine is lower by more than 1 order of magnitude in both systems. This observation, coupled with unexpectedly slow ring closure to give (tmpa)Cu^{II}-S,N-pen, reflects geometric rearrangements in the first coordination sphere of copper required to accommodate the steric demands of the penicillamine β,β-dimethyl substituents. Redox decay rate constants of Cu(tmpa)$^{2+}$ adducts with S-cys, S-cme, and S-glu are remarkably small at low pH as well ($<10^{-3}$ s^{-1} at 25 °C below pH 4) although S,O chelation does not contribute appreciably to the activation barrier.

Introduction

Interest in the kinetic stability of the copper(II)-mercaptide sulfur bond has been spurred by synthetic efforts to model the physical properties of the blue copper proteins.[1] While synthetic analogues that exhibit both the spectroscopic and stability characteristics of type 1 Cu(II) remain elusive, suppression of internal electron transfer in (thiolato)copper(II) compounds has been achieved, primarily through steric restrictions that specifically stabilize the cupric oxidation state.[1,2] We have undertaken kinetic studies of reactions between mercapto amino acids and copper(II)-polyamine complexes in order to quantitatively evaluate the relative contributions of coordination geometry, electronic characteristics of ligand donor atoms, Cu(II) oxidizing strength, mercaptan substituents, and steric effects to Cu(II)-S bond stability.[3-5] Considerable mechanistic flexibility exists within this class of mercapto amino acid oxidations, as changes in the rate law may be induced by modest variations in reductant concentration (at fixed $[Cu(II)]_0$),[3,5] pH,[5] Cu(II) oxidizing strength,[4] and the structures of the redox partners.[4,5]

First-order decay of Cu^{II}-SR adducts should be regarded as atypical,[5] except when the oxidizing strength of the Cu(II) center permits the formation of metal-stabilized thiyl or disulfide radicals.[4] Second-order reactivity with respect to inner-sphere, S-bonded intermediates, leading to concerted two-electron transfer and S-S bond formation, is far more common.[5-7] Nevertheless, a slow, pH-dependent unimolecular redox reaction was observed within the 1:1 S-bonded cysteine (cys-SH) complex of [tris(2-pyridylmethyl)amine]copper(II) (Cu(tmpa)$^{2+}$), a weak oxidant ($E°$ = -147 mV).[3] This latter characteristic, coupled with chelation (S,O) of copper by the mercaptan and steric crowding about the coordinated sulfur atom, was proposed to account for the remarkable kinetic stability of aqueous [(tmpa)Cu-S-cys]$^+$ in the pH range 4-6.[3]

We report here comparative kinetic studies of the intracomplex redox reactions of cysteine, cysteine methyl ester (cme-SH), penicillamine (β,β-dimethylcysteine, pen-SH), and glutathione (γ-L-glutamyl-L-cysteinylglycine, glu-SH) with the trigonal-bipyramidal[8,9] oxidants Cu(tmpa)$^{2+}$ and [2,2',2''-tris(dimethylamino)triethylamine]copper(II) (Cu(Me_6tren)$^{2+}$). The importance of aromatic nitrogen ligands to Cu(II)-S bond stability should be apparent from this comparison, and structural variations within the family of cysteine derivatives will permit a critical assessment of mercaptan substituent and chelation effects.

Experimental Section

Materials. Mercaptans were obtained and checked for purity as previously described.[4] Literature methods were used to prepare Cu(tmpa)$(ClO_4)_2$[10] and Cu(Me_6tren)$(ClO_4)_2$.[9] Ionic strength 0.1 M ($NaNO_3$) buffer (5 mM) solutions in the pH range 5.6-11.1 were prepared from BES (*N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid) or sodium carbonate and triply distilled water.[3] Anaerobic reductant and oxidant solutions in the same buffer were prepared, and $[H^+]$ was evaluated as before.[3]

Kinetic and Stoichiometry Measurements. Kinetic measurements were performed at 25 °C on a Durrum D-110 stopped-flow apparatus, with inlet of anaerobic solutions through Teflon needles.[3] Data were transmitted to an Apple II computer, stored on floppy disks, and quantitatively interpreted through an interface system to be described elsewhere.[11] A 40% excess of oxidant (0.14 mM) over mercaptan (0.10 mM) was used in most kinetic runs. Reduction of Cu(tmpa)$^{2+}$ was followed at the 387-, 401-, and 396-nm charge-transfer absorptions of intermediates formed with cysteine methyl ester, glutathione, and penicillamine, respectively. Monitoring at 350 nm was convenient for all four mercaptide adducts with Cu(Me_6tren)$^{2+}$. Observed first- or second-order rate constants (k_{obsd}), reported as the average of at least three trials, were calculated as before.[3] Copper(I) in product mixtures was assayed spectrophotometrically after conversion to $Cu(dpmp)_2^{3-}$.[3,12]

Results

Mercaptan Reactions with Cu(tmpa)$^{2+}$. Stoichiometric measurements on the reactions of excess Cu(tmpa)$^{2+}$ and Cu(Me_6tren)$^{2+}$ (0.30 mM) with mercaptans (0.10 mM) indicated the formation of disulfide products (1 mol of Cu(I) formed/mol of RSH), as expected. Intense transient sulfur-to-copper(II) charge-transfer maxima at 396, 387, and 401 nm are observed upon mixing cysteine, cysteine methyl ester, and glutathione, respectively, with Cu(tmpa)$^{2+}$ at pH 5.[3] In contrast, no such transient absorption was detected with penicillamine (pH 5.0)[3] and *S*-methyl-L-cysteine (pH 7.3). An absorbing intermediate does form in the penicillamine-Cu(tmpa)$^{2+}$ reaction above pH

