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An S-Bonded Adduct of Cysteine with the [Tris(2-pyridylmethyl)amine]copper(II) Ion

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Received March 2, 1983

A 1:1, S-bonded adduct rapidly forms upon mixing cysteine with the [tris(2-pyridylmethyl)amine]copper(II) ion ($Cu(tmpa)^{2+}$) at pH 4.0–11.2, resulting in an intense absorption maximum at 396 nm ($\Delta\epsilon$ 6.0 × 10^3 M^{-1} cm^{-1}). The formation constant of this RS^-–Cu(II) complexation reaction is 4.7 × 10^5 M^{-1} (25.0 °C, I = 0.1 M ($NaNO_3$)). With excess $Cu(tmpa)^{2+}$ present, first-order decay curves were observed for the reduction of copper(II) by the coordinated mercaptide group, yielding $Cu(tmpa)^+$ and cystine. The pH dependence of this redox rate (pH 6.4–11.2) may be understood in terms of three distinct reactant species (I, II, III; decay rate constants k_1, k_2, k_3, respectively) related through two ionization equilibria (K_{a1}: I ⇄ II + H^+; K_{a2}: II ⇄ III + H^+; $k_1 \simeq 0$, k_2 = 2.0 s^{-1}, k_3 = 4.1 × 10^{-2} s^{-1}, pK_{a1} = 8.33, pK_{a2} = 8.52 (25.0 °C, I = 0.1 M ($NaNO_3$))). Species I and III, assigned as S,O- and S,N-bonded cysteine chelates, respectively, are considerably more resistant to internal electron transfer than is complex II, thought to contain monodentate cysteinato-*S* and hydroxo ligands. Activation parameters of $k = k_2K_{a1}$ ($\Delta H^{\ddagger}$ = 17.1 kcal/mol, $\Delta S^{\ddagger}$ = −39 eu) indicate large enthalpic and entropic barriers to the conversion of complex I to II, followed by internal electron transfer. The remarkable stability of S-bonded cysteine–$Cu(tmpa)^{2+}$ adducts in aqueous solution is accounted for in terms of the weak oxidizing strength of the Cu(II) center ($E°$ ($Cu(tmpa)^{2+/+}$) = −147 mV vs. NHE (25 °C, pH 6.0, I = 0.1 M (MES)) by cyclic voltammetry) and hindrance of mercaptide radical coupling linked to steric crowding about the coordinated sulfur atom.

Introduction

The fascinating chemistry of the blue copper proteins has inspired considerable interest in the electronic structure and reactivity of compounds having a copper(II)–mercaptide sulfur bond.[1] While numerous attempts have been made to model the distinctive[2] electronic and ESR spectra of metalloprotein type-1 cupric sites, Cu(II)–thiolate complexes are generally unstable with respect to internal oxidation–reduction in aqueous solution.[3] Some stabilization of the Cu(II)–mercaptide S bond has been achieved in complexes where structural constraints suppress the formation of Cu(I).[3-6] Thus, the X-ray crystal structure of [Cu(tetb)(*o*-$SC_6H_4CO_2$)]·H_2O has been determined,[4] and reasonably stable thiolate adducts in copper(II) cyclam,[5] *N*-(mercaptoacetyl)-L-histidine,[6] and 1-methoxy-2-methyl-1,1-bis(1-methylimidazol-2-yl)-2-propanethiol[3] complexes were identified in nonaqueous solutions through characteristic, intense S(σ) → Cu(II) LMCT transitions in the near-ultraviolet region.

Reactivity and equilibrium studies of the formation and decay of (L-cysteinato)[tris(2-pyridylmethyl)amine]copper(II) complexes are presented in this paper. Our primary objective is to determine the influence of other donor atoms and coordination geometry on the kinetic stability of the Cu(II)–S bond. The tris(2-pyridylmethyl)amine (tmpa) ligand was chosen with the expectation that steric crowding about the coordinated S atom[3] and poor oxidizing strength of the Cu(II) center[3,7] would stabilize the S-bonded cysteine–$Cu(tmpa)^{2+}$ adduct in aqueous solution. In fact, it was possible to measure both the equilibrium formation constant of this complex and its rate of redox decay, yielding $Cu(tmpa)^+$ and the disulfide cystine. While the cysteine–$Cu(tmpa)^{2+}$ adduct is not presented as a blue copper protein model, a structural analogy may be drawn between the nonplanar coordination geometry imposed by the tmpa ligand and the low-symmetry type-1 copper sites of metalloproteins.[7]

Experimental Section

Materials. Reagent grade chemicals were used throughout. L-Cysteine (cys-SH), DL-penicillamine, L-cysteine methyl ester hydrochloride, glutathione, *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES), and the disodium salt of 2,9-dimethyl-1,10-phenanthrolinediyl-4,7-bis(benzenesulfonic acid) (Na_2dpmp) were supplied by Sigma. (Hydroxyethyl)ferrocene (Strem Chemicals) and 2-morpholinoethanesulfonic acid (MES) (Sigma) were used as supplied. Tris(2-pyridylmethyl)amine[8] and $Cu(tmpa)(ClO_4)_2$[9] were prepared by literature methods. The purity of the latter compound was confirmed through the excellent agreement between its visible spectrum and that reported by Anderegg et al. [λ_{max} 872 nm (ε 214 M^{-1} cm^{-1})].[9] All solutions were prepared with triply distilled water.

