## References and Notes


(1) We wish to express our appreciation to the National Science Foundation for its support of this work.
(2) R. Steinberger and F. H. Westheimer, *J. Am. Chem. Soc.*, **73**, 429 (1951).
(3) J. V. Rund and R. A. Plane, *J. Am. Chem. Soc.*, **86**, 367 (1964).
(4) J. V. Rund and K. G. Claus, *J. Am. Chem. Soc.*, **89**, 2256 (1967).
(5) M. Munakata, M. Matsui, M. Tabushi, and T. Shigematsu, *Bull. Chem. Soc. Jpn.*, **43**, 114 (1970).
(6) Y. Yamane, M. Miyazaki, N. Yamaji, and T. Ogashiwa, *Chem. Pharm. Bull.*, **19**, 2342 (1971).
(7) P. R. Bontchev and V. Michaylova, *Proc. Symp. Coord. Chem.*, 1, 405 (1970).
(8) G. W. Kosicki and F. H. Westheimer, *Biochemistry*, **7**, 4303 (1968).
(9) N. V. Raghavan and D. L. Leussing, *J. Am. Chem. Soc.*, **98**, 723 (1976).
(10) E. Gelles and R. W. Hay, *J. Chem. Soc.*, 3673 (1958).
(11) E. Gelles and A. Salama, *J. Chem. Soc.*, 3864, 3869 (1958).
(12) G. Schwarzenbach and H. Flaska, "Complexometric Titrations", 2nd English ed, Nethven and Co. Ltd., London, 1969.
(13) V. S. Sharma and D. L. Leussing, *Talanta*, **18**, 1137 (1971).
(14) Owing to variations in pH it was not feasible to plot the remainder of the data on this type of graph. However, all of the data points for each series of investigations were employed in the numerical analyses. The agreement between observed and calculated rates for the final fit is as good in general as is shown in the figures. All of the data are given in the microfilm version.
(15) V. S. Sharma and D. L. Leussing, *Inorg. Chem.*, 11, 138, 1955 (1972).
(16) H. Sigel, *J. Inorg. Nucl. Chem.*, **37**, 507 (1975).
(17) H. Sigel, "Metal ions in Biological Systems", Vol. 2, Marcel Dekker, New York, N.Y., 1974, p 63–125.
(18) There is some correlation between the stability constants of $Cu_2(L)(H_{-1}oxac)$ and $Cu_2(L)_2(H_{-1}oxac)$. A high value chosen for the former will yield a low value for the latter, and vice versa. Regardless of the uncertainties in the absolute values of the formation constants, the calculations show that the mixed ligand dinuclear complexes are relatively unstable with respect to the mononuclear complexes.
(19) Of the metal ions studied in ref 5 only Cu(II) has a sufficiently high affinity for $oxac^{2-}$ that the bis complex is likely to be formed under the conditions described. Inhibition at the higher pH examined indicates the formation of mononuclear enolate complexes. In experiments employing dilute solutions, we have found the $pK_a$ of Cu(oxac) to be around 5.8. $Cu(oxac)_2^{2-}$ is expected to exhibit a value of $pK_{1a}$ slightly lower than this, roughly in agreement with the results given in ref 5. We are not able to account for the slight rate enhancement in $CO_2$ evolution reported when histidine is added to Cu(II)–$oxac^{2-}$ solutions. Our results show that coordinated histidine has about the same influence on coordinated $oxac^{2-}$ as $H_2O$. Perhaps, because protonated histidine complexes can be formed, $oxac^{2-}$ is merely distributed over a greater number of species that are able to undergo decarboxylation, Cu(oxac), Cu(his)$oxac^-$, and Cu(Hhis)oxac. This explanation invokes a simple statistical effect as the cause.
(20) H. Speck, *J. Biol. Chem.*, **178**, 315 (1949).
(21) G. Eichhorn, *Adv. Chem. Ser.*, **No. 37**, Chapter 3 (1963).
(22) R. J. P. Williams, *Biol. Rev. Cambridge Philos. Soc.*, **28**, 381 (1953), cited in ref 21.
(23) W. D. Covey and D. L. Leussing, *J. Am. Chem. Soc.*, **96**, 3860 (1974).
(24) M. Emlý, has found the formation constants of Mn(oxac) and $Mn_2(H_{-1}oxac)^+$ to be 36 and $2 \times 10^{-4}$ $M^{-1}$, respectively, in aqueous media. $K_{enol}$ is about 1 (unpublished results obtained in these laboratories).
(25) The model predicts that Cu(II) should show more enzymatic activity than is observed. The deactivation could arise from (1) the binding of Cu(II) to a second enzyme site, (2) to an unusually high binding constant of the enzyme toward $Cu_2(H_{-1}oxac)^+$, or (3) to the binding of the 1:1 enolate complex, $Cu(H_{-1}oxac)^-$, which was neglected in the calculations.
(26) Rates of decarboxylation have been found to increase in mixed solvent systems of low dielectric constant, ref 3, 7, 27.
(27) C. S. Tsai, Y. T. Lin, C. Reyes-Zamora, and J. A. Fraser, *Bioinorg. Chem.*, **4**, 1 (1974).
(28) B. Vallee and W. E. C. Wacker, "The Proteins", Vol. V, 2nd ed, H. Neurath, Ed., Academic Press, New York, N.Y., 1970.
(29) L. G. Sillen and A. E. Martell, *Spec. Publ., Chem. Soc.*, **No. 17** (1964), and **25** (1971).


