MECHANISTIC FLEXIBILITY IN THE REDUCTION OF COPPER(II)
COMPLEXES OF ALIPHATIC POLYAMINES BY
MERCAPTO AMINO ACIDS

by

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CHAPTER I

INTRODUCTION

The stability of the copper(II)-mercaptide sulfur bond in oxidized blue copper proteins [1] should not be taken for granted, as this unit is highly susceptible to both substitutional and redox decay in model systems [2]. Indeed, the variety of redox decay pathways available to RS^- -Cu(II) adducts has not been fully appreciated [3]. Several contributions to the kinetic stability of the Cu(II)-mercaptide S bond, including steric crowding about the coordinated sulfur atom, chelation of copper by the mercaptan, and weak oxidizing strength of the Cu(II) center, were elucidated in a recent study of 1:1 adducts between cysteine (cys-SH) and the [tris(2-pyridylmethyl)amine] copper(II) ion ($\text{Cu}(\text{tmpa})^{2+}$) [3]. First order kinetics was observed (pH 6.4-11.2) with excess $\text{Cu}(\text{tmpa})^{2+}$ present, indicative of simple, intra-complex electron transfer. In contrast, [cys-SH]-dependent, second order decay curves pertain in the reaction of excess cysteine with $\text{Cu}(\text{tmpa})^{2+}$ at pH 7.8 [3]. Similar second order patterns, characteristic of binuclear Cu(II) intermediates, have been reported for the reactions of 2-mercaptosuccinic acid [4] and cysteine [5] with $\text{Cu}^{2+}(\text{aq})$.

Second order reactivity with respect to inner-sphere, S-bonded intermediates is reasonable in copper(II)-mercaptan redox reactions, as S-S bond formation and two-electron transfer may be concerted

within a binuclear[Cu(II)-SR]₂ activated complex [2,4]. On this basis, first order decay of Cu(II)-SR adducts should be regarded as atypical, except when oxidizing strength of the Cu(II) center permits the formation of metal-stabilized thiyl(RS[•]) radicals, i.e., in oxidations by Cu(dmp)₂²⁺ [6,7]. Understanding the relationship between RS⁻-Cu(II) adduct structure and preferred redox decay pathway (first or second order) is the motivation for the research described in this paper. Of particular interest are mechanism changes induced by variations in mercaptan concentration (at fixed [Cu(II)]₀), pH, and the copper(II) coordination sphere.

Copper(II)-aliphatic polyamine oxidants are considered here, including Cu(dien)²⁺, Cu(Me₅dien)²⁺, Cu(en)₂²⁺, Cu(dien)₂²⁺, and Cu(Me₆tren)²⁺. Reactions with excess cysteine, cysteine methyl ester, penicillamine, and glutathione are reported in the pH range 4-7. Emphasis is placed on cupric complexes with tridentate ligands (dien, Me₅dien, terpy), in which a single equatorial coordination position is available to attack by the mercaptan.

CHAPTER II

EXPERIMENTAL

Reagents and Solution Preparation

Reagent grade chemicals were used throughout, and solutions were prepared from triply distilled water. L-cysteine, free base (Sigma), DL-penicillamine (Aldrich), L-cysteine methyl ester hydrochloride (Sigma), and reduced glutathione (Sigma) were used as received after confirming their purity through iodometric titrations [8]. BES and Na_2dpmp^1 were supplied by Sigma. Literature methods were used to prepare $\text{Na}_4\text{Cu}(\text{tsp}) \cdot 2\text{H}_2\text{O}^1$ [9], $\text{Cu}(\text{dien})(\text{NO}_3)_2$ [10] $\text{Cu}(\text{dien})_2(\text{ClO}_4)_2$ [10], $[\text{Cu}(\text{terpy})(\text{H}_2\text{O})](\text{ClO}_4)_2$ [11], $\text{Cu}(\text{en})_2(\text{ClO}_4)_2$ [12], $\text{Cu}(\text{Me}_6\text{tren})(\text{ClO}_4)_2$ [13], and $\text{Cu}(\text{Me}_5\text{dien})(\text{ClO}_4)_2 \cdot 2\text{CH}_3\text{CN}$ [14]. The latter compound was converted to $\text{Cu}(\text{Me}_5\text{dien})(\text{ClO}_4)_2$ before use by washing with cold butanol and ether, followed by vacuum drying for 24 h. The purity of these copper complexes was confirmed through elemental analyses and comparisons of UV-visible spectra (Cary 17 or Perkin-Elmer Lambda 5 spectrophotometers) with those in the literature.

Anaerobic (N_2 -purged) mercaptan and oxidant solutions in $\text{I} = 0.2 \text{ M}$ sodium acetate or $\text{I} = 0.1 \text{ M}(\text{NaNO}_3)$, 5 mM BES buffers [3,7] were used immediately after preparation. Transfers of anaerobic solutions were made with Hamilton gastight syringes. Hydrogen ion concentrations were derived from pH readings (Brinkmann pH-104 meter) by

correcting for the activity coefficient of 0.2 M NaOAc or 0.1 M NaNO₃ [3].

Analyses

Copper(II) produced in the reaction of 1.0 mM Cu(dien)²⁺ with 0.10 mM cysteine at pH 5.03 (I = 0.2 M NaOAc) was converted to Cu(dpmp)₂³⁻ and assayed spectrophotometrically, as before [3].

Differential potentiometric titrations were performed to determine the extent to which the ligands of Cu(dien)²⁺ and Cu(Me₅dien)²⁺ dissociate through protonation within the pH range 4-6. Aliquots (50 mL) of 5.0 mM copper complex and distilled water were titrated with 0.02 M HClO₄. The hydrogen ion concentration consumed through ligand protonation was calculated at each volume of titrant added by comparison of pH readings for blank and copper complex solutions. After correcting for dilution, these [H⁺]-consumed figures could be expressed as plots of mole H⁺ consumed/mole Cu vs. pH of the complex solution.