Formation Constant Measurements. Formation of a $Cu(tmpa)^{2+}$–cysteine adduct was followed through the increase in absorbance at 396 nm (Cary 17 and 219 spectrophotometers). Thermostated solutions (25.0 ± 0.2 °C) of cysteine and $Cu(tmpa)^{2+}$ in I = 0.1 M ($NaNO_3$) sodium acetate (5 mM) buffers (pH 3.99–5.80) were mixed in a 1-cm quartz cell, such that $[Cu(tmpa)^{2+}]_0$ was fixed at 50 μM and 0.1 mM ≤ [cysteine] ≤ 0.1 M. Spectra were recorded within 1 min. after mixing (cell block thermostated at 25 °C), and the pH of product mixtures was obtained from a Brinkmann pH-104 meter. Hydrogen ion concentrations were derived from pH readings by using eq 1, based on an activity coefficient of 0.762 for 0.1 M $NaNO_3$.[10]

$$-\log [H^+] = \mathrm{pH} - 0.12 \quad (1)$$

The apparent formation constant (at constant pH) of a 1:1 $Cu(tmpa)^{2+}$–cysteine complex (K_f = $[complex]_e/[Cu(tmpa)^{2+}]_e[cysteine]_e$) was calculated as the ratio of least-squares intercept to slope in linear plots of $(A_e - A_0)^{-1}$ vs. $[cysteine]^{-1}$, based on the relationship[11]

$$(A_e - A_0)^{-1} = (lC_0(\Delta\epsilon))^{-1} + (lC_0(\Delta\epsilon)K_f)^{-1}[cysteine]^{-1} \quad (2)$$

where A_e, A_0, l, C_0, and $\Delta\epsilon$ correspond respectively to the equilibrium absorbance, the initial absorbance, the spectrophotometric path length, the total copper concentration, and the differential extinction coefficient associated with adduct formation. In the above relationships, $[cysteine]_e$ = [cys-SH], as virtually all (>99%) of the free mercaptan is present in the un-ionized thiol form throughout the pH range covered in the formation constant studies (vide infra).

Kinetic Measurements. Reduction of copper(II) was conveniently followed at 396 nm, λ_{max} of an intense absorption peak. Kinetic runs

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were performed on a Durrum D-110 stopped-flow spectrophotometer. Absorbance–time traces were displayed on a Hewlett-Packard Model 7004 B X-Y recorder. Anaerobic (N_2-purged) solutions of cysteine and $Cu(tmpa)^{2+}$ were transferred to the stopped-flow apparatus through Teflon needles, and a minimum of 30 min was allowed for temperature equilibration to occur before kinetic runs were initiated. A 40% excess of $Cu(tmpa)^{2+}$ (0.14 mM) over cysteine (0.10 mM) was used in most kinetic runs.

Both reactants were prepared in BES (pH 6.4–8.7) or sodium carbonate (pH 8.7–11.2) buffers (5 mM) containing 0.1 M $NaNO_3$. Hydrogen ion concentrations were calculated from eq 1.

Observed first-order rate constants (k_{obsd}) of the cysteine–$Cu(tmpa)^{2+}$ redox reaction (excess $Cu(tmpa)^{2+}$) were derived from the least-squares slopes of ln $(A_t - A_\infty)$ vs. time plots. The Guggenheim method[12] was applied in a few runs where A_∞ was poorly defined. Second-order kinetics was apparent in some runs where $[\text{cysteine}]_0 \gg [Cu(tmpa)^{2+}]_0$, as plots of $(A_t - A_\infty)^{-1}$ vs. time were found to be linear. Second-order k_{obsd} values were calculated as slope $-$ ((intercept)$[Cu(tmpa)^{2+}]_0)^{-1}$ from the least-squares analysis of these plots. Most reported rate constants are the average of three trials.