(1) Kuehn, C. G.; Isied, S. S. *Prog. Inorg. Chem.* **1980**, *27*, 153.
(2) Hughey, J. L.; Fawcett, T. G.; Rudlich, S. M.; Lalancette, R. A.; Potenza, J. A.; Schugar, H. T. *J. Am. Chem. Soc.* **1979**, *101*, 2617.
(3) Baek, H. K.; Holwerda, R. A. *Inorg. Chem.* **1983**, *22*, 3452.
(4) Stevens, G. D.; Holwerda, R. A. *Inorg. Chem.* **1984**, *23*, 2777.
(5) Anderson, C. H.; Holwerda, R. A. *J. Inorg. Biochem.* **1985**, *23*, 29.
(6) Lappin, A. G.; McAuley, A. *J. Chem. Soc., Dalton Trans.* **1978**, 1606.
(7) Deutsch, E.; Root, M. J.; Nosco, D. L. *Adv. Inorg. Bioinorg. Mech.* **1982**, *1*, 269.
(8) Karlin, K. D.; Hayes, J. C.; Juen, S.; Hutchinson, J. P.; Zubieta, J. *Inorg. Chem.* **1982**, *21*, 4106.
(9) Ciampolini, M.; Nardi, N. *Inorg. Chem.* **1966**, *5*, 45.
(10) Anderegg, G.; Hubmann, E.; Podder, N. G.; Wenk, F. *Helv. Chim. Acta* **1977**, *60*, 123.
(11) Johnston, R. J.; Holwerda, R. A., submitted for publication.
(12) $dpmp^{2-}$ = 2,9-dimethyl-1,10-phenanthrolinediyl-4,7-bis(benzenesulfonate).

0020-1669/85/1324-1077$01.50/0

Table I. Observed Rate Constants for Redox Decay of $Cu(tmpa)^{2+}$ Complexes with Mercapto Amino Acids[a]

mercaptan	$[H^+]$, M	$10^2 k_{obsd}$,[b] s^{-1}	$10^2 k_{calcd}$,[c] s^{-1}
cysteine methyl ester	1.82×10^{-6}	8.7 (0.4)	7.4
	1.07×10^{-6}	10.2 (0.1)	11.2
	1.05×10^{-6}	10.5 (1.6)	11.3
	7.59×10^{-7}	10.4 (0.7)	14.2
	3.31×10^{-7}	21.2 (0.5)	22.4
	2.69×10^{-7}	25.1 (1.2)	24.4
	1.95×10^{-7}	27.8 (1.0)	27.4
	1.12×10^{-7}	36.4 (1.5)	31.8
	4.90×10^{-8}	39.7 (0.1)	35.9
	4.37×10^{-8}	38.3 (0.2)	36.2
	2.40×10^{-8}	36.0 (1.2)	37.5
	1.38×10^{-8}	33.4 (0.2)	37.6
	1.05×10^{-8}	35.4 (0.3)	37.4
	2.75×10^{-9}	32.3 (2.0)	32.8
	1.66×10^{-9}	31.3 (0.3)	29.3
	1.15×10^{-9}	23.1 (1.1)	26.3
	4.27×10^{-10}	18.5 (0.3)	17.5
	7.58×10^{-11}	12.6 (0.9)	7.5
	3.31×10^{-11}	6.7 (0.7)	5.7
	1.15×10^{-11}	4.1 (0.8)	4.7
penicillamine	2.51×10^{-7}	8.6 (0.4)	9.0
	1.07×10^{-7}	8.5 (0.2)	8.5
	4.57×10^{-8}	8.4 (0.3)	7.7
	1.45×10^{-8}	5.5 (0.3)	5.6
	8.91×10^{-9}	4.1 (0.1)	4.5
	3.98×10^{-9}	2.4 (0.1)	2.8
	2.82×10^{-9}	2.7 (0.1)	2.2
	2.04×10^{-9}	1.5 (0.1)	1.8
	9.77×10^{-10}	1.9 (0.1)	1.1
	2.34×10^{-10}	0.37 (0.02)	0.52
	8.32×10^{-11}	0.25 (0.02)	0.39
glutathione	1.86×10^{-6}	0.074 (0.001)	0.075
	5.62×10^{-7}	0.075 (0.001)	0.076
	2.29×10^{-7}	0.077 (0.004)	0.078
	1.58×10^{-7}	0.081 (0.018)	0.079
	3.98×10^{-8}	0.096 (0.001)	0.092
	2.14×10^{-8}	0.10 (0.01)	0.11
	1.29×10^{-8}	0.13 (0.01)	0.13
	7.59×10^{-9}	0.15 (0.01)	0.15
	2.63×10^{-9}	0.18 (0.01)	0.18
	1.38×10^{-9}	0.17 (0.01)	0.16
	6.61×10^{-10}	0.13 (0.01)	0.14
	5.62×10^{-10}	0.14 (0.01)	0.14
	4.37×10^{-10}	0.13 (0.01)	0.13
	3.55×10^{-11}	0.13 (0.01)	0.12
	2.09×10^{-11}	0.12 (0.01)	0.12

[a] Conditions: $[Cu(tmpa)^{2+}]_0 = 0.14$ mM; $[RSH]_0 = 0.10$ mM; 25.0 °C; $I = 0.1$ M ($NaNO_3$). [b] Average deviations from the mean shown in parentheses. [c] Rate constant calculated from nonlinear least-squares rate parameters (see Table III and text) by using eq 2 or 3 (penicillamine).

6, however, with λ_{max} at 338 nm and pronounced shoulders near 300 and 380 nm (pH 12.0). The spectrophotometric method[3] used to calculate the formation constant of $[(tmpa)Cu\text{-}S\text{-}cys]^+$ was not successful in the case of penicillamine at pH 7.1 and 8.1. Initial fast absorbance increases (396 nm), prior to redox decay, were found to be essentially independent of [pen-SH] at $[Cu(tmpa)^{2+}]_0 = 0.14$ mM.

