a normal geometry. In **1b**, it lies on an inversion center; thus the chlorine atom is statistically disordered over six positions.

Both compounds **1** and **2** which are diamagnetic six-coordinate cobalt(III) porphyrins present a d-type hyperporphyrin electronic spectrum[22,23] (Figure 5) with split Soret bands lying close to 384 and 465 nm for which there is no precedent for in cobalt porphyrin systems.[1,24] These spectra also closely resemble the spectra of dimercaptoiron(III)[25] -ruthenium(III)[26] and -chromium(III)[27] porphyrin derivatives. d-Type hyperporphyrin spectral properties seem to be a characteristic feature of dimercapto metalloporphyrins. Moreover, these spectral properties of the dimercaptocobalt compounds are essentially identical with those of the species obtained by treatment of the native state of cobalt-substituted cytochrome $P450_{CAM}$ with dithiothreitol[1] (Figure 5) which are also very similar to those of the ferric counterpart.[28]

Thus, the spectral properties of the dimercaptocobalt porphyrins that we have synthesized indicate that the treatment of Co $P450_{CAM}$ with dithiothreitol yields most probably a dimercaptocobalt(III) species and confirms indirectly what is now commonly accepted, that is, cysteinate axial ligation to the metal in the resting state of cytochrome P450.

Registry No. 1a, 91128-01-7; **1b**, 91128-02-8; **2**, 91128-04-0; [Co^{III}(Cl)(TPP)], 60166-10-1; [$Co^{II}(SC_6HF_4)$(TPP)][Na⊂222], 91157-01-6; cryptand 222, 23978-09-8; sodium 2,3,5,6-tetrafluorobenzenethiolate, 91156-99-9; sodium 2,4,5-trichlorobenzenethiolate, 91157-00-5.

Supplementary Material Available: Listings of hydrogen atom parameters for **1a** (Table V) and **1b** (Table VI), thermal parameters for non-hydrogen atoms of **1a** (Table VII) and **1b** (Table VIII), and observed and calculated structure factors (× 10) for all observed reflections for **1a** (Table IX) and **1b** (Table X) (71 pages). Ordering information is given on any current masthead page.

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Removal of Glycine from Cu(II) upon Peptide Proton Ionization in a Mixed-Ligand Cu(II)-Peptide-Amino Acid Chelate. A Possible Model for Substrate Removal at Metalloenzyme Centers

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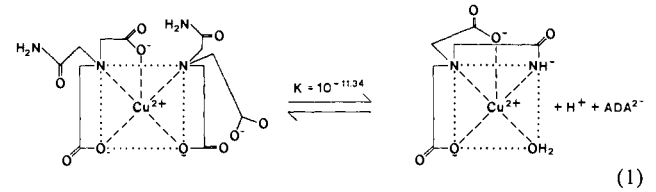
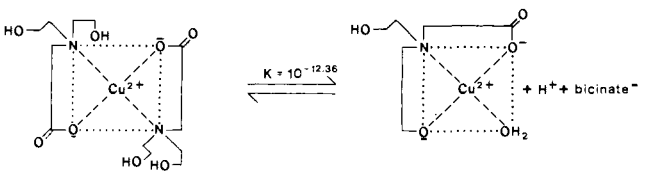
Abstract: Potentiometric, visible, and electron spin resonance studies indicate that the mixed-ligand metal complex, (glycylglycine-*N*,*N*-diacetato)(glycinato)copper(II), [Cu(DGDA)gly^{2-}] undergoes loss of an amino acidate ligand upon peptide proton ionization, [Cu(DGDA)gly^{2-}] ⇄ [Cu(H_1DGDA)$^{2-}$] + gly^- + H^+, with a constant of $10^{-11.68\pm0.02}$. The possible importance of this reaction to substrate release at metalloenzyme centers is discussed.