Kinetic Measurements

Kinetic measurements were performed on a Durrum D-110 stopped flow apparatus, with inlet of anaerobic solutions through teflon needles. In most reactions investigated, intensely colored intermediates were formed such that 400 nm was a convenient monitoring wavelength. The reactions of Cu(dien)²⁺ (615 nm) and Cu(Me₅dien)²⁺ (632 nm) with glutathione were followed at the Cu(II) d-d band maxima, as no intense transient absorbance could be detected. Essentially colorless Cu(I) products were formed in all reactions examined.

Most absorbance-time traces were preserved as photographs from a Tektronix Model 564 B oscilloscope. Towards the end of these studies, data were transmitted to an Apple II Plus computer and stored on floppy disks through an interface system to be described elsewhere [15]. Copper(II) was the limiting reagent in all kinetic studies. Reported rate constants are the average of at least three trials. Clear criteria must be set out for the distinction between pseudo-first and second order decay curves, since authentic second order reactions may be mistaken for first order on the basis of slightly curved $\ln(A_t - A_\infty)$ vs. time correlations. Traces were accepted as unambiguously first order only when $\ln(A_t - A_\infty)$ vs. time plots were linear (minimum correlation coefficient of 0.995) over at least 90% of the overall absorbance change (0.1 minimum). Analogous linearity criteria were applied to second order analyses, based on eq. 1, where C_0 , l , $\Delta\epsilon$, and k_{obsd} represent the initial reactant concentration, the spectrophotometric path length (2 cm), the differential extinction coefficient associated with the redox reaction, and the second order observed rate constant, respectively. Pseudo first and second order rate constants (K_{obsd}) were calculated as linear least squares $-(\text{slope}) (\ln(A_t - A_\infty) \text{ vs. time})$ and $\text{slope} ((\text{intercept}) C_0)^{-1} (A_t - A_\infty)^{-1} \text{ vs time})$, respectively.

$$(A_t - A_\infty)^{-1} = (C_0 l \Delta\epsilon)^{-1} + K_{\text{obsd}} t / l \Delta\epsilon \quad (1)$$

CHAPTER III

RESULTS

Mercaptan Oxidations at pH 5.0

Dark black transient coloration was typically observed upon mixing copper(II) complexes of aliphatic polyamines with mercaptans. Decay of these transients at 25.0°C, $I = 0.2\text{ M}$ (NaOAc), pH 5.0 was investigated for the reactions of excess cysteine with $\text{Cu}(\text{dien})^{2+}$, $\text{Cu}(\text{Me}_5\text{dien})^{2+}$, $\text{Cu}(\text{terpy})^{2+}$, $\text{Cu}(\text{en})_2^{2+}$, $\text{Cu}(\text{dien})_2^{2+}$, and $\text{Cu}(\text{Me}_6\text{tren})^{2+}$. The oxidations of penicillamine, cysteine methyl ester, and glutathione by $\text{Cu}(\text{dien})^{2+}$ and $\text{Cu}(\text{Me}_5\text{dien})^{2+}$ were also examined under these conditions. Stoichiometric measurements showed that 1.0 molecule of cysteine is oxidized per $\text{Cu}(\text{dien})^{2+}$ ion reduced, as expected. Essentially no change in the intense 601 and 318 nm bands of $\text{Cu}(\text{tsp})^{4-}$ was observed upon adding very large excesses of cysteine to a 26 μM , pH 5.0 solution. Table 1 presents the cysteine oxidation rate data, while a comparison of the reactivities of $\text{Cu}(\text{dien})^{2+}$ and $\text{Cu}(\text{Me}_5\text{dien})^{2+}$ with the other mercaptans is set out in Table 2. Decay curves were cleanly first order in the reactions of cysteine with all oxidants except $\text{Cu}(\text{Me}_6\text{tren})^{2+}$, for which pseudo-second order kinetics pertained over the entire concentration range examined. No strongly-absorbing intermediate was detected in the cysteine- $\text{Cu}(\text{terpy})^{2+}$ reaction, and measurements at the d-d maximum of 675 nm showed that the reaction is too fast to follow by stopped flow techniques. Linear $(A_0 - A_\infty)^{-1}$ vs.

TABLE 1

Observed Rate Constants for the Oxidation of Cysteine by
Copper(II)-polyamine Complexes at pH 5.0^{a,b}

Oxidant	[cys-SH](mM)	$k_{\text{obsd}}(\text{s}^{-1} \text{ or } \text{M}^{-1}\text{s}^{-1})^{\text{c}}$
$\text{Cu}(\text{dien})^{2+ \text{d}}$	0.50	2.58(0.01)
	1.0	5.36(0.13)
	2.5	13.6(0.7)
	5.0	30.6(0.1)
	10.0	60.5(1.3)
	5.0	16.8(0.2); 18.9°C
	5.0	41.3(0.8); 31.9°C
	5.0	66.2(3.4); 39.4°C
$\text{Cu}(\text{Me}_5\text{dien})^{2+ \text{e}}$	5.0	17.1(0.4)
	10.0	35.8(0.7)
	20.0	65.8(2.1)
	30.0	86.2(3.2)
	40.0	117.0(7)
	50.0	138.0(13)
$\text{Cu}(\text{en})_2^{2+ \text{f}}$	4.0	37.0(0.4)
	8.0	46.6(2.2)
	12.0	55.9(1.6)
	16.0	61.8(2.4)
	20.0	70.9(4.7)
$\text{Cu}(\text{dien})_2^{2+ \text{f}}$	4.0	22.1(0.1)
	8.0	42.7(0.5)
	12.0	63.8(1.3)
	16.0	81.7(2.3)
	20.0	97.2(3.5)
$\text{Cu}(\text{Me}_6\text{tren})^{2+ \text{g}}$	1.0	5.1(0.2) $\times 10^4$
	2.5	1.5(0.1) $\times 10^5$
	5.0	3.2(0.3) $\times 10^5$
	5.0	3.2(0.3) $\times 10^5$
	7.5	5.6(0.8) $\times 10^5$
	10.0	7.1(0.5) $\times 10^5$

a. 25.0°C (unless otherwise noted), pH 5.0, I = 0.2 M (NaOAc).