First-order analytical plots were found to be linear over ≥90% of the absorbance decrease in redox decay reactions of $Cu(tmpa)^{2+}$ adducts with all mercaptans examined (40% excess of oxidant). The dependence of k_{obsd} on $[Cu(tmpa)^{2+}]_0$ (0.08–0.14 mM) at fixed $[RSH]_0 = 0.10$ mM was examined to verify that these electron-transfer processes are strictly unimolecular, with rates independent of small concentrations of excess oxidant or reductant. We have already established that a change in rate law to second order in the absorbing intermediate occurs when greater than a 10-fold excess of cysteine is present (pH 7.8).[3] Experiments at pH 7.6–8.2 showed no variation in k_{obsd} between the above concentration limits, but distinct rate increases were noted with $[Cu(tmpa)^{2+}]_0 \geq 0.20$ mM.

Table II. Penicillamine–$Cu(tmpa)^{2+}$ Complex Formation Rate Constants[a]

$[H^+]$, M	$10^{-2} k_{obsd}$,[b] s^{-1}	$10^{-2} k_{calcd}$,[c] s^{-1}
7.59×10^{-7}	2.05 (0.27)	2.02
4.47×10^{-7}	2.03 (0.12)	2.08
2.51×10^{-7}	2.07 (0.05)	2.19
1.07×10^{-7}	2.60 (0.04)	2.49
4.57×10^{-8}	3.13 (0.09)	3.05
8.91×10^{-9}	4.17 (0.01)	4.31
2.82×10^{-9}	3.73 (0.27)	3.52
9.77×10^{-10}	1.80 (0.06)	1.95
2.34×10^{-10}	0.584 (0.013)	0.690
8.32×10^{-11}	0.499 (0.014)	0.357

[a] Conditions: $[Cu(tmpa)^{2+}]_0 = 0.14$ mM; $[pen\text{-}SH]_0 = 0.10$ mM; 25.0 °C; $I = 0.1$ M ($NaNO_3$). [b] Average deviations from the mean shown in parentheses. [c] Rate constant calculated from nonlinear least-squares rate parameters (see text) by using eq 2.

Table I displays the $[H^+]$ dependence of (tmpa)Cu–SR redox decay rate constants (pH 5.6–11.1). The bell-shaped k_{obsd}–pH profile of (tmpa)Cu–S-cys was understood in terms of three distinct reactant species (I, II, III) related through two rapid ionization equilibria (eq 1).[3]

$$\mathrm{I} \overset{K_{a1}}{\rightleftharpoons} \mathrm{II} + \mathrm{H^+}$$

$$\mathrm{II} \overset{K_{a2}}{\rightleftharpoons} \mathrm{III} + \mathrm{H^+}$$

$$\mathrm{I} \xrightarrow{k_1} \mathrm{Cu(tmpa)^+} + \text{mercaptan oxidation product} \quad (1)$$

$$\mathrm{II} \xrightarrow{k_2} \mathrm{Cu(tmpa)^+} + \text{mercaptan oxidation product}$$

$$\mathrm{III} \xrightarrow{k_3} \mathrm{Cu(tmpa)^+} + \text{mercaptan oxidation product}$$

Relationship 2 describes the $[H^+]$ dependence of k_{obsd} for decay of $[adduct]_{tot}$ implied by this mechanism. When only one ion-

$$k_{obsd} = \frac{k_1[H^+]^2 + k_2K_{a1}[H^+] + k_3K_{a1}K_{a2}}{[H^+]^2 + K_{a1}[H^+] + K_{a1}K_{a2}} \quad (2)$$

ization equilibrium pertains (K_{a1}), this rate expression simplifies to relationship 3. In the case of cysteine, II was found to be

$$k_{obsd} = \frac{k_1[H^+] + k_2K_{a1}}{[H^+] + K_{a1}} \quad (3)$$

considerably more reactive than either I or III.[3]

Bell-shaped k_{obsd}–pH curves were also found in the decay of $Cu(tmpa)^{2+}$ complexes with cysteine methyl ester and glutathione. As compared with the values for cysteine, the maximum rate and corresponding pH exhibited by the ester complex are shifted from 8×10^{-1} s^{-1} and 8.6 to 4×10^{-1} s^{-1} and 7.9, respectively. In contrast to the cys-S^- and cme-S^- oxidation results, the electron-transfer rate within (tmpa)Cu–S-glu varies within only a 2-fold range between pH 5.8 and 10.8, and k_{obsd}(max) (1.8×10^{-3} s^{-1}, pH 8.7) is smaller by more than 2 orders of magnitude.

Complexation of $Cu(tmpa)^{2+}$ by all mercaptans except penicillamine was complete within the stopped-flow mixing time (ca. 3 ms). With $[Cu(tmpa)^{2+}]_0 = 0.14$ mM and $[pen\text{-}SH] = 0.10$ mM, the fast initial 396-nm absorbance increase was barely measurable in the pH 6.2–10.2 interval. Considering the relative slowness of the subsequent redox decay reaction, A_∞ could be reliably assigned as the maximum in biphasic absorbance–time traces. The pH dependence of first-order $Cu(tmpa)^{2+}$–pen-SH complexation rate constants, displayed in Table II, shows a maximum at pH 8.2.