Electrochemical Measurements. Cyclic voltammetry was utilized to measure the $Cu(tmpa)^{2+/+}$ reduction potential in $I = 0.1$ M, pH 6.0 MES buffer. A three-electrode system composed of a carbon paste working electrode, a platinum wire auxiliary electrode, and a Ag/AgCl (3 M NaCl) reference electrode was used. The $Cu(tmpa)(ClO_4)_2$ concentration was 1.0 mM; variable concentrations of (hydroxyethyl)ferrocene (HEF) (E° = 402 mV vs. NHE),[13] a water-soluble internal calibrant,[13,14] were included in some runs. Voltammograms were generated with a Bioanalytical Systems CV-1B apparatus, and output was obtained from a Hewlett-Packard Model 7004B recorder. The potential of the $Cu(dmp)_2^{2+/+}$ (dmp = 2,9-dimethyl-1,10-phenanthroline) couple was measured to verify the accuracy of the internal calibrant method. The E° value obtained (+213 mV relative to HEF, +615 mV vs. NHE) is in excellent agreement with the literature value.[15]

Stoichiometry of the $Cu(tmpa)^{2+}$–Cysteine Oxidation–Reduction Reaction. Copper(I) produced in the reduction of $Cu(tmpa)^{2+}$ was converted to $Cu(dpmp)_2^{3-}$ and assayed spectrophotometrically (ϵ_{483} 1.225×10^4 M^{-1} cm^{-1}).[16] An anaerobic solution of 0.65 mM cysteine and 65 μM $Cu(tmpa)^{2+}$ at pH 5.6 and 25 °C (0.5 mM NaOAc buffer, I = 0.1 M ($NaNO_3$)) was allowed to stand until the 396-nm absorbance decrease was complete (30 min). A 5-fold excess of Na_2dpmp was then injected anaerobically into the product solution, resulting in the immediate formation of the orange $Cu(dpmp)_2^{3-}$ complex.

Results

Equilibrium Constant of the Cysteine–$Cu(tmpa)^{2+}$ Complexation Reaction. An intense, symmetric absorption band with λ_{max} at 396 nm was observed upon mixing cysteine with $Cu(tmpa)^{2+}$ throughout the pH range 4.0–6.0. Although extinction coefficients cannot be defined precisely, the d–d spectrum of the cysteine–$Cu(tmpa)^{2+}$ adduct may be described in semiquantitative fashion. A shallow absorption maximum at 835 nm falls off slightly to a broad plateau between 650 and 800 nm in pH 5 $Cu(tmpa)^{2+}$ solutions containing large excesses of cysteine. The absorbance falls off sharply below 650 nm, reaching a minimum at 530 nm. Aside from the shift in λ_{max} from 872 to 835 nm, the most notable change in the visible spectrum of the adduct relative to that of $Cu(tmpa)^{2+}$ is the substantial increase in 600–750-nm absorbance. Thus, $A_{872}(max)/A_{700}$ in $Cu(tmpa)^{2+}$ is 2.4, while $A_{835}(max)/A_{700}$ in the cysteine adduct is only 1.3.

Stopped-flow studies at 396 nm indicated that complexation was complete within the mixing time (3 ms), even at low cysteine concentrations. Formation constants (K_f) were obtained at pH 3.99, 4.29, 4.59, 4.99, 5.54, and 5.80. Although reduction of copper occurred on long standing, A_{396} of solutions used in equilibrium studies was found to be invariant over 1 (high pH and [cys-SH]) to 60 (low pH and [cys-SH]) min after mixing. The time stability of A_{396} could be extended to several hours in unbuffered solutions at pH < 4.

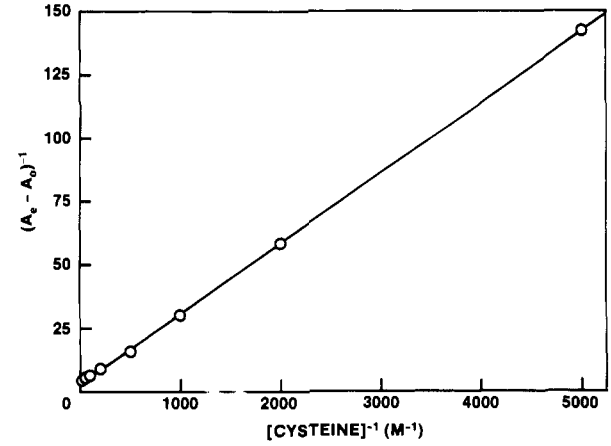

Figure 1. Plot providing a basis for the calculation of K_f (25 °C, I = 0.1 M, pH 4.99, C_0 = 50 μM, l = 1 cm, λ = 396 nm).