Previously, we have described amino acidate dechelation upon amide deprotonation[1] in the bis[*N*-acetamidoiminodiacetato]-copper(II) chelate (eq 1) and upon hydroxy group ionization[2] in

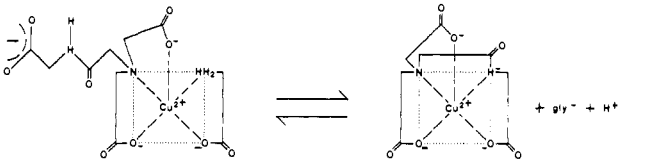


(1)



(2)

the bis(*N*,*N*-bis(2-hydroxyethyl)glycinato)copper(II) chelate. In a continuing study of dechelation reactions in metal complexes, the mixed-ligand chelate (*N*,*N*-bis(carboxymethyl)glycylglycinato)(glycinato)copper(II) (I) was found to undergo loss of the glycinate ion upon peptide proton ionization (eq 3). The



(3)

discrovery of this reaction completes our initial goal of showing that (when ionized) the three strongest σ-donor groups present at the active sites of metalloenzymes, i.e., amide groups (asparagine, glutamine), alcohol groups (serine, threonine), and peptide groups, are capable of initiating dechelation reactions.

Experimental Section

Reagents. Baker Analyzed reagent grade $Cu(NO_3)_2 \cdot 3H_2O$ was used for all metal solutions, which were standardized via standard ion-exchange techniques. Aliquots of the metal ion solution were passed through Dowex 50W-X8 strongly acidic cation-exchange resin, and the

(1) Paar, D. P.; Rhodes, C. R., III; Nakon, R. *Inorg. Chim. Acta* **1983**, *80*, L11.
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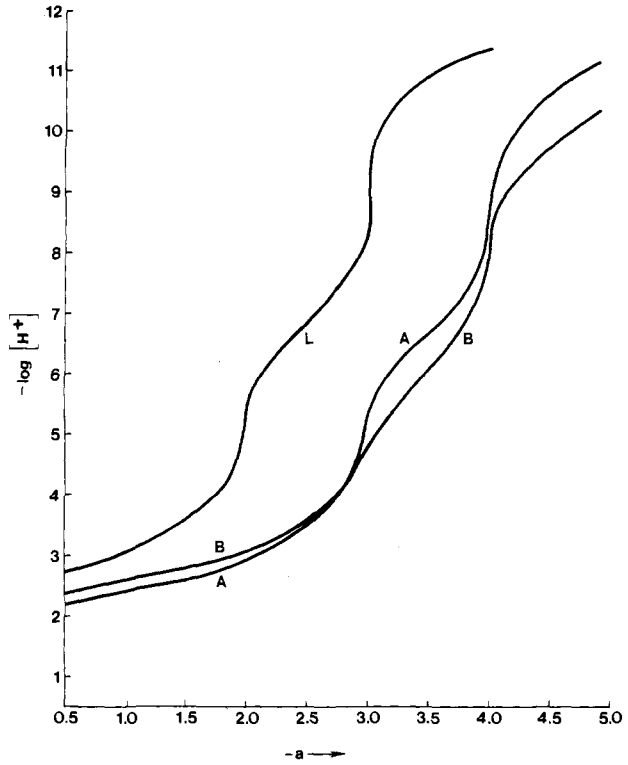


gure 1. Potentiometric formation curves for H_3DGDA (L): 1:1 Cu(II) to H_3DGDA (A) and 1:1:1 Cu(II) to H_3DGDA to HGly (B). H_3DGDA $\sim 3 \times 10^{-3}$ M and a is moles of base per mole of H_3DGDA.

effluent solutions were titrated with standard NaOH solutions using phenolphthalein as an indicator.

Solutions of H_3DGDA (supplied by Prof. A. E. Martell) and glycine (Reagent grade, Fisher Scientific) (gly, G) were standardized via potentiometric titration.