b. Average deviations from the mean shown in parentheses.

c. Units are inverse seconds for all oxidants except $\text{Cu}(\text{Me}_6\text{tren})^{2+}$.

d. $C_0 = 0.10$ mM, $\lambda = 400$ nm.

e. $C_0 = 1.00$ mM, $\lambda = 330$ nm.

f. $C_0 = 0.40$ mM, $\lambda = 400$ nm.

g. $C_0 = 0.20$ mM, $\lambda = 400$ nm.

TABLE 2

Observed Rate Constants for the Oxidation of Penicillamine,
Cysteine Methyl Ester, and Glutathione by $\text{Cu}(\text{dien})^{2+}$
and $\text{Cu}(\text{Me}_5\text{dien})^{2+}$ at pH 5.0^{a,b}

<u>Mercaptan</u>	<u>[mercaptan](mM)</u>	$10^{-4}k_{\text{obsd}}(\text{M}^{-1}\text{s}^{-1})$	
		<u>$\text{Cu}(\text{dien})^{2+}$</u>	<u>$\text{Cu}(\text{Me}_5\text{dien})^{2+}$</u>
penicillamine	1.0	9.4(3.1)	0.90(0.06)
	2.0	1.4(0.1)	0.85(0.02)
	3.0	1.5(0.1)	0.78(0.05)
	4.0	1.5(0.1)	0.77(0.01)
	5.0	1.5(0.2)	0.77(0.02)
	10.0	2.1(0.1)	0.80(0.05)
cysteine methyl ester	1.0	52(5)	7.0(0.6)
	2.0	57(1)	7.9(0.6)
	3.0	49(1)	10.0(0.3)
	4.0	49(1)	
	5.0	48(1)	
	10.0	39(1)	
	10.0	39(1)	
glutathione	16.0	1.7(0.1)	0.72(0.02)
	24.0	1.0(0.1)	0.95(0.03)
	32.0	0.66(0.03)	1.2(0.1)
	40.0	0.55(0.01)	1.7(0.1)

- a. 25.0°C., pH 5.0, $I = 0.2 \text{ M}(\text{NaOAc})$. $C_0 = 0.10 \text{ mM}$, $\lambda = 400 \text{ nm}$
except in glutathione studies where $C_0 = 0.80 \text{ mM}$, $\lambda = 615 \text{ nm}$
($\text{Cu}(\text{dien})^{2+}$) or 632 nm ($\text{Cu}(\text{Me}_5\text{dien})^{2+}$).
- b. Average deviations from the mean shown in parentheses.

$[\text{cys} - \text{SH}]^{-1}$ plots demonstrated 1:1 mercaptan:Cu(II) stoichiometry [3,16] for the absorbing intermediates in the $\text{Cu}(\text{dien})^{2+}$ and $\text{Cu}(\text{Me}_5\text{dien})^{2+}$ reactions. Formation constants of $(1.3 \pm 0.2) \times 10^2 \text{M}^{-1}$ and $(5.5 \pm 0.1) \times 10^1 \text{M}^{-1}$ derived for cysteine adducts with $\text{Cu}(\text{dien})^{2+}$ and $\text{Cu}(\text{Me}_5\text{dien})^{2+}$, respectively, may be compared with that of $1.21 \times 10^2 \text{M}^{-1}$ characteristic of $(\text{tmpa}) \text{Cu}(\text{S-cys})^+$ under the same conditions [3].

Designating the absorbing intermediate as I, the results in Table 1 may be understood in terms of the generalized rate law:

$$\begin{aligned} -d[\text{I}]/dt &= (k_0 + k_1 [\text{cys} - \text{SH}]) [\text{I}]^a \\ &= k_{\text{obsd}} [\text{I}]^a \end{aligned} \quad (2)$$

where a is 1 in all reactions except that of $\text{Cu}(\text{Me}_6\text{tren})^{2+}$ ($a = 2$). Least squares analysis of linear k_{obsd} vs. $[\text{cys} - \text{SH}]$ correlations gave k_1 rate constants of $(6.1 \pm 0.1) \times 10^3 \text{M}^{-1} \text{s}^{-1}$ ($\text{Cu}(\text{dien})^{2+}$), $(2.7 \pm 0.1) \times 10^3 \text{M}^{-1} \text{s}^{-1}$ ($\text{Cu}(\text{Me}_5\text{dien})^{2+}$), $(2.1 \pm 0.1) \times 10^3 \text{M}^{-1} \text{s}^{-1}$ ($\text{Cu}(\text{en})_2^{2+}$), $(4.7 \pm 0.2) \times 10^3 \text{M}^{-1} \text{s}^{-1}$ ($\text{Cu}(\text{dien})_2^{2+}$), and $(7.5 \pm 0.3) \times 10^7 \text{M}^{-2} \text{s}^{-1}$ ($\text{Cu}(\text{Me}_6\text{tren})^{2+}$). Only in the case of $\text{Cu}(\text{en})_2^{2+}$ was the intercept of this analysis outside of experimental error from zero, yielding $k_0 = (3 \pm 1) \times 10^1 \text{s}^{-1}$. A temperature variation study of the $\text{Cu}(\text{dien})^{2+}$ - cysteine redox reaction rate was carried out in the interval 18.9 - 39.4°C (Table 1). Activation parameters based on an excellent linear Eyring plot of $\ln k(=k_{\text{obsd}}/[\text{cys} - \text{SH}])/T$ vs. $1/T$ are: $H^\ddagger = 11.6 \pm 0.5 \text{ kcal/mole}$, $S^\ddagger = -3 \pm 2 \text{ eu}$ (pH 5.0).