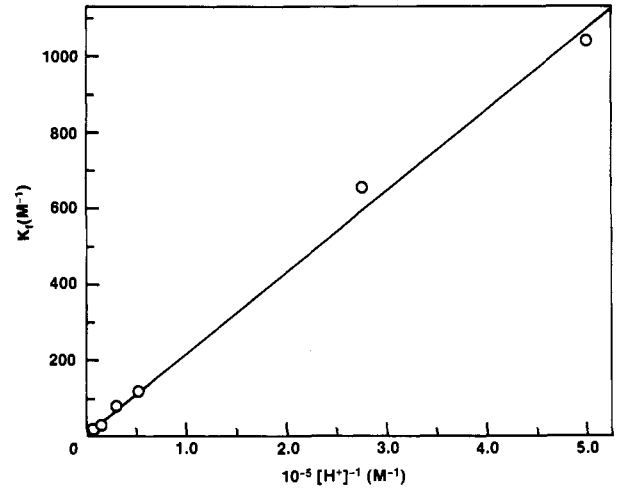

Figure 2. Plot of K_f vs. $[H^+]^{-1}$ supporting eq 4 (25.0 °C, I = 0.1 M).

Excellent linear $(A_e - A_0)^{-1}$ vs. $[\text{cys-SH}]^{-1}$ correlations were found within the pH range 3.99–5.80, indicating the exclusive formation of a 1:1 cysteine–$Cu(tmpa)^{2+}$ adduct. Parameters derived from a representative plot (Figure 1) are $K_f = (1.21 \pm 0.06) \times 10^2$ M^{-1} and $\Delta\epsilon_{396} = (6.0 \pm 0.3) \times 10^3$ M^{-1} cm^{-1} (pH 4.99). The pH dependence of K_f is consistent with the formation of a thiolate sulfur–copper(II) bond:

$$\text{cys-SH} + Cu(tmpa)^{2+} \underset{}{\overset{K_f'}{\rightleftharpoons}} [(tmpa)Cu\text{-S-cys}]^+ + H^+ \quad (3)$$

$$K_f = K_f'/[H^+] \quad (4)$$

Thus, K_f varies linearly with $[H^+]^{-1}$ (Figure 2), as anticipated from eq 4. The concentration equilibrium constant K_f', derived from the least-squares slope of Figure 2, is $(2.2 \pm 0.2) \times 10^{-3}$ (25.0 °C, I = 0.1 M).

Glutathione (λ_{max} 401 nm) and cysteine methyl ester (λ_{max} 387 nm) rapidly form strongly absorbing adducts with $Cu(tmpa)^{2+}$, but the redox decay rates of these species are substantially faster than that of $[(tmpa)Cu\text{-S-cys}]^+$, preventing the measurement of K_f' by our method. The redox reactivity advantage of cysteine methyl ester over cysteine is at least 2 orders of magnitude at pH 5, while the glutathione adduct is somewhat less reactive. No change in the 320–500-nm absorption spectrum was observed immediately after mixing large

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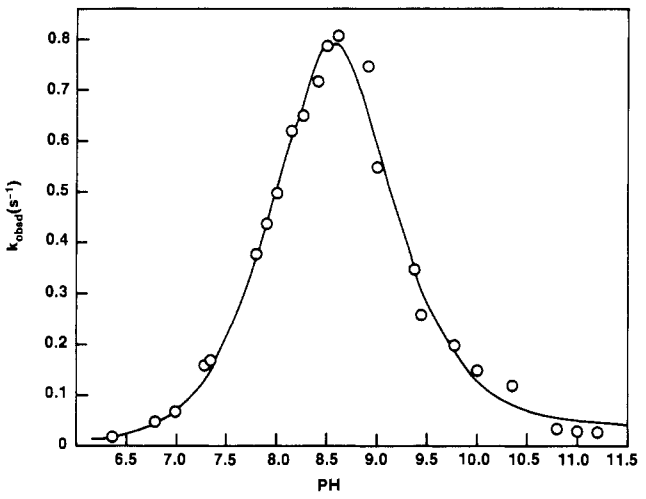

Figure 3. pH dependence of k_{obsd} for the cysteine–Cu(tmpa)$^{2+}$ oxidation–reduction reaction (25.0 °C, I = 0.1 M ($NaNO_3$), [cys-SH]$_0$ = 0.10 mM, [Cu(tmpa)$^{2+}$]$_0$ = 0.14 mM). The solid curve was drawn on the basis of nonlinear least-squares rate parameters obtained from the fit of k_{obsd} – [H$^+$] points to eq 6 (see text).

excesses of penicillamine with Cu(tmpa)$^{2+}$ at pH 5. A slow increase in the 300–400-nm absorbance was noted, however, over a period of several days after mixing. A poorly resolved shoulder near 350 nm, but no new near-UV absorption maximum, was found in these penicillamine–Cu(tmpa)$^{2+}$ solutions.