Because of insufficient adduct formation at lower pHs, redox decay of $(tmpa)Cu^{II}$–S-pen could only be followed in the pH 6.5–10.2 range. Reduction of copper(II) in the penicillamine adduct, characterized by a decreasing sigmoidal k_{obsd}–pH profile, is substantially faster than in (tmpa)Cu–S-glu but still is 1 order

Table III. Comparison of Rate Parameters for the Redox Decay of Mercaptide Complexes with $Cu(tmpa)^{2+}$ and $Cu(Me_6tren)^{2+}$

oxidant	mercaptan	k_1, s^{-1}	k_2, s^{-1}	k_3, s^{-1}	pK_{a1}	pK_{a2}
$Cu(tmpa)^{2+}$	cysteine[b]	$<1 \times 10^{-3}$	2.0	4.1×10^{-2}	8.33	8.52
	cysteine methyl ester	$<1 \times 10^{-3}$	$4.1\ (0.2) \times 10^{-1}$	$4\ (1) \times 10^{-2}$	6.39 (0.06)	9.13 (0.10)
	penicillamine	$9.3\ (0.4) \times 10^{-2}$	$3\ (1) \times 10^{-3}$		7.98 (0.08)	
	glutathione[c]	$7.5\ (0.2) \times 10^{-4}$	0.36–40.8	$1.2\ (0.1) \times 10^{-3}$	10.7–8.7	6.0–8.0
$Cu(Me_6tren)^{2+}$	cysteine	7.0 (0.2)	$5\ (1) \times 10^{-1}$		7.44 (0.06)	
	cysteine methyl ester	1.8 (0.1) × 10	2.5 (0.3)		7.24 (0.07)	
	penicillamine	$2.1\ (0.2) \times 10^{-1}$	$3.9\ (0.5) \times 10^{-2}$		6.36 (0.11)	

[a] Conditions: 25.0 °C; I = 0.1 M ($NaNO_3$). Standard deviations are shown in parentheses. See text and eq 1 for definitions of parameters obtained from nonlinear least-squares fits to eq 2 or 3. [b] Reference 3. [c] See text for discussion of k_2, pK_{a1}, pK_{a2} uncertainties.

of magnitude slower than the analogous reactions of cys-S^- and cme-S^-.

A nonlinear least-squares fit[13] of the rate data to eq 2 was performed for cme-S^- and glu-S^- adducts with $Cu(tmpa)^{2+}$, basing initial parameter estimates on limiting rate constants at high- and low-pH extremes and inflection points in k_{obsd}–pH plots. Parameters derived from these fits are contained in Table III. A satisfactory fit of the cysteine methyl ester data was achieved, as indicated by the good agreement between observed rate constants and those calculated from eq 2 (k_{calcd}) with the least-squares parameters (see Table I). As was previously reported for $[(tmpa)Cu\text{-}S\text{-}cys]^+$, k_1(cme) is experimentally indistinguishable from zero; only an upper limit may be given.

The precise extraction of five rate parameters for redox decay of (tmpa)Cu–S-glu is not possible, considering the narrow range of k_{obsd} values observed in the pH-dependence study. Indeed, even the applicability of mechanism 1 and eq 2 to this system is in doubt. Only k_1 and k_3 (Table III) are well-defined from the low- and high-pH limits, respectively, of k_{obsd}. While the best-fit results, $k_2 = 0.37\ s^{-1}$, $pK_{a1} = 10.7$, $pK_{a2} = 6.0$, give excellent agreement between k_{obsd} and k_{calcd} (Table I), extraordinarily large uncertainties in all three parameters dictate the reporting of ranges rather than specific values (Table III). A virtual continuum of k_2, K_{a1}, K_{a2} combinations that satisfactorily fit the rate data may be drawn from these ranges. This continuum is defined by compensating changes in pK_{a1} and pK_{a2} (constant $(pK_{a1} + pK_{a2})/2$), coupled with increases in k_2 as the pK's move closer together.[14]

The bell-shaped kinetic pH dependence of $Cu(tmpa)^{2+}$–penicillamine complexation suggests a mechanism similar to eq 1, where the K_{a1}, K_{a2} ionizations may apply to either reactant. Three different weakly absorbing Cu(II)–mercaptan precursors (I, II, III) would then rearrange to give strongly absorbing, inner-sphere, (*S*-penicillaminato)copper(II) products. Nonlinear least-squares parameters based on eq 2 for the complexation reaction are as follows: $k_1 = (1.9 \pm 0.1) \times 10^2$, $k_2 = (7.5 \pm 1.6) \times 10^2$, $k_3 = (1.6 \pm 1.7) \times 10\ s^{-1}$; $pK_{a1} = 7.9 \pm 0.2$, $pK_{a2} = 8.5 \pm 0.2$. The (tmpa)Cu–S-pen intracomplex electron-transfer data were fit to eq 3 in straightforward fashion (Table III), assuming only two reactive intermediates. Identical rate constants were obtained from pH 7.60 measurements at 340 and 396 nm.

Mercaptan Reactions with $Cu(Me_6tren)^{2+}$. Black transients were observed upon mixing excess $Cu(Me_6tren)^{2+}$ with mercapto amino acids (pH 5.6–11.0). Point-by-point 330–400-nm spectra of these transients were obtained from initial absorbance measurements after mixing 0.14 mM $Cu(Me_6tren)^{2+}$ with 0.10 mM mercaptan on the stopped-flow apparatus (pH 7.0 (BES), I = 0.1 M ($NaNO_3$)). Broad bands with λ_{max} at 345 ± 5, 355 ± 10, and 338 ± 5 nm and ϵ's on the order of $10^3\ M^{-1}\ cm^{-1}$ were found for adducts with cysteine, cysteine methyl ester, and penicillamine, respectively. Excellent linear first-order plots of ln $(A_t - A_\infty)$ vs. time could be derived from A_{350} in most runs where 0.14 mM $Cu(Me_6tren)^{2+}$ was mixed with 0.10 mM mercaptan. It should be noted, however, that decay curves could be better understood through a second-order analysis when larger than 2-fold excesses of $Cu(Me_6tren)^{2+}$ over cme-SH, glu-SH, and pen-SH were employed.