Potentiometric Measurements. A Corning Digital 112 Reseach Model pH meter was used to determine hydrogen ion concentration for potentiometric titrations. The concentration of all solutions was approximately 3×10^{-3} M in Cu^{2+}. All titrations were performed in a double-walled titration cell of 50-mL capacity. The temperatures of all solutions were maintained at 25.00 ± 0.05 °C by circulating thermostated water through the outer jacket of the cell. The cell was fitted with glass and calomel extention electrodes, a microburet delivery tube, and a nitrogen inlet tube. Ionic strengths of all solutions were maintained at 0.1 M by the addition of an appropriate amount of 1.0 M KNO_3. The solutions were stirred with a magnetic stirrer, and all titrations were done in triplicate. The glass electrode was calibrated to read directly $-\log [H^+]$ according to the method of Rajan and Martell[3] using standard HCl and NaOH solutions. All formation and protonation constants were calculated via Bjerrum's method.[4]

Visible Spectra. All visible spectral data were obtained on a Bausch and Lomb Spectronic 2000 spectrophotometer using a matched set of quartz cells (1.0 cm) at ambient temperatures. Spectra at various a values (moles of base per mole of H_3DGDA) were obtained for 1:1 Cu(II) to H_3DGDA and 1:1:1 Cu(II) to H_3DGDA to gly solutions ($\sim 3 \times 10^{-3}$ M).

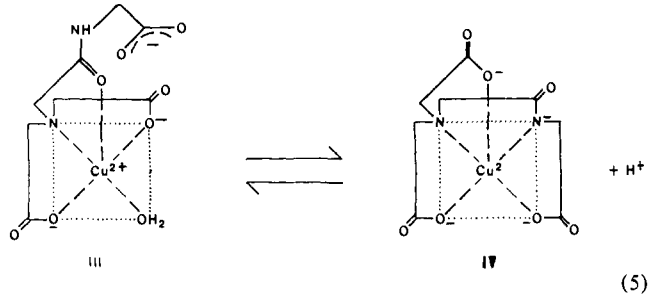
ESR Spectra. All ESR spectra were recorded on a Brucker ER200D SRC spectrometer at ambient temperatures. Spectra at various a values for 1:1 Cu(II) to H_3DGDA and 1:1:1 Cu(II) to H_3DGDA to gly solutions ($\sim 2 \times 10^{-3}$ M) were recorded.

Results

Protonation and Formation Constants. Potentiometric formation curves for H_3DGDA, 1:1 Cu(II) to H_3DGDA and 1:1:1 Cu(II) to H_3DGDA to glycine, are shown in Figure 1. The H_3DGDA curve (L) consists of two buffer zones terminated by inflections at $a = 2.0$ and 3.0, moles of base per mole of H_3DGDA. In the low pH region the two carboxylate protons of H_3DGDA are titrated, while the zwitterionic proton reacted in the higher buffer region. The pK_a of the zwitterionic proton (eq 4) was determined to be 6.89 ± 0.01, in good agreement with the literature value[5] of 6.92

$$HDGDA^{2-} \rightleftarrows DGDA^{3-} + H^+ \quad (4)$$

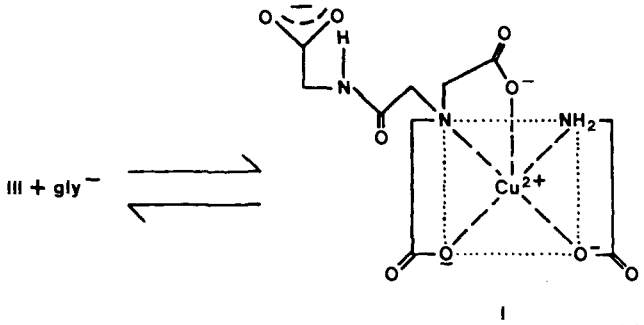
The 1:1 Cu(II) to H_3DGDA curve (A) was found to have inflections at $a = 3.0$ and 4.0. The reaction occurring in the low buffer zone is the formation of $[Cu(DGDA)H_2O^-]$ (III), while that in higher buffer region is the formation of II (eq 5). The



(5)

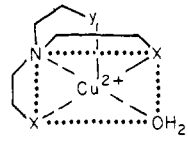
logarithm of the ionization constant of the peptide proton in $[Cu(DGDA)H_2O^-]$ (eq 5) was determined to be −6.58 ± 0.01, in agreement with the literature value of −6.61 ± 0.04.[5]