Kinetics and Stoichiometry of the Cysteine–Cu(tmpa)$^{2+}$ Redox Reaction. Copper(II) is quantitatively converted to Cu(I) (0.9 ± 0.1 mol of Cu(I) produced per mole of Cu(II) reactant) in the reaction of excess cysteine with Cu(tmpa)$^{2+}$. Excellent first-order plots were derived from A_{396} when cysteine was the limiting reagent (pH 6.4–11.2). With [Cu(tmpa)$^{2+}$]$_0$ fixed at 0.14 mM, k_{obsd} was found to be independent of [cys-SH]$_0$ in the interval 0.025–0.14 mM (25.2 °C, pH 7.8). In contrast, with [Cu(tmpa)$^{2+}$]$_0$ = 50 μM and [cys-SH]$_0$ ≥ 0.5 mM, redox decay was second order with respect to the absorbing intermediate (25 °C, pH 7.8). A detailed study was not made with Cu(tmpa)$^{2+}$ as the limiting reagent, but representative second-order rate constants of 4.9 × 10^3 M^{-1} s^{-1} (0.50 mM cys-SH) and 2.6 × 10^4 M^{-1} s^{-1} (5.0 mM cys-SH) may be given.

The first-order decay of the 1:1 cysteine–Cu(tmpa)$^{2+}$ adduct was studied at 23 pHs in the interval 6.4–11.2. These results (Figure 3) show that k_{obsd} increases to a maximum near pH 8.6 and then falls off rapidly in more alkaline solutions. The data may be understood in terms of three distinct reactant species (I, II, III) related through two rapid ionization equilibria.

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

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

$$\mathrm{I} \xrightarrow{k_1} \mathrm{Cu(tmpa)^+} + \text{cystine}$$

$$\mathrm{II} \xrightarrow{k_2} \mathrm{Cu(tmpa)^+} + \text{cystine}$$

$$\mathrm{III} \xrightarrow{k_3} \mathrm{Cu(tmpa)^+} + \text{cystine} \qquad (5)$$

On this basis, k_{obsd} for the decay of [adduct]$_{tot}$ = [I] + [II] + [III] is given by relationship 6. Considering the very small

$$k_{obsd} = \frac{k_1[\mathrm{H^+}]^2 + k_2K_{a1}[\mathrm{H^+}] + k_3K_{a1}K_{a2}}{[\mathrm{H^+}]^2 + K_{a1}[\mathrm{H^+}] + K_{a1}K_{a2}} \qquad (6)$$

decay rate at pH <6, we have performed a nonlinear least-

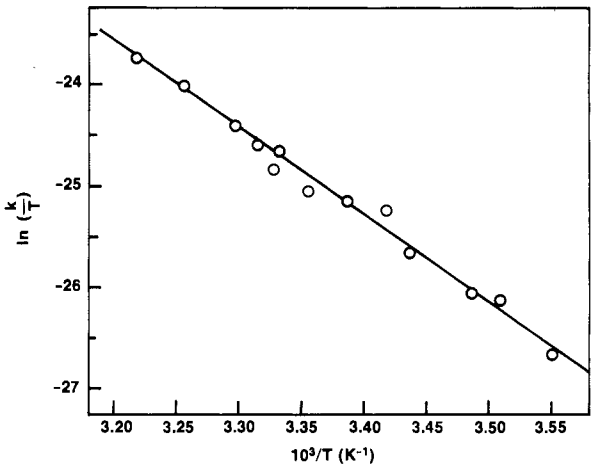

Figure 4. Eyring plot illustrating the temperature dependence of $k = k_{obsd}[\mathrm{H^+}] = k_2K_{a1}$ (I = 0.1 M, pH 7.8).

squares fit[17] of the data to eq 6 with the assumption that k_1 = 0. This fit was successful, as shown by the curve calculated from the least-squares parameters:[18] k_2 = 2.0 ± 0.1 s^{-1}, k_3 = (4.1 ± 0.2) × 10^{-2} s^{-1}, pK_{a1} = 8.33 ± 0.05, pK_{a2} = 8.52 ± 0.05. It should be noted that K_{a1} and K_{a2} are concentration, not activity constants.

The limiting form of eq 6 at low pH is

$$k_{obsd} = k_2K_{a1}/[\mathrm{H^+}] \qquad (7)$$

provided that k_1 = 0. This simplified expression is appropriate between pH 6.7 and 7.8, as a k_{obsd} – [H$^+$]$^{-1}$ plot is linear with intercept of zero. The temperature dependence of k_{obsd} was evaluated between 8.6 and 37.8 °C at pH 7.8.[19] An Eyring plot of ln (k/T) vs. $1/T$ is shown in Figure 4, where $k = k_{obsd}[\mathrm{H^+}] = k_2K_{a1}$. Activation parameters derived from this analysis are $\Delta H^\ddagger$ = 17.1 ± 0.6 kcal/mol and $\Delta S^\ddagger$ = −39 ± 3 eu (k (M^{+1} s^{-1})).