Table IV. Observed Rate Constants for Redox Decay of $Cu(Me_6tren)^{2+}$ Complexes with Mercapto Amino Acids[a]

mercaptan	$[H^+]$, M	k_{obsd},[b] s^{-1}	k_{calcd},[c] s^{-1}
cysteine	8.37×10^{-7}	6.76 (0.44)	6.72
	6.01×10^{-7}	6.85 (0.55)	6.62
	2.92×10^{-7}	6.19 (0.13)	6.27
	1.32×10^{-7}	5.60 (0.08)	5.59
	7.41×10^{-8}	4.62 (0.18)	4.85
	3.70×10^{-8}	3.30 (0.26)	3.78
	1.87×10^{-8}	3.00 (0.15)	2.71
	9.91×10^{-9}	1.95 (0.26)	1.91
	5.38×10^{-9}	1.85 (0.05)	1.36
	4.53×10^{-9}	1.69 (0.22)	1.24
	2.60×10^{-9}	0.91 (0.09)	0.96
	7.41×10^{-10}	0.50 (0.13)	0.66
	6.47×10^{-11}	0.43 (0.02)	0.54
	3.89×10^{-11}	0.41 (0.04)	0.54
	2.40×10^{-11}	0.40 (0.05)	0.53
	1.50×10^{-11}	0.36 (0.04)	0.53
cysteine methyl ester	7.94×10^{-7}	$1.77\ (0.07) \times 10$	1.70×10
	3.72×10^{-7}	$1.59\ (0.05) \times 10$	1.60×10
	1.00×10^{-7}	$1.10\ (0.05) \times 10$	1.24×10
	5.01×10^{-8}	$1.00\ (0.05) \times 10$	9.78
	2.14×10^{-8}	7.12 (0.06)	6.74
	9.77×10^{-9}	5.56 (0.10)	4.76
	7.41×10^{-9}	4.42 (0.06)	4.27
	5.01×10^{-9}	4.01 (0.06)	3.74
	2.34×10^{-9}	3.84 (0.06)	3.09
	1.91×10^{-9}	3.38 (0.08)	2.98
	3.80×10^{-10}	2.35 (0.04)	2.58
penicillamine	3.72×10^{-6}	$1.94\ (0.01) \times 10^{-1}$	1.88×10^{-1}
	8.32×10^{-7}	$1.34\ (0.06) \times 10^{-1}$	1.48×10^{-1}
	3.39×10^{-7}	$1.17\ (0.02) \times 10^{-1}$	1.12×10^{-1}
	1.20×10^{-7}	$7.70\ (0.04) \times 10^{-2}$	7.51×10^{-2}
	5.13×10^{-8}	$6.07\ (0.10) \times 10^{-2}$	5.66×10^{-2}
	1.70×10^{-8}	$5.76\ (0.01) \times 10^{-2}$	4.53×10^{-2}
	1.15×10^{-8}	$5.10\ (0.01) \times 10^{-2}$	4.33×10^{-2}
	3.31×10^{-9}	$4.56\ (0.01) \times 10^{-2}$	4.03×10^{-2}
	2.09×10^{-10}	$3.86\ (0.18) \times 10^{-2}$	3.91×10^{-2}
	1.29×10^{-10}	$3.07\ (0.21) \times 10^{-2}$	3.91×10^{-2}
glutathione	7.94×10^{-7}	$6.3\ (0.1) \times 10^{-1}$	
	3.63×10^{-7}	$5.1\ (0.2) \times 10^{-1}$	
	1.02×10^{-7}	1.00 (0.04)	
	5.62×10^{-8}	1.18 (0.12)	
	2.63×10^{-8}	1.40 (0.09)	
	1.32×10^{-8}	1.54 (0.09)	
	1.26×10^{-8}	1.87 (0.01)	
	8.32×10^{-9}	2.61 (0.12)	
	1.55×10^{-10}	$7.36\ (0.15) \times 10^4$ [d]	

[a] Conditions: $[Cu(Me_6tren)^{2+}]_0 = 0.14$ mM; $[RSH]_0 = 0.10$ mM; 25.0 °C; I = 0.1 M ($NaNO_3$). [b] Average deviations from the mean shown in parentheses. [c] Rate constant calculated from nonlinear least-squares rate parameters (see Table III) by using eq 3. [d] Second order rate constant ($M^{-1}\ s^{-1}$).

The pH dependence of $(Me_6tren)Cu^{II}$–SR redox decay rate constants (pH 5.6–10.8) is described in Table IV. As compared with the corresponding $Cu(tmpa)^{2+}$ reductions, these reactions are uniformly much faster. Decreasing sigmoidal k_{obsd}–pH profiles with only small differences in inflection points characterize the

(13) Program NLLSQ (CET Research Group Ltd., Norman, OK). This Apple II+ program uses an optimum seeking routine based on the Levenburg–Marquardt algorithm. See: Christian, S. D.; Tucker, E. E. *Am. Lab.* **1982**, *14* (9), 31.

(14) This statement is based, in part, on the prediction from eq 2 that k_{obsd} is maximal when $-\log [H^+] = (pK_{a1} + pK_{a2})/2$.