In marked contrast to the cysteine oxidations, second order decay curves pertained in the reactions of all other mercaptans with $\text{Cu}(\text{dien})^{2+}$ and $\text{Cu}(\text{Me}_5\text{dien})^{2+}$ at pH 5.0. Furthermore, second order

penicillamine and cysteine methyl ester oxidation rate constants are largely insensitive to the reductant concentration above 2 mM. It should be noted that cysteine methyl ester is more reactive than penicillamine by factors of ca. 30 and 10 in adducts with $\text{Cu}(\text{dien})^{2+}$ and $\text{Cu}(\text{Me}_5\text{dien})^{2+}$, respectively. Consistent with the cysteine oxidation results, $\text{Cu}(\text{dien})^{2+}$ is reduced more rapidly than $\text{Cu}(\text{Me}_5\text{dien})^{2+}$, by factors of ca. 2 (penicillamine) and 5 (cysteine methyl ester). The limited data set reported for the cysteine methyl ester - $\text{Cu}(\text{Me}_5\text{dien})^{2+}$ reaction reflects poor second order analyses at reductant concentrations larger than 3 mM. There was some indication that first order decay of the absorbing intermediate is favored at very high concentrations of this mercaptan. Surprisingly, no strongly-absorbing intermediates were observed in the reactions of glutathione with $\text{Cu}(\text{dien})^{2+}$ and $\text{Cu}(\text{Me}_5\text{dien})^{2+}$ at pH 5.0, making it necessary to follow the kinetics at d-d band maxima, with very large excesses of mercaptan present. At $[\text{glutathione}] = 8.0 \text{ mM}$, first order reduction of $\text{Cu}(\text{dien})^{2+}$ ($k_{\text{obsd}} = 1.25 \pm 0.03 \text{ s}^{-1}$) occurs, as indicated by excellent linear $\ln (A_t - A_\infty)$ vs. time plots. When glutathione was in greater than 10-fold excess over the oxidant, however, a second order analysis was appropriate, yielding k_{obsd} values that are more sensitive to mercaptan concentration than the analogous penicillamine and cysteine methyl ester parameters. Considering the limited mercaptan concentration range which could be covered, a more detailed quantitative analysis of k_{obsd} - $[\text{glutathione}]$ profiles is not justified.

pH Dependence Studies

Decay of intermediates formed between cysteine and $\text{Cu}(\text{dien})^{2+}$ or $\text{Cu}(\text{Me}_5\text{dien})^{2+}$ was found to be first order with respect to both cysteine and the intermediate throughout the pH range 4-6, $I = 0.2 \text{ M}$ (NaOAc), 25°C . The overall (400 or 330 nm) absorbance change falls off sharply with decreasing pH in both reactions (Table 3). In contrast, k_1 decreases only modestly with increasing $[\text{H}^+]$. The comparison of k_1 rate constants with potentiometric differential titration data in Table 3 shows that reactivity decreases reflect the partial protonation of the polyamine ligand. Thus, the 60% decrease in k_1 ($\text{Cu}(\text{dien})^{2+}$) between pH 5.68 and 4.00 correlates with an increase in protonation fraction from 0.04 to 1.28. Similarly, the attenuation in k_1 ($\text{Cu}(\text{Me}_5\text{dien})^{2+}$) of only 30% from pH 6.00 to 4.00 reflects the greater resistance of the Me_5dien ligand towards protonation ($\text{PF}(\text{pH } 4.00) = 0.27$). A simple mechanism involving the formation of only two intermediates (parent and protonated) in the pH 4-6 range is not adequate for the quantitative understanding of $k_1 - [\text{H}^+]$ profiles, as non-linear least squares fits of the data to the appropriate rate expression [17] were not successful. The reduction of $\text{Cu}^{2+}(\text{aq})$ ($1.0 \text{ mM Cu}(\text{OAc})_2$) by 10.0 mM cysteine at pH 4.0, $I = 0.2 \text{ M}$, 25°C proceeds through an intermediate with second order decay rate constant of $3.6 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$. We conclude, therefore, that the dien and Me_5dien ligands are retained in all $\text{Cu}(\text{II})$ -S-cys intermediates even at the low pH limit of our studies.

TABLE 3

pH Dependence of Cysteine Oxidation Rate Constants^{a,b}

pH	—Cu(dien) ²⁺ —			—Cu(Me ₅ dien) ²⁺ —		
	10 k_1 (M ⁻¹ s ⁻¹)	ΔA_{400}^c	P _F ^d	10 k_1 (M ⁻¹ s ⁻¹)	ΔA_{330}^c	P _F ^d
4.00	2.92(0.08)	0.235	1.28	2.1 (0.1)	0.109	0.27
4.30	3.09(0.04)	0.287	0.78	2.2 (0.1)	0.171	0.12
4.67	3.83(0.03)	0.404	0.37	2.2 (0.3)	0.312	0.06
5.00	6.1(0.1)		0.19	2.7 (0.1)	0.450	0.04
5.23	6.6(0.3)	0.856	0.12	2.6 (0.1)	0.772	0.03
5.68	7.3(0.1)	1.31	0.04	3.0 (0.1)	1.11	0.02
6.00				3.0 (0.1)	1.25	0.01

a. 25.0°C, I = 0.2 M, C₀ = 1.0 mM, [cys-SH] = 10.0 mM. k_1 calculated as $k_{\text{obsd}}/[\text{cys-SH}]$. $\lambda = 400$ nm (Cu(dien)²⁺) or 330 nm (Cu(Me₅dien)²⁺).

b. Average deviations from the mean shown in parentheses.

c. $A = (A_0 - A_\infty)$ calculated as the intercept of $\ln(A_t - A_\infty)$ vs. time plots. Pathlength = 2 cm.

d. P_F = mole H⁺ consumed/mole Cu(II) (protonation fraction), obtained from potentiometric differential titrations (see Experimental section).