Reduction Potential of Cu(tmpa)$^{2+}$. Quasi-reversible cyclic voltammograms were obtained through the cathodic reduction of Cu(tmpa)$^{2+}$, followed by reoxidation of Cu(tmpa)$^+$ in the anodic wave. Peak-to-peak separations of 137 and 181 mV were found at sweep rates of 100 and 200 mV/s, respectively. The reduction potential (−549 mV vs. HEF, −147 ± 8 mV vs. NHE, 25.0 °C, pH 6.0, I = 0.1 M) was calculated as $(E_A + E_C)/2$. As a check of this calculation, $E°$ was found to be invariant with sweep rate at six values between 100 and 210 mV/s. Our $E°$ value may be compared with that of −0.39 V vs. NHE reported for [Cu(tmpa)Cl]PF_6 in DMF (0.11 M N(n-C_4H_9)$_4PF_6$).[7]

Discussion

The kinetic stability of our Cu(tmpa)$^{2+}$–cysteine adduct, although limited, is remarkable for a mercaptide S–Cu(II) complex in aqueous solution. While strongly absorbing S-bonded transients have been observed in many spectroscopic[3-6] and kinetic[20,21] investigations of RS$^-$–Cu(II) interactions, equilibrium characteristics of the complexation step have not been reported previously. The intense absorption peak at 396 nm may be assigned as a RS$^-$(σ) → Cu(II) LMCT band by comparison with the spectra of analogous complexes in the

(17) "Science and Engineering Programs Apple II Edition"; Heilborn, J., Ed.; Osborne/McGraw-Hill: Berkeley, 1981; p 19.
(18) The predicted [H$^+$] at which k_{obsd} is maximal may be calculated from $[\mathrm{H^+}]_{max} = (K_{a1}K_{a2})^{1/2}$ = 3.78 × 10^{-9} M (pH 8.54).
(19) Correction was made for the temperature dependence of pH when calculating [H$^+$] from eq 1.
(20) Lappin, A. G.; McAuley, A. *J. Chem. Soc., Dalton Trans.* **1978**, 1606.
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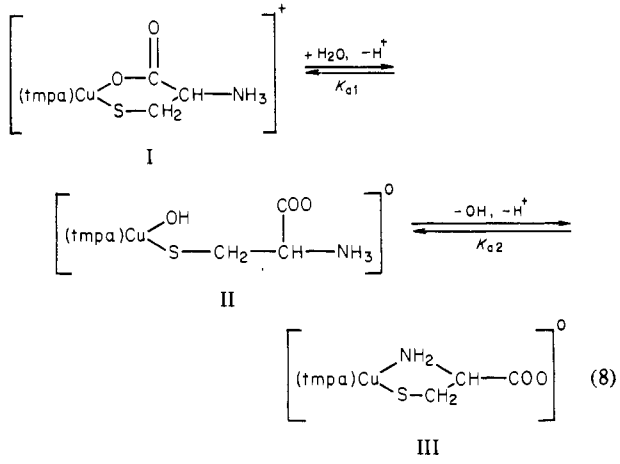
330–440-nm region.[3-6] Our equilibrium data clearly demonstrate that $Cu(tmpa)^{2+}$–cysteine complex formation at low pH proceeds with 1:1 stoichiometry and the ionization of one proton per adduct molecule. The formula $[(tmpa)Cu\text{-}S\text{-}cys]^+$ therefore is justified under these conditions. Considering adduct formation to be the sum of two steps, cysteine S-H ionization (pK_a(25 °C) = 8.33)[22] and mercaptide anion–copper(II) complexation, one may calculate the formation constant of the latter step (K_c) (4.7×10^5 M^{-1}, $\Delta G_c° = -7.7$ kcal/mol) from $K_f' = K_a K_c$.

When cysteine is the limiting reagent, reduction of copper(II) proceeds through intracomplex electron transfer from the ligated sulfur atom. This conclusion is supported by the first-order decay curves and independence of k_{obsd} on $[cysteine]_0$ observed under these conditions. The existence of three structurally distinct 1:1 $Cu(tmpa)^{2+}$–cysteine complexes is indicated by the pH dependence of k_{obsd}. Analytically impure solids and oils have resulted from attempts to isolate a crystalline $Cu(tmpa)^{2+}$–cysteine adduct from nonaqueous solutions.[23] On this basis, we will not attempt a detailed assignment of the structures of species I, II, and III.