Table V. Comparison of Free Energy Changes Resulting from S,N Chelation in $Cu(tmpa)^{2+}$ and $Cu(Me_6tren)^{2+}$ Complexes (25 °C)

parameter	cysteine	cysteine methyl ester	penicillamine
pK_a[a]	10.37[e]	8.99[f]	10.45[e]
$\Delta pK_a(tmpa)$[b]	−1.85	+0.14	−2.47
$\Delta\Delta G_a°(tmpa)$[c]	−2.5	+0.2	−3.4
$\Delta pK_a(Me_6tren)$[b]	−2.93	−1.75	−4.09
$\Delta\Delta G_a°(Me_6tren)$[c]	−4.0	−2.4	−5.6
$\Delta\Delta G_a°(Me_6tren - tmpa)$[d]	−1.5	−2.6	−2.2

[a] Ammonium group ionization constant of free amino acid. [b] Kinetically determined ammonium group pK_a in S-bonded Cu(II) complex minus pK_a of free amino acid. [c] Free energy difference (kcal/mol) corresponding to ΔpK_a. [d] $\Delta\Delta G_a°(Me_6tren) - \Delta\Delta G_a°(tmpa)$. [e] Reference 17. [f] Reference 19.

oxidations of cys-S^-, cme-S^-, and pen-S^- coordinated to $Cu(Me_6tren)^{2+}$. Relationship 3 accounts well for these results, supporting a two-reactant, single-ionization mechanism. Again, the nonlinear least-squares parameters k_1, k_2, and K_{a1} may be found in Table III, and a comparison of k_{obsd} with k_{calcd} is included in Table IV. A notable similarity between the $Cu(tmpa)^{2+}$ and $Cu(Me_6tren)^{2+}$ reductions is the relatively low reactivity of penicillamine.

Electron transfer within $(Me_6tren)Cu$–S-glu is unique in several respects. A modest *increase* in k_{obsd} from 0.63 to 2.61 s^{-1} occurs between pH 6.2 and 8.4. Neither first- nor second-order analysis was satisfactory in the pH 8.5–9.5 interval. In more alkaline solutions, up to at least pH 11, decay rates are $[H^+]$ independent and cleanly second order with respect to the absorbing intermediate. Neither eq 2 nor eq 3 offers an appropriate quantitative interpretation of the $(Me_6tren)Cu$–S-glu kinetic data.

Discussion

Rate-limiting unimolecular decay of Cu^{II}–SR adducts, to give Cu(I) and a thiyl radical as the initial organic product, is indicated by our kinetic results for all reactions considered except that of glu-SH with $Cu(Me_6tren)^{2+}$ at high pH. The three-reactant, two-ionization formalism (eq 1) adequately accounts for the pH dependence of $(tmpa)Cu^{II}$–S-cys, –S-cme, and –S-glu electron-transfer rates. The mechanistic resemblance of the last reaction to the first two may only be superficial, however, considering the uniformly low and nearly pH-independent redox reactivity of $(tmpa)Cu^{II}$–S-glu.

In our initial study of $(tmpa)Cu^{II}$–S-cys complexes, the resistance of species I and III to internal electron transfer (see Table III) was attributed in part to S,O and S,N chelation, respectively.[3] The absence of such chelation (monodentate OH^- and *S*-cysteinato ligands) was proposed for the more reactive complex II. This hypothesis is supported by the decrease in pK_{a1} from 8.33 to 6.39 associated with esterification of the cysteine carboxylate group. Thus, the transformation of $(tmpa)Cu^{II}(RS)$ into $(tmpa)Cu^{II}(RS)(OH)$ should be facilitated when the competition between $-COO^-$ and OH^- for a common binding site is eliminated. Esterification of the carboxylate group has only a moderate effect on k_2, decreasing the reactivity of II by a factor of 5. This observation also is consistent with the formulation of II as a hydroxo, monodentate thiolato complex, since a noncoordinated $-COO^-$ or $-COOCH_3$ substituent remote from the Cu(II)–S bond should have little effect on the activation process.[4]

Although suppression of S,O chelation markedly affects K_{a1}, k_1 upper limit estimates for $(tmpa)Cu^{II}$ adducts with cys-S^- and cme-S^- demonstrate that such chelation is not primarily responsible for their remarkable kinetic stabilities at low pH. The actual k_1 value reported for the glu-S^- adduct further underscores this point. Surprisingly large thermodynamic *stabilization* of the cupric oxidation state by coordinated thiolate sulfur (typical $\Delta E_{1/2}$ of −0.33 V)[15] should not be underestimated.[15,16] Considering the already unfavorable Cu(II,I) conversion in $Cu(tmpa)^{2+}$ ($E° = -147$ mV),[3] ligation of Cu(II) by RS^- ironically may reduce thermodynamic driving force to such an extent that thiolate oxidation no longer is favored.

Formation of the $(tmpa)Cu^{II}$–S-pen complex was the only reaction of this type slow enough to be observable on the stopped-flow apparatus. The mechanism of eq 4 is offered to account

$$^+H_3N\text{-pen-SH}\cdot(tmpa)Cu^{II} \xrightarrow{k_1} (tmpa)Cu\text{-S-pen-}NH_3^{2+} + H^+$$

$$^+H_3N\text{-pen-SH} \underset{\text{fast}}{\overset{K_{a1}}{\rightleftharpoons}} {}^+H_3N\text{-pen-}S^- + H^+$$

$$^+H_3N\text{-pen-}S^-\cdot(tmpa)Cu^{II}OH^+ \xrightarrow{k_2} (tmpa)(OH)Cu\text{-S-pen-}NH_3^+ \quad (4)$$

$$^+H_3N\text{-pen-}S^- + (tmpa)CuOH^+ \underset{\text{fast}}{\overset{K_{a2}}{\rightleftharpoons}} (tmpa)Cu\text{-}NH_2\text{-pen-}S^+ + H_2O$$

$$(tmpa)Cu\text{-}NH_2\text{-pen-}S^+ \xrightarrow{k_3} (tmpa)Cu\begin{smallmatrix}\diagup S^+ \\ \diagdown NH_2\end{smallmatrix}pen$$

pen = $(CH_3)_2\overset{|}{C}-\overset{|}{C}H-COO^-$

for the strong pH dependence of this complexation rate. The prevalent form of the $(tmpa)Cu^{II}$ reactant will be $Cu(tmpa)(OH)^+$ above pH 8, as ionization of coordinated water occurs with a pK_a of 7.40 (25 °C, I = 0.1 M);[10] the reactant species shown in the k_1 and k_2 steps represent outer-sphere complexes. Good agreement between kinetically determined (7.9) and literature (7.95)[17] penicillamine pK_{a1} values may be cited in support of this mechanism, and the $k_2 > k_1$ ordering is as expected for either I_a or I_d substitution processes. The relative slowness of the k_3 ring-closure step presumably reflects geometric rearrangements in the first coordination sphere required to accommodate the steric demands of the $-C(CH_3)_2S^-$ donor unit.