Cysteine Oxidations at pH 7.0

The oxidations of cysteine by $\text{Cu}(\text{dien})^{2+}$, $\text{Cu}(\text{Me}_5\text{dien})^{2+}$, and $\text{Cu}(\text{terpy})^{2+}$ were examined at 25.0°C, pH 7.0, $I = 0.1 \text{ M NaNO}_3$. Under these conditions, the reactivities of all three oxidants differ drastically from the pH 4-6 results. Second order decay of black intermediates were found in each case throughout the entire mercaptan concentration range of 0.20 - 100 mM. The contrast in $\text{Cu}(\text{terpy})^{2+}$ behavior is particularly notable, considering the apparent absence of a strongly-absorbing intermediate below pH 6. Rate and spectrophotometric data pertaining to these pH 7.0 cysteine oxidations are presented in Table 4.

Unlike the pH 5.0 observations presented earlier, 400nm ($\text{Cu}(\text{dien})^{2+}$) and 465 nm ($\text{Cu}(\text{Me}_5\text{dien})^{2+}$, $\text{Cu}(\text{terpy})^{2+}$) absorbance changes at pH 7.0 did not vary with $[\text{cys-SH}]$ as expected for the intermediacy of 1:1 mercaptan $\text{Cu}(\text{II})$ complexes. These ΔA_λ values generally were significantly larger than the analogous pH 5 results, and were, for the most part, insensitive to cysteine concentration changes. On this basis, several intermediates may contribute to the observed kinetics at any particular $[\text{cys-SH}]$. This contingency is not covered by the second order analysis of eq. 1, in which the initial concentration of a single intermediate (C_0) is assumed to be equal to that of the $\text{Cu}(\text{II})$ parent ion. Because of this complication, trends in k_{obsd} with $[\text{cys-SH}]$ and C_0 variations may reflect perturbations in the distribution of inner-sphere, S-bonded intermediates as well as the participation of non-coordinated cysteine as a reductant. The uncertainties in second order k_{obsd} values are proportional to ΔA_λ , as

TABLE 4

Cysteine Oxidation Rate Constants, pH 7.0^{a,b}

[cyc-SH] (mM)	C_O (mM) ^c	λ (nm) ^d	ΔA_{λ}^e	$10^{-4}k_{\text{obsd}}(\text{M}^{-1}\text{s}^{-1})$	$10^{-4}k_{\text{obsd}}(\text{M}^{-1}\text{s}^{-1})$	$10^{-4}k_{\text{obsd}}(\text{M}^{-1}\text{s}^{-1})$
					$[\text{Cu}(\text{dien})^{2+}]$	$[\text{Cu}(\text{Me}_5\text{dien})^{2+}]$
						$[\text{Cu}(\text{terpy})^{2+}]$
0.20	0.20	400		2.14(0.05)		0.12(0.01)
2.0	0.20	400		8.1(0.4)		4.9(0.3)
5.0	0.20	400		6.8(0.8)		0.66(0.05)
10.0	0.20	400		6.8(0.3)		3.0(0.1)
15.0	0.20	400		6.5(0.1)		3.1(0.6)
20.0	0.20	400		8.0(0.3)		3.8(0.1)
50.0	0.20	400		10.1(0.6)		9.5(0.2)
100.0	0.20	400		16.2(5.3)		20.0(1.8)
10.0	0.01	400		51.0(9)		
10.0	0.10	400		8.0(1.7)		0.30(0.01)
10.0	0.20	400		6.1(2.9)		11.5(1.7)
10.0	0.30	400		8.4(4.8)		8.2(1.1)
10.0	0.40	400		9.0(1.2)		6.1(1.1)
10.0	0.60	400		4.1(0.5)		7.5(1.3)
10.0	1.00	400		4.7(0.8)		6.8(0.5)
10.0	5.00	400		0.011(0.001)		14.7(3.1)
10.0	10.00	400		0.005(0.001)		27.7(2.7)
1.0	0.10	350	3.52	7.9(0.4)		
1.0	0.10	375	0.68	8.9(1.5)		
1.0	0.10	400	1.76	8.3(1.4)		
1.0	0.10	425	0.29	10.0(1.0)		
1.0	0.10	450	0.25	8.7(0.8)		
1.0	0.10	500	0.22	7.4(1.8)		
1.0	0.10	600	0.12	4.2(0.1)		

TABLE 4 - Continued

-
- a. 25.0°C, pH 7.0, I = 0.1 M (NaNO₃).
 - b. Average deviations from the mean shown in parentheses.
 - c. Initial oxidant concentration.
 - d. Monitoring wavelength.
 - e. $\Delta A_{\lambda} = (A_0 - A_{\infty})$ calculated from the intercept of $(A_t - A_{\infty})^{-1}$ vs. time plots. Pathlength = 2 cm.
 - f. $\lambda = 465$ nm, $C_0 = 0.20$ mM.

the small $(C_0/\Delta\epsilon)^{-1}$ term in eq. 1 and corresponding standard deviation may become comparable when C_0 and $\Delta\epsilon$ are large. As a result, the uncertainties in pH 7.0 k_{obsd} values are significantly larger than those characteristic of pH 5 second order rate constants.