From Figure 3 it is clear that the intermediate species I and III are considerably more stable with respect to internal electron transfer than is intermediate II. Chelation of Cu(II) by cysteine in complexes I (S^-, COO^- donor groups) and III (S^-, NH_2 donor groups) could significantly stabilize these species toward reduction to Cu(I). A carboxylate group is available in the predominant zwitterionic form of cysteine at low pH (pK(COOH) = 1.71, 25 °C), while $pK(NH_3^+)$ = 10.78.[22] Since the cysteine SH group is already ionized in the adduct with $Cu(tmpa)^{2+}$, we propose that the K_{a1} and K_{a2} equilibria correspond to the formation of a coordinated hydroxide ion and ionization of the cysteine NH_3^+ group, respectively (eq 8). From the assumption that tmpa consistently



(8)

functions as a tetradentate ligand, all of the adduct species would have six-coordinate, distorted-octahedral structures. The possibility that cysteine functions as a tridentate (S, N, O) ligand in III cannot presently be ruled out. The coordination environment in $[Cu(tmpa)Cl]PF_6$ is trigonal bipyramidal,[7] but space-filling models suggest no steric restraint in the rearrangement to an octahedral structure. Indeed, an octahedral or square-pyramidal structure evidently is preferred when the tmpa hydrogen atoms ortho to pyridyl N atoms are replaced by methyl groups;[9] thus, the most intense d–d band of the [tris(6-methyl-2-pyridylmethyl)amine]copper(II) ion [λ_{max} 695 nm (ϵ 130 M^{-1} cm^{-1})] is strongly blue-shifted relative to that of $Cu(tmpa)^{2+}$ at 872 nm, and a low energy shoulder is observed at 800 nm (ϵ 110 M^{-1} cm^{-1}). The d–d spectrum of I strongly resembles that of the former ion, consistent with our structural hypothesis.

Our pK_{a1} value of 8.33 may be compared with that of 7.40 (20 °C, I = 0.1 M (KNO_3))[9] for the ionization of $Cu(tmpa)^{2+}$(aq) to give $[Cu(tmpa)OH]^+$. This order of magnitude difference in ionization constants probably reflects competition between the cysteine carboxylate group and hydroxide ion for a coordination position. Comparison of our pK_{a2} value with that of the free cysteine ammonium group indicates a decrease of ca. 3.1 kcal/mol in $\Delta G°$ resulting from coordination of the NH_2 function in complex III.

The activation requirements of $k = k_2 K_{a1}$ indicate large enthalpic and entropic barriers to the conversion of complex I to II, followed by internal electron transfer. The contribution of the K_{a1} equilibrium to $\Delta H^‡$ may be estimated at 12 kcal/mol, by analogy to the ionization of Cu^{2+}(aq) (pK_1 = 7.3).[24] The $\Delta S^‡$ value of −39 eu must primarily reflect inner-sphere rearrangement in II prior to electron transfer, as ionization entropies of bivalent aquo cations typically fall in the range −12 to +8 eu.[24]

The criteria described by Bosnich and co-workers[3] are useful in understanding the unusual kinetic stability of the Cu(II)–S bond in complex I. Our $Cu(tmpa)^{2+/+}$(aq) reduction potential of −147 mV clearly shows that a nonplanar coordination geometry alone does not necessarily enhance the oxidizing strength of a Cu(II) center relative to that of Cu^{2+}(aq) ($E°$ + 153 mV).[25] Other factors, particularly differences in coordination number and in the strengths of Cu(II) and Cu(I) donor-atom bonds, must be considered. Part of the stability of complex I at low pH may therefore be attributed to the small driving force of the cysteine–$Cu(tmpa)^{2+}$ redox reaction under these conditions. The effective oxidation potential of the cysteine/cystine couple at pH 4 is only +163 mV.[26]

Perhaps the most important contribution to the stabilization of the Cu(II)–S bond in the $Cu(tmpa)^{2+}$–cysteine system is hindrance of mercaptide radical coupling linked to steric crowding about the coordinated sulfur atom. Chelation of copper(II) by the reducing agent may also retard the rate of Cu–S bond cleavage in a reductive elimination process. The importance of chelation is suggested by the instability of complex II and the $Cu(tmpa)^{2+}$–cysteine methyl ester adduct, in which such stabilization is not possible. Steric crowding about the sulfur atom is readily apparent in a space-filling model of complex I. The failure of penicillamine (α,α-dimethylcysteine) to rapidly form an adduct analogous to complex I is a further indication of the severe spatial restrictions within the metal-containing cavity of tmpa. Facile formation of S-bonded complexes occurs upon mixing both cysteine and penicillamine with Cu^{2+}(aq), in which such spatial restrictions do not exist.[27]

Reduction of copper(II) by mercaptans typically is second order with respect to S-bonded intermediates. This behavior is well documented in the reactions of 2-mercaptosuccinic acid[20] and cysteine[21] with Cu^{2+}(aq), and the oxidation of cysteine by 1:1 copper(II) complexes of tridentate nitrogen-donor ligands (diethylenetriamine; 1,1,4,7,7-pentamethyldiethylenetriamine; 2,2′,2″-terpyridine)[28] exhibits second-order kinetics under conditions similar to those employed in the present study. Binuclear, two-electron-accepting copper(II) intermediates are preferred in these reactions, permitting the