Except for the glutathione complexes, all of the $Cu(tmpa)^{2+}$ and $Cu(Me_6tren)^{2+}$ thiolate adducts considered here are greatly stabilized at the high-pH limit, where S,N or S,N,O chelation should be favored. Before the kinetic results are interpreted, a comparison of kinetically determined and literature $-NH_3^+$ substituent ionization constants will be useful (Table V). For this purpose, we assign all $(Me_6tren)Cu$–SR and the $(tmpa)Cu$–S-pen K_{a1} values to ionization of ammonium substituents with subsequent S,N, or S,N,O chelation; the K_{a2} ionization is attributed to this reaction in the $(tmpa)Cu^{II}$–S-cys and –S-cme adducts. We assume, therefore, that the difference between the ammonium group pK_a value in a Cu(II)-mercapto amino acid complex and the free amino acid (ΔpK_a with corresponding free energy change $\Delta\Delta G_a°$) is mainly due to Cu(II) ligation. The thermodynamic driving force associated with S,N chelation is largest for penicillamine in both the $Cu(tmpa)^{2+}$ and $Cu(Me_6tren)^{2+}$ series, and $\Delta\Delta G_a°$ is consistently more negative, by 1.5–2.6 kcal/mol, in the $(Me_6tren)Cu$–SR ammonium group ionization reactions.[18] Although $\Delta\Delta G_a°$ is notably less favorable for cysteine methyl ester,

(15) Addison, A. W.; Rao, T. N.; Sinn, E. *Inorg. Chem.* **1984**, *23*, 1957.
(16) Anderson, O. P.; Perkins, C. M.; Brito, K. K. *Inorg. Chem.* **1983**, *22*, 1267.
(17) Wilson, E. W., Jr.; Martin, R. B. *Arch. Biochem. Biophys.* **1971**, *142*, 445. To be precise, pK_{a1} corresponds to predominant, but not exclusive, ionization of –SH rather than a $-NH_3^+$ proton.
(18) The overall reaction for S,N chelation in $(tmpa)Cu^{II}$ adducts is thought to be

$$(tmpa)(OH)Cu\text{-}S\overset{\diagup NH_3^+}{\diagdown} \rightarrow (tmpa)Cu\begin{smallmatrix}\diagup S^+ \\ \diagdown NH_2\end{smallmatrix} + H_2O$$

whereas displacement of coordinated hydroxide ion is unlikely in the $(Me_6tren)Cu^{II}$ system.
(19) Li, N. C.; Manning, R. A. *J. Am. Chem. Soc.* **1955**, *77*, 5225.

a strong case cannot be made for S,N,O chelation by cysteine and penicillamine.

A substantial contribution of S,N chelation to kinetic Cu(II)–S bond stability is readily apparent from the k_2((Me$_6$tren)Cu–SR, (tmpa)Cu–S-pen) and k_3((tmpa)Cu–S-cys and –S-cme) rate constants. The presence of an optimal, five-membered S,N chelate unit certainly would stabilize the Cu(II) oxidation state and retard Cu(II)–S bond breaking in a reductive-elimination pathway leading to Cu(I) and a monodentate, N-bonded thiyl radical. Oxidation rate constants of S,N-bonded cysteine and its methyl ester are not greatly different in complexes with both (tmpa)Cu^{II} and (Me$_6$tren)Cu^{II}, but the reactivity of penicillamine is lower by more than 1 order of magnitude in both systems. This observation lends further support to a reductive-elimination mechanism requiring Cu(II)–S bond cleavage in the rate-determining step. Considering the surprisingly small rate of ring closure within (tmpa)Cu-NH$_2$-pen-S$^-$ and this reaction's ample thermodynamic driving force, even slower ring opening would be anticipated from microscopic reversibility considerations alone. The enhanced kinetic stability of Cu^{II}–S-pen certainly is not entirely related to S,N chelation, as shown most dramatically by the 30-fold difference in k_1(Cu(Me$_6$tren)$^{2+}$) for oxidation of cys-S$^-$ and pen-S$^-$.

Although stable five- or six-membered rings would not be readily formed from S-bonded glutathione (except through deprotonation of glycine or cysteine peptide nitrogens), the redox decay rate of (tmpa)Cu–S-glu is remarkably small and insensitive to pH. Extraordinarily large uncertainties in k_2, pK_{a1}, and pK_{a2} preclude a quantitative analysis of these parameters, but it is clear that the unusual relationship pK_{a1} > pK_{a2} must apply to achieve a good fit of the kinetic data to eq 3. In any case, the incorporation of cysteine into a protein environment seemingly is more important to Cu(II)–S bond stability than the reactions corresponding to the K_{a1}, K_{a2} ionizations. Partial encapsulation of the Cu(tmpa)$^{2+}$ unit by the polypeptide would hinder reductive elimination of thiyl sulfur in much the same way as chelation.