The 1:1:1 Cu(II) to H_3DGDA to gly curve (B) has inflections at $a = 3.0$ and 4.0, corresponding to the formation of III and I, respectively. The logarithm of the mixed-ligand formation constant (eq 6) is 5.95 ± 0.01, which is about halfway between that



(6)

for $[Cu(NTA)gly^{2-}]$ (5.46)[6] and $[Cu(IMDA)gly^-]$ (6.42).[7] It has been shown that the Lewis acidity for a series of complexes of the type IV are dependent upon the donor strength of the group



IV

coordinated to the axial position.[8] The coordinated amide carbonyl oxygen in $[Cu(DGDA)H_2O^-]$ is a weaker donor than the carboxylate of $[Cu(NTA)^-]$ and therefore would lower the Lewis acidity of the remaining equatorial site of IV to a lesser degree than with a bound carboxylate group. Accordingly, the $[Cu(IMDA)H_2O]$ complex with no chelated group in the axial position should be the best binder of glycine as is observed.

Although the metal complex formation reactions in the low pH buffer zones of curves A and B are identical, i.e., the formation of $[Cu(DGDA)H_2O^-]$, the curves are not identical. Curve B has a consistently higher pH value due to the presence of glycine, which contains an unprotonated carboxylate group. It is this group with a pK_a of 2.36 that buffers the protons released in the formation

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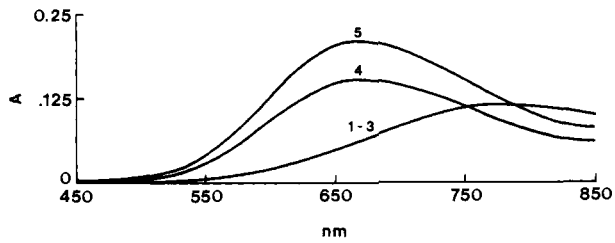

Figure 2. Visible spectra of 1:1:1 Cu(II) to H_3DGDA to HGly at a = 1.0 (1), 2.0 (2), 3.0 (3), 4.0 (4), and 5.0 (5), where a is moles of base per mole of DGDA. [Cu(II)] $\sim 1 \times 10^{-3}$ M.

of $[Cu(DGDA)H_2O^-]$, leading to higher pH values in the region a = 0.0 to 3.0 (eq 7).

$$NH_3^+CH_2COO^- + H^+ \rightleftarrows NH_3^+CH_2COOH \quad (7)$$

An unusual and interesting feature of the formation curve is that the formation of $[Cu(DGDA)gly^{2-}]$ occurs over a pH range which is not all that much lower than that of $[Cu(H_{-1}DGDA)^{2-}]$. In order to obtain a constant for eq 6, it was necessary to include $[Cu(H_{-1}DGDA)^{2-}]$ in the calculations. Therefore, the following mass (eq 8) and charge balance (eq 10) equations had to be used.

$$T_{gly} = [HG] + [G^-] + [Cu(DGDA)G^{2-}] \quad (8)$$

$$T_{Cu} = [Cu(DGDA)H_2O^-] + [Cu(H_{-1}DGDA)^{2-}] + [Cu(DGDA)G^{2-}] \quad (9)$$

$$[Na^+] + [H^+] = [OH^-] + [NO_3^-] + [G^-] + [Cu(DGDA)H_2O^-] + 2[Cu(H_{-1}DGDA)^{2-}] + 2[Cu(DGDA)G^{2-}] \quad (10)$$

Appropriate manipulation of the equations led to $[G^-] = ([Na^+] + [H^+] - [OH^-] - 4T_{Cu})/(1 - Z/X)$ and $K = [Cu(DGDA)G^{2-}]/([Cu(DGDA)][G]) = (T_G - AGZ)/[(AGZ/X)[G^-]]$, where $Z = 1 + [H]/K_a$, $X = 1 + [H]/K_{1a}$, and K_a is the K_a of glycine (determined to be $10^{-9.55\pm0.01}$, in good agreement with the literature value[9] of $10^{-9.57}$, while K_{1a} is the peptide ionization constant of $[Cu(DGDA)H_2O^-]$ (eq 5).