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concerted formation of the disulfide S-S bond without prior generation of high-energy mercaptide radicals.[20] The first-order decay of 1:1 $Cu(tmpa)^{2+}$-cysteine adducts therefore provides further confirmation that radical coupling is strongly hindered in this system. Even here, a bimolecular redox pathway is preferred when large excesses of cysteine are present. The mechanistic aspects of this and other copper(II) reductions by excess cysteine are still under investigation.[23,28]

Acknowledgment. Support of this research by the Robert A. Welch Foundation (Grant D-735) is gratefully acknowledged. We thank Professor David McMillin for helpful criticisms of the manuscript.

Registry No. $Cu(tmpa)^{2+}$, 87207-95-2; cysteine, 52-90-4.

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Complexes Containing the $Mo_2O_5^{2+}$ Core: Preparation, Properties, and Crystal Structure of $Mo_2O_5[(CH_3)_2NCH_2CH_2NHCH_2C(CH_3)_2S]_2$

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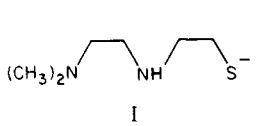
Received December 28, 1982

Mo(VI) complexes of the form $Mo_2O_5L_2$ have been prepared by reaction of $MoO_2(acac)_2$ with LH in methanol solution. The ligand L is a tridentate monoanion with one thiolate donor (L = $(CH_3)_2NCH_2CH_2NHCH_2CH_2S^-$, $(CH_3)_2NCH_2CH_2NHCH_2C(CH_3)_2S^-$, $(C_5H_4N)CH_2NHC_6H_4S^-$). ^{17}O NMR and IR spectra are consistent with a monooxo-bridged dinuclear structure, which is confirmed by X-ray crystallography in one case. Intensity data collected by using counter methods have led to the determination of the crystal and molecular structure of (μ-oxo)bis[dioxo(2,7-dimethyl-2,5-diazaoctane-7-thiolato)molybdenum(VI)], $Mo_2O_5(C_8H_{19}N_2S)_2$. The compound crystallizes in the orthorhombic space group *Pbca* with a = 11.711 (2) Å, b = 17.233 (6) Å, c = 24.778 (7) Å, V = 5000.8 (22) Å^3, and Z = 8. Molybdenum centers are bridged by an oxo group, forming discrete dinuclear species in the unit cell. The molecule possesses a pseudo-twofold symmetry axis that passes through the bridging oxo group. The Mo-O_b-Mo angle is 143.8 (3)°; $(Mo—O_b)_{av}$ = 1.923 (5) Å, and $(Mo=O)_{av}$ = 1.713 (6) Å. The complex $Mo_2O_5(C_6H_{15}N_2S)_2$ reacts with C_6H_5SH and with $P(C_6H_5)_3$ to give the dinuclear Mo(V) complexes $Mo_2O_3(C_6H_{15}N_2S)_2(SC_6H_5)_2$ and $Mo_2O_4(C_6H_{15}N_2S)_2$, respectively.

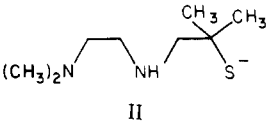
Introduction

Oxo complexes of Mo(VI) have been under intense study[2-4] in part due to their potential relevance as model systems for Mo sites in enzymes. Ligands containing sulfur donors have been prominent in these studies as there is strong evidence that S is present in the enzymic Mo coordination sphere. Almost all of the attention with regard to ligands has focused on bidentate and tetradentate ligands,[5-13] which, respectively, form Mo(VI) complexes of the form MoO_2L_2 and MoO_2L containing the *cis*-MoO_2^{2+} core. In this paper we report complexes of some related tridentate ligands.

Tridentate ligands are of intrinsic interest because their complexes with the MoO_2^{2+} core should be of the form MoO_2L^+, leaving one open coordination site to potentially bind a substrate, a product, or an inhibitor.[14-16] Alternatively, the available site could be involved in bridging to a second molybdenum in a dinuclear complex. In this paper we report dinuclear Mo(VI) complexes of the tridentate ligands I-III and of a related potentially tetradentate ligand IV. These



I



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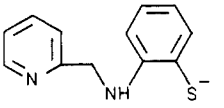
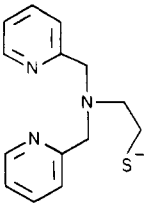


III



IV

ligands each form dinuclear complexes of the form $Mo_2O_5L_2$ whose IR and NMR spectra are consistent with a bent Mo-

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