The consistently greater kinetic instability of (Me$_6$tren)Cu^{II}–SR adducts and the accessibility of only one kinetically relevant ionization in these complexes are the main points of contrast between the Cu(Me$_6$tren)$^{2+}$ and Cu(tmpa)$^{2+}$ kinetic results. The oxidizing strengths of the two Cu(II) centers appear to be similar, although a rigorous comparison is excluded by the irreversible cathodic wave of Cu(Me$_6$tren)$^{2+}$.[20] Steric interactions among the Me$_6$tren dimethylamino groups strongly hinder the rearrangement of Cu(Me$_6$tren)$^{2+}$ coordination geometry from trigonal bipyramidal toward square pyramidal or octahedral.[9,21] In contrast, four equatorial donor atoms are easily accommodated in complexes of tmpa and related polypyridylamine ligands.[10] For this reason, rearrangement of an *S*-thiolato, trigonal-bipyramidal (Me$_6$tren)Cu^{II}–SR complex into a six-coordinate thiolato, hydroxo species analogous to that proposed for the tmpa system (II) is unlikely, as is S,O chelation by the mercaptan. Considering the well-known high affinity of Cu(II) for nitrogen donor ligands,[22] the (Me$_6$tren)Cu–SR K_{a1} ionization most logically corresponds to S,N chelation of copper. Such chelation need not involve displacement of a Me$_6$tren dimethylamino group, as five-coordination could be retained by displacement of Cu(II) below the plane of these –N(CH$_3$)$_2$ donors.

Structural differences among the various mercaptide adducts of Cu(tmpa)$^{2+}$ and Cu(Me$_6$tren)$^{2+}$ complicate comparisons of rate constants between the two systems. Nevertheless, impressive relative stabilizations of the (tmpa)Cu^{II}–SR species by factors of ca. 10^4 and 10 are apparent at the low- and high-pH limits, respectively. Although increases in S(σ) → Cu(II) LMCT transition energies correlate in some instances with negatively tending $E°$(Cu(II,I)),[16] the (Me$_6$tren)Cu^{II}–SR rate data clearly show that such blue shifts do not necessarily result in greater *kinetic* stability of the Cu(II) oxidation state. Delocalization of thiolate sulfur negative charge over the tmpa pyridyl π systems could contribute to the reactivity difference between complexes with aliphatic and aromatic nitrogen donor atoms. Such delocalization would of course be analogous to that available through the imidazole group of physiological histidine ligands in the blue copper proteins.

Acknowledgment. Support of this research by the Robert A. Welch Foundation (Grant D-735) is gratefully acknowledged.

(20) Voltammograms generated from 1 mM Cu(Me$_6$tren)$^{2+}$ in pH 6.0, I = 0.1 M (MES) buffer showed a cathodic current maximum at –217 mV vs. NHE (50 mV/s sweep rate). $E°$(Cu(tmpa)$^{2+/+}$) is –147 mV under these conditions.[3] MES = morpholinoethanesulfonic acid.
(21) Coates, J. H.; Collins, P. R.; Lincoln, S. F. *J. Chem. Soc., Faraday Trans. 1* **1979**, *75*, 1236.
(22) Angelici, R. J. In "Inorganic Biochemistry"; Eichhorn, G. L., Ed.; Elsevier: Amsterdam, 1973; Vol. 1, p 63.

Contribution from the Department of Chemistry, Washington University, St. Louis, Missouri 63130

Electron Self-Exchange in Dicyanoiron Porphyrins

DABNEY WHITE DIXON,* MICHAEL BARBUSH, and ATAOLLAH SHIRAZI

Received August 15, 1984

Electron self-exchange rate constants have been measured for a series of dicyanoiron porphyrins by ^{1}H NMR. The rate constant for the Fe$^{II/III}$TPP(CN)$_2^{2-/-}$ system in Me$_2$SO-d_6 is 5.8 × 10^7 M^{-1} s^{-1} at 37 °C. Substituted tetraphenylporphyrins have slightly lower rate constants. Iron(II, III) protoporphyrin and deuteroporphyrin have self-exchange rate constants of ~1 × 10^7 M^{-1} s^{-1}. Assignments are given for the ^{1}H NMR resonances of the Fe(II) synthetic porphyrins and the ^{13}C resonances of CN$^-$ bound to Fe(II) porphyrins. The rate constants for cyanide exchange in the Fe(II) and Fe(III) systems are both <15 s^{-1}.

Introduction

The factors that control the rates of electron-transfer reactions of transition-metal complexes have been the focus of substantial interest.[1-3] In recent years this interest has been extended to biological systems.[4-6] One area of intense study has been the pathway of electron transfer in heme proteins.[7-9]

Most heme proteins have one edge of the heme exposed to solvent, and current thinking is that electron transfer generally takes place between the exposed heme edges of two proteins. The

(1) Lippard, S. J., Ed. "Progress in Inorganic Chemistry"; Wiley-Interscience: New York, 1983; Vol. 30.
(2) Cannon, R. D. "Electron Transfer Reactions"; Butterworths: Boston, 1980.
(3) Eberson, L. *Adv. Phys. Org. Chem.* **1982**, *18*, 79–185.
(4) Adman, E. *Biochim. Biophys. Acta* **1979**, *549*, 107–144.
(5) Handford, P. M.; Lee, W. K. *Inorg. Biochem.* **1981**, *2*, 78–150.
(6) Chance, B., DeVault, D. C., Frauenfelder, H., Marcus, R. A., Schrieffer, J. R., Sutin, N., Eds. "Tunneling in Biological Systems"; Academic Press: New York, 1979.
(7) (a) Ferguson-Miller, S.; Brautigan, D. L.; Margoliash, E. In "The Porphyrins"; Dolphin D., Ed.; Academic Press: New York, 1979; Vol. 7, pp 149–240. (b) Timkovich, R. *Ibid.* pp 241–294.
(8) Meyer, T. E.; Kamen, M. D. *Adv. Protein Chem.* **1982**, *35*, 105–212.
(9) Capaldi, R. A.; Darley-Usmar, V.; Fuller, S.; Millett, F. *FEBS Lett.* **1982**, *138*, 1–7.

0020-1669/85/1324-1081$01.50/0