Rate variations with increasing [cys-SH] are complicated, but similar in the reductions of $\text{Cu}(\text{dien})^{2+}$, $\text{Cu}(\text{Me}_5\text{dien})^{2+}$ and $\text{Cu}(\text{terpy})^{2+}$. An initial rate increase in the $[\text{cys-SH}]_0/C_0 = 1-10$ interval is followed by a moderate decrease to a plateau when this ratio is 10-100. Finally, k_{obsd} again increases with [cys - SH] at very high reductant concentrations. In the plateau region, $\text{Cu}(\text{dien})^{2+}$ and $\text{Cu}(\text{Me}_5\text{dien})^{2+}$ reduction rates are identical to within experimental error and approximately twice that of $\text{Cu}(\text{terpy})^{2+}$. Measurements on $\text{Cu}(\text{dien})^{2+}$ at $[\text{cys - SH}]_0 = 1.0 \text{ mM}$ showed that the adduct with cysteine exhibits an absorbance maximum near 400 nm; k_{obsd} is wavelength-independent between 350 and 500 nm. Holding $[\text{cys - SH}]_0$ constant at 10.0 mM, k_{obsd} was found to be independent of C_0 ($\text{Cu}(\text{dien})^{2+}$) only within a narrow range (0.10 - 0.40 mM), with much smaller rates being found at high C_0 values approaching the stoichiometric level for cysteine oxidation. From this it is clear that several absorbing intermediates contribute to the observed second order rate constant, as k_{obsd} would be independent of C_0 (at constant [cys - SH]) if only a single transient was involved. Considering the $[\text{cys - SH}]_0$ and C_0 variation results together, it is apparent that a single intermediate, which decays with [cys - SH]-independent rate constant of $(8 \pm 1) \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$, predominates at 10 to 100-fold excesses of reductant over $\text{Cu}(\text{dien})^{2+}$.

Cysteine oxidation by $\text{Cu}(\text{dien})^{2+}$ at pH 6.5 ($I = 0.1 \text{ M}$ (NaOAc)) proceeds with $k_{\text{obsd}} = (4.0 \pm 0.4) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ (25°C) when $[\text{cys} - \text{SH}]_0 = 3.0\text{--}10.0 \text{ mM}$ ($C_0 = 0.10 \text{ mM}$). Neither first nor second order analytical plots were acceptably linear at lower reductant concentrations. A similar result was obtained from the cysteine- $\text{Cu}(\text{dien})_2^{2+}$ reaction under these conditions ($k_{\text{obsd}} = 3.2 \pm 0.3) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$; $[\text{cys} - \text{SH}] = 6.0 - 15.0 \text{ mM}$, $C_0 = 0.30 \text{ mM}$). Both first and second order analytical plots were non-linear at all reductant concentrations in pH 6.0 and 6.3 studies of the cysteine- $\text{Cu}(\text{dien})^{2+}$ reaction, indicative of a transition region to the clean first order behavior observed at lower pH.

CHAPTER IV

DISCUSSION

Our kinetic results demonstrate a high degree of mechanistic flexibility in mercaptan oxidations by copper(II) complexes. Indeed, major changes in the rate law could be induced by relatively minor variations in mercaptan or oxidant structure, pH, or even reductant concentration. The intensely-colored transients observed in most reactions are readily assigned as S-bonded, innersphere complexes between the mercapto amino acids and copper(II) polyamine oxidants. Similar $S(3p\sigma) - Cu(II) (3d\sigma)$ charge transfer transitions have been reported in numerous other studies of the $RS^- - Cu(II)$ bond [3,18]. Before discussing mechanisms, it will be useful to summarize the various rate laws observed for the decay of $RS^- - Cu(II)$ transients in the presence of excess mercaptan:

1. first order in transient, zeroth order in mercaptan
(cysteine- $Cu(en)_2^{2+}$ and $Cu(dmp)_2^{2+}$ [7] reactions at pH 5);
2. first order in transient and mercaptan (cysteine- $Cu(dien)^{2+}$, $Cu(Me_5dien)^{2+}$, $Cu(en)_2^{2+}$, $Cu(dien)_2^{2+}$ reactions at pH 5);
3. second order in transient (approximately), zeroth order in mercaptan (penicillamine, cysteine methyl ester, glutathione reactions with $Cu(dien)^{2+}$ and $Cu(Me_5dien)^{2+}$ at pH 5; cysteine- $Cu(dien)^{2+}$, $Cu(Me_5dien)^{2+}$, $Cu(terpy)^{2+}$ reactions at pH 7);

4. first order in mercaptan, second order in transient
 (cysteine-Cu(Me₆tren)²⁺ reaction at pH 5; 2-mercaptosuccinic
 acid reaction with Cu²⁺(aq) [4].

Uncertainty concerning the composition and distribution of RS⁻ - Cu(II) intermediates unfortunately precludes a detailed mechanistic discussion of some reactions reported here. Nevertheless, the (A₀ - A_∞)⁻¹ vs. [cys - SH]⁻¹ analyses of cysteine oxidations by Cu(dien)²⁺ and Cu(Me₅dien)²⁺ demonstrate that 1:1 adducts are formed at pH 5. Two cysteine molecules per Cu(II) unit are present, therefore, in the activated complexes associated with first order mercaptan and transient dependences. The reactions of coordinated thiols with one-equivalent oxidants typically proceed through relatively stable disulfide anion radical intermediates [2]. This principle is applied in the mechanism proposed for cysteine oxidations by all copper(II) polyamine complexes (LCu(II)) except Cu(Me₆tren)²⁺ at pH 4-6 (eq. 3).