The reaction in the high pH buffer zone (pH >9) was found to be dechelation of glycine upon peptide proton ionization (eq 3), and the logarithm of the constant was determined to be −11.68 ± 0.02, which is similar to those determined for eq 1 (−11.34)[1] and eq 2 (−12.36).[2] The charge (eq 13) and mass (eq 11) balance equations and K_d expression (eq 14) are given.

$$T_{Cu} = [Cu(DGDA)G^{2-}] + [Cu(H_{-1}DGDA)^{2-}] \quad (11)$$

$$T_G = [HG] + [G^-] + [Cu(DGDA)G^{2-}] \quad (12)$$

$$[Na^+] + [H^+] = [OH^-] + 2T_{Cu} + [G^-] + 2[Cu(DGDA)G^{2-}] + 2[Cu(H_{-1}DGDA)^{2-}] \quad (13)$$

$$K_d = [(T_{Cu} - T_G + [G^-] + [HG])(H^+)(G^-)]/(T_G - [G^-] - [HG]) \quad (14)$$

Visible Spectra. The visible spectra of 1:1:1 Cu(II) to H_3DGDA to gly solutions at various a values are shown in Figure 2. From a = 0.0 to 3.0, there is little change in λ_{max} (767 nm) or ϵ_{max} (61 M^{-1} cm^{-1}), which are identical with those of 1:1 Cu(II) to H_3DGDA solutions from a = 0.0 to a = 3.0 (Figure 3), indicating that $[Cu(DGDA)H_2O^-]$ is the only Cu(II) species present in both solutions. From a = 3.0 to 4.0 (1:1:1 system), there is a shift in λ_{max} to higher energy (664 nm) and an increase in ϵ_{max} (80 M^{-1} cm^{-1}), indicating at a = 4.0 the complete formation of $[Cu(DGDA)G^{2-}]$. While the λ_{max} value of 668 nm for $[Cu(H_{-1}DGDA)^{2-}]$ (Figure 3) is similar to that of $[Cu(DGDA)G^{2-}]$, the ϵ_{max} value of the former, 123 M^{-1} cm^{-1}, is sufficient to differentiate the two metal complexes. From a = 4.0 to 5.0 (1:1:1 system) there is a slight shift to lower energy (672 nm) and an

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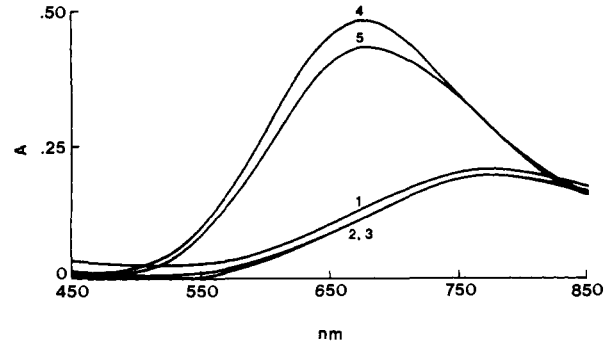


Figure 3. Visible spectra of 1:1 Cu(II) to H_3DGDA at a = 1.0 (1), 2.0 (2), 3.0 (3), 4.0 (4), and 5.0 (5), where a is moles of base per mole of ligand. [Cu(II)] $\sim 2 \times 10^{-3}$ M.

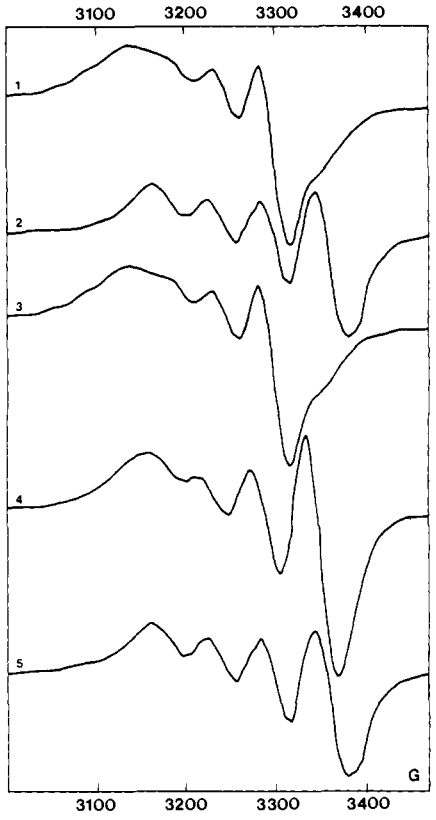
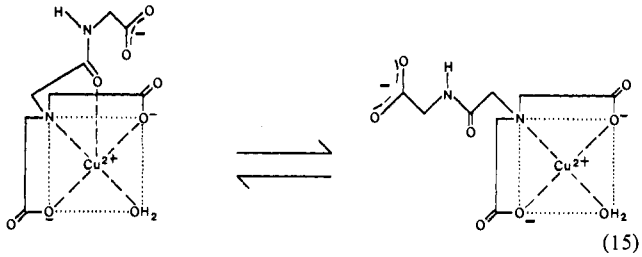


Figure 4. Electron spin resonance spectra of 1:1 Cu(II) to H_3DGDA at a = 3.0 (1) and 4.0 (2) and 1:1:1 Cu(II) to H_3DGDA to HGly at a = 3.0 (3), 4.0 (4) and 5.0 (5), where a is moles of base per mole of ligand. [Cu(II)] $\sim 1 \times 10^{-3}$ M.

increase in λ_{max} (120 M^{-1} cm^{-1}), which is the same within experimental error as that of $[Cu(H_{-1}DGDA)^{2-}]$, indicating the loss of chelated glycine and concomitant ionization and coordination of the peptide moiety (eq 3).

ESR Spectra. ESR spectra of 1:1:1 Cu(II) to H_3DGDA to gly solutions at various a values are shown in Figure 4, as are those of 1:1 Cu(II) to H_3DGDA solutions. The ESR spectra at a = 3.0 in both systems are identical and appear to represent two species with "g" values of 2.164 and 2.119. The presence of two ESR signals at a = 3.0, an inflection point in the potentiometric formation curve, is probably due to the following equilibrium (eq 15). Apparently the equilibrium is slow compared to that of the ESR measurement and involves no protons as required by the formation curves. At a = 4.0, (Figure 4) the ESR spectra of $[Cu(DGDA)G^{2-}]$ (g = 2.132) is different that that at a = 4.0 of the 1:1 Cu(II) to H_3DGDA solution (g = 2.125). However, at a = 5.0 the ESR spectra of the 1:1:1 system was found to be the same (g = 1.126) as that for the $[Cu(H_{-1}DGDA)^{2-}]$ species. The above complements the visible and potentiometric data in supplying further evidence for mixed-ligand chelate formation (eq 6, a =



(15)

3.0–4.0) followed by dechelation (eq 3, a = 4.0 to 5.0).

Discussion

As in the previous papers in this series, the mechanism of the substitution reaction appears to be of the S_N1CB variety.[10] In each case the product of the reaction contains the conjugate base responsible for the substitution reaction. In eq 1–3, the metal chelate products contain an ionized amide group, an ionized alcohol group, and an ionized peptide group, respectively. Unlike the initial studies which led Basolo and Pearson[10] to postulate the conjugate base species, $[Co(NH_3)_4(NH_2)X^+]$ and $[Co(NH_3)_4(NH_2)^{2+}]$ (eq 16), as transient intermediates in Co(III) substi-

$$[Co(NH_3)_5X^{2+}] \underset{-OH^-}{\overset{+OH^-}{\rightleftharpoons}} [Co(NH_3)_4(NH_2)X^+] + H_2O \rightleftharpoons$$