The near-zero S[‡] characteristic of the cysteine-Cu(dien)²⁺ reaction implies that the associative contribution from the incoming cysteine molecule balances dissociative terms owing to proton release or reductive elimination of the coordinated mercaptan.

The pH 5 k₁ rate constants for reduction of Cu(dien)²⁺, Cu(Me₅dien)²⁺, Cu(en)₂²⁺, and Cu(dien)₂²⁺ by cysteine are remarkably

similar, falling within only a three-fold range of $(2.1 - 6.1) \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$. Particularly surprising is the very small reactivity advantage of $\text{Cu}(\text{dien})^{2+}$ over $\text{Cu}(\text{dien})_2^{2+}$, in which all six octahedral coordination positions are initially blocked by chelate nitrogen atoms [10]. Substitution of N for S in the coordination sphere of $\text{Cu}(\text{dien})_2^{2+}$ must be both fast and thermodynamically favorable in order for the above mechanism to pertain. The steric influence of $\text{Cu}(\text{Me}_5\text{dien})^{2+}$ N-methyl substituents evidently is very small in the approach of cys-SH towards the coordinated S atom in $(\text{Me}_5\text{dien})\text{Cu-S-cys}^+$.

The bimolecular rate-determining step in cysteine oxidations by $\text{Cu}(\text{dien})^{2+}$ and related complexes at pH 5 contrasts with the simple unimolecular decay of $\text{Cu}(\text{dmp})(\text{S-cys})^+$ and $\text{Cu}(\text{dmp})_2(\text{cys-SH})^{2+}$, with respective rate constants of 4.8×10^1 and $4 \times 10^1 \text{ s}^{-1}$, under the same conditions [7]. The larger oxidizing strength of Cu(II)-dmp complexes [7] presumably permits the direct formation of thiyl radicals without the necessity to stabilize the leaving group through disulfide radical formation. The observations of Davies and Loose on Cu(II)-dmp oxidations of thiocyanate ion and thiourea [6] are consistent with this reasoning, as a pathway similar to eq. 3 is followed by these weak, sulfur-containing reductants.

The modest kinetic pH dependences observed in the cysteine reductions of $\text{Cu}(\text{dien})^{2+}$ and $\text{Cu}(\text{Me}_5\text{dien})^{2+}$ most likely reflect partial protonation of the polyamine ligands. Thus, the larger rate decrease for $\text{Cu}(\text{dien})^{2+}$ from pH 5.68 to 4.00 correlates with a pH 4.00 protonation fraction of 1.28 as compared with only 0.27 for $\text{Cu}(\text{Me}_5\text{dien})^{2+}$.

Comparatively high sensitivity of ΔA_λ to pH is anticipated from the effect of $[H^+]$ on the K_f pre-equilibrium position. Stability constant measurements have provided no evidence for partially protonated dien or Me_5dien ligands in $Cu(II)$ complexes [19,20]. Singly-protonated polyamines could reasonably occur in mixed complexes with cysteine, however, with mercaptan S and O (carboxylate) donor atoms occupying the equatorial coordination positions left open by bidentate $dien-H^+$ or $Me_5dien-H^+$ ligands. Full release of the tridentate amine ligands clearly does not occur to an appreciable extent even at pH 4, as the first order transient decay patterns at this limit conflict with the second order reactivity of Cu^{2+} (aq) with cysteine.

The sensitivity of preferred $Cu(II)$ reduction pathway to small modifications in mercaptan structure is dramatically illustrated by the pH 5.0 oxidations of cysteine methyl ester, penicillamine, and glutathione, in which adducts formed with $Cu(dien)^{2+}$ and $Cu(Me_5dien)^{2+}$ undergo second order decay at rates insensitive, rather than proportional, to mercaptan concentration. An additional important contrast lies in the absence of a strongly-absorbing transient in the glutathione oxidations. Two-electron reduction of a binuclear $Cu(II)$ center concerted with disulfide formation apparently is preferred over the intermediacy of $RSSR^-$ when cysteine is esterified, alkylated at the β carbon atom, or incorporated into a tripeptide. Within this class of reductants, the unique capability of cysteine to chelate (S, O) effectively under acidic conditions without sacrificing the steric accessibility of the coordinated sulfur atom is notable. Such

chelation is thought to contribute strongly towards the stability of $\text{Cu}(\text{tmpa})(\text{S-cys})^+$ within the pH 4-6 range [3]. The , -dimethyl groups of penicillamine should strongly inhibit attack at the coordinated sulfur atom in a disulfide radical-forming mechanism such as eq. 3. Di- -mercapto bridging in a binuclear $\text{Cu}(\text{II})$ activated complex would be sterically preferable. Such bridging is even more attractive in the cysteine methyl ester and glutathione oxidations, as carboxylate groups would no longer compete well for the two coordination positions per $\text{Cu}(\text{II})$ unit needed to establish a strong di- -mercapto bridge. The 30-fold second order reactivity advantage of cysteine methyl ester over penicillamine in reactions with $\text{Cu}(\text{dien})^{2+}$ contrasts with the factor of 2 difference between first order redox decay rate constants of adducts with $\text{Cu}(\text{dmp})^{2+}$ [7]. This observation, coupled with rate attenuations induced by the N-methyl groups of Me_5dien , confirms the expected sensitivity of the binuclear activated complex to steric interactions among the $\text{Cu}(\text{II})$ monomer and mercaptide bridging units. Steric inhibition of complexation between sarcosine (N-methylglycine) and $\text{Cu}(\text{dien})^{2+}$ derivatives with two or more alkyl substituents on the terminal (1,7) nitrogen atoms has been reported [21].

Weak sulfur-to-copper(II) charge transfer bands are characteristic of mercaptide complexes when rigid, tetradentate, macrocyclic ligands monopolize the equatorial coordination positions [22-24]. Our observation that cysteine causes no change in the absorption spectrum of $\text{Cu}(\text{tsp})^{4-}$ further confirms this behavior. Although completely unexpected, axial sulfur coordination presumably accounts for the

absence of an optically-detectable transient in reactions of glutathione with $\text{Cu}(\text{dien})^{2+}$ and $\text{Cu}(\text{Me}_5\text{dien})^{2+}$.

The trigonal bipyramidal [13] complex $\text{Cu}(\text{Me}_5\text{tren})^{2+}$ stands out as the only oxidant to form a cysteine adduct which decays at a rate second order in transient and first order in excess cysteine. In this respect, the reaction resembles that of $\text{Cu}^{2+}(\text{aq})$ with 2-mercaptosuccinic acid [4]. Interestingly, straightforward first order redox decay of $(\text{Me}_6\text{tren})\text{Cu-S-cys}^+$ occurs at higher pH (7-10) with excess oxidant present [25]. Steric repulsions among N-methyl equatorial substituents make it difficult for $\text{Cu}(\text{Me}_6\text{tren})^{2+}$ to accept more than one additional ligand donor atom, in an axial position, or to rearrange into a pseudo-octahedral structure analogous to that documented for complexes of the parent tren ligand [13]. Because of these steric restraints, cysteine cannot easily chelate Cu(II) in $\text{Cu}(\text{Me}_6\text{tren})^{2+}$, and a coordinated sulfur atom would be protected from attack by a second cysteine molecule, as is necessary in the RSSR^- -forming pathway preferred by the less hindered oxidants. One mechanism consistent with the observed rate law (eq. 4) involves formation of a coordinated disulfide in the rate-determining step, coupled with attack of a non-coordinated cysteine molecule on a vacated coordination position to stabilize the $(\text{Me}_6\text{tren})\text{Cu(I)}$ product.

The structure of the activated complex is quite uncertain, as mercaptide bridging between Cu(II) centers is ruled out by the above-mentioned steric restraints.

A transition from first to second order transient dependence in cysteine oxidations by $\text{Cu}(\text{dien})^{2+}$ and $\text{Cu}(\text{Me}_5\text{dien})^{2+}$ accompanies a pH increase from 5 to 7. Potentiometric [19] and spectrophotometric [20] evidence has been reported for the ionization of coordinated water above pH 6, to give $\text{Cu}(\text{dien})(\text{OH})^+$ and $\text{Cu}(\text{Me}_5\text{dien})(\text{OH})^+$; $\log K_{\text{OH}}$ values are 5.17 and 5.14 (25°C. $I = 0.13 \text{ M}$), respectively [19]. Dimerization of $\text{RS}^- - \text{Cu}(\text{II})$ adducts through mono or di- -hydroxo bridging would account for the favorability of second order decay at high pH. Speciation unfortunately cannot be defined from the pH 7 kinetic results, but C_0 and $[\text{cys} - \text{SH}]$ variation experiments demonstrated that single intermediates, with $[\text{cys-SH}]$ -independent decay rates, predominate with 10 to 100-fold excesses of reductant present.

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APPENDIX A
ABBREVIATIONS

dmp:	2,9-dimethyl-1,10-phenanthroline
dien:	diethylenetriamine
Me ₅ dien:	1, 1, 4, 7, 7-pentamethyldiethylenetriamine
en:	ethylenediamine
Me ₆ tren:	2,2', 2''-tris(N,N-dimethylamino)triethylamine
terpy:	2, 2', 2''-terpyridine
BES:	N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid
Na ₂ dpmp:	disodium 2,9-dimethyl-1,10-phenanthroline-4,7-bis(benzenesulfonic acid)
tsp:	4, 4', 4'', 4'''-tetrasulfophthalocyanine.

APPENDIX B

k_{obsd} VERSUS CONCENTRATION FIGURES

FIGURE 1. k_{obsd} Versus Concentration for the Reaction of $\text{Cu}(\text{dien})^{2+}$ with Cysteine at $\text{pH} = 5.0$. $C_0 = 0.10 \text{ mM}$, $\lambda = 400 \text{ nM}$, 25.0°C , $I = 0.2 \text{ M (NaOAC)}$.

FIGURE 2. k_{obsd} Versus Concentration for the Reaction of $\text{Cu}(\text{Me}_5\text{dien})^{2+}$ With Cysteine at $\text{pH} = 5.0$. $C_0^2 = 0.10\text{mM}$, $\lambda = 400 \text{ nm}$, 25.0°C , $I = 0.2 \text{ M}$ (NaOAc).

FIGURE 3. k_{obsd} Versus Concentration for the Reactions of Cu(en)_2^{2+} and Cu(dien)_2^{2+} With Cysteine at pH = 5.0. 25.0°C, I = 0.2 M (NaOAC), $C_0 = 0.40$ mM, $\lambda = 400$ nm.

FIGURE 4. k_{obsd} Versus Concentration for the Reactions of $\text{Cu}(\text{Me}_6\text{tren})^{2+}$ With Cysteine at pH = 5.0.
 $C_0^2 = 0.20 \text{ mM}$, $\lambda = 400 \text{ nm}$, 25°C , $I = 0.2$ (NaOAC)

FIGURE 5. k_{obsd} Versus Concentration for the Reactions of $\text{Cu}(\text{dien})^{2+}$ With Cysteine at $\text{pH} = 7.0$. $C_0 = 0.20$ (mM), $\lambda = 400$ nm, 25°C , $I = 0.1$ M (NaNO_3).

APPENDIX C
EYRING PLOT FIGURE

FIGURE 6. Eyring Plot for the Reaction of $\text{Cu}(\text{dien})^{2+}$ With Cysteine at $\text{pH} = 5.0$. ($k = k_{\text{obsd}}/[\text{cys-SH}]/T$).