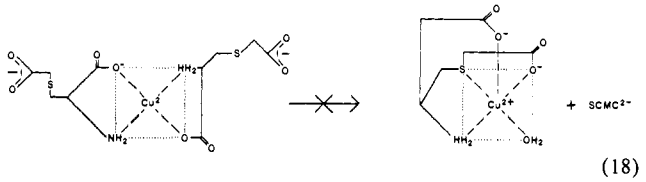
$$[Co(NH_3)_4(NH_2)^{2+}] + X^+ \underset{-H_2O}{\overset{H_2O}{\rightleftharpoons}} [Co(NH_3)_4(NH_2)(OH_2)^{2+}] \rightleftharpoons [Co(NH_3)_5OH)^{2+}] \quad (16)$$

tution reactions, the stable metal-containing products of these reactions are the conjugate base species. However, there are remarkable similarities in the nature of these reactions and those studied by Basolo and Pearson.[10] The reactions are pH dependent, require the presence of a very strong σ-donor (NH_2^-, RO^-, $-CONH^-$, and $-CON^--$), and involve the removal of a strongly coordinated group. The dissimilarities are retention of the conjugate base in the metal species product and the facility speed with which these newly discovered reactions occur (instantaneous).

The removal of amino acidate ligands from metal centers in the above reactions (eq 1–3) is quite remarkable in that such binding is quite strong (log K = 8.2, eq 17)[11] and has been shown

$$Cu^{2+} + L^- \rightleftharpoons CuL^+ \quad (17)$$

to prevent tetradentate chelate formation in bis(3-((2-aminoethyl)thio)-L-alaninato)- and bis(3-((carboxymethyl)thio)-L-alaninato)copper(II) chelates (eq 18, 19).[12]



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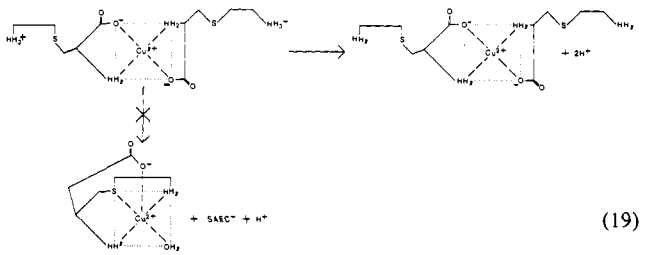

(19)

Biological Significance. The removal of substrates from metalloenzyme centers is a neglected area of research, perhaps due to the fact that step is fast and is most often preceded by the slow step. The one generally acknowledged feature is that conformational changes in the protein often accompany substrate loss. Since the reactions in eq 1–3 are instantaneous, involve the loss of a coordinated aminoacidate group, and contain functional groups found at the active site of metalloenzymes [amide (asparagine, glutamine), alcohol (serine, threonine), and peptide linkages], the reactions in eq 1–3 could be models for the loss of substrate from metalloenzyme centers. It has been well established that the ionization of peptide protons in Ni(II) polypeptide complexes induces a stereochemical change about Ni(II), i.e., octahedral to square planar.[13] An octahedral to tetrahedral stereochemical change has also been shown[14] to occur upon amide proton ionization in a Zn(II) chelate. Such stereochemical changes about metal ions would undoubtedly induce conformational changes within a protein. Finally, there are functional groups located at the activate site of some metalloenzymes for which there is no known purpose, even though their removal, (via replacement of one amino acid by another) deactivates the enzyme. Perhaps, the function of such groups is to aid in substrate removal.

While there is no direct evidence that eq 1–3 are models for substrate removal at the active site of any metalloenzyme, the mechanism as outlined above does explain many observed data. The reaction times of eq 1–3 are instantaneous, a strongly coordinated group is removed in a facile manner from a metal center, the coordination of a strong σ-donor can lead to stereochemical changes about the metal center which would induce conformational changes in the protein, and the presence of certain functional groups with unknown purpose at metalloenzyme centers may be explained as aiding in substrate removal. The latter point is especially appealing in that if functional groups at the active site are thought to aid in substrate binding, why should they not aid in substrate removal. Reactions 1–3 occur just within the physiological pH range; if such reactions do occur in proteins, facile exchange may render it difficult to retain and detect.

Acknowledgment. We express our gratitude to Professor A. E. Martell for the gift of DGDA and to Dr. D. Nettar for his helpful discussions concerning the ESR spectra. Funds for this study were provided by the National Science Foundation (PRM 8011453).

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