# Ligand Effects on the Thermodynamic Stabilization of Copper(III)–Peptide Complexes


**Frank P. Bossu, K. L. Chellappa, and Dale W. Margerum***

*Contribution from the Department of Chemistry, Purdue University, West Lafayette, Indiana 47907.*

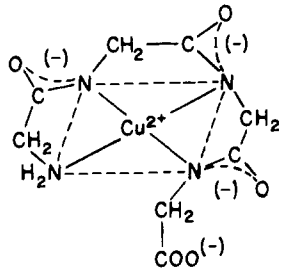
*Received August 16, 1976*


**Abstract:** Electrode potentials are measured for 40 $Cu^{III,II}$–peptide couples (including peptide amides) in aqueous solution. The potentials are very sensitive to changes in the nature of the ligand and span a range from 1.02 to 0.45 V. The values of $E^0$ decrease with an increase in the number of deprotonated-peptide groups. Bound hydroxide groups in the place of equatorial carboxylate groups and C-substituents in the chelated amino acid residues also decrease the value of $E^0$, but to a lesser extent. Histidine-containing peptide complexes have relatively high values of $E^0$. The additivity of the individual ligand effects allows the electrode potential of a copper complex to be estimated on the basis of the nature of the ligand. The triply deprotonated peptide complexes and the highly C-substituted tripeptide complexes of copper have effective potentials at physiological pH such that $O_2$ oxidation to Cu(III) is thermodynamically possible. From a correlation between the potentials and visible absorption maxima of the copper(II) peptides it is concluded that the relative gain in the crystal field stabilization energy for the change from $d^9$ Cu(II) to $d^8$ Cu(III) is an important factor in the overall thermodynamic stability of the Cu(III)–peptide complexes.


Trivalent copper has generally been considered to be an uncommon oxidation state, although a limited number of Cu(III) compounds have been identified in the solid state[1,2] and a few complexes have been prepared in nonaqueous solutions.[3,4] In aqueous solution Cu(III)–aquo and Cu(III)–amine complexes, generated by pulse radiolysis, are transient species which decay rapidly.[5] In the work of Levitski, Anbar, and Berger,[6] $IrCl_6^{2-}$ was used to oxidize Cu(II)-tetra-L-alanine to a Cu(III) complex which was proposed as an intermediate species prior to further oxidation and fragmentation of the peptide. Bour and Steggerda[2] isolated crystalline Cu(III) complexes of biuret and oxamide, providing some of the first evidence that Cu(III) could be stabilized by deprotonated amide groups. The copper(II) complexes of oligopeptides promote ionization of the peptide hydrogens upon complexation and the resulting deprotonated-peptide groups are strong in-plane donors. Systematic studies of the solution behavior of oligopeptide complexes using potentiometric and spectrophotometric methods have given copper(II)–peptide stability constants and have provided evidence for coordination of the deprotonated-peptide group.[7-15]

The copper complexes of diglycine, triglycine, and tetraglycine were studied by infrared ($D_2O$ solutions) in addition to the other techniques and also gave evidence for deprotonated-peptide nitrogen bonding.[16-18] ESR has been used to study several copper(II)–peptide complexes,[19-21] while ORD-CD has been used to examine nickel(II)–peptide complexes.[22] In addition both divalent nickel and copper complexes have been studied with proton NMR.[23] In a complex with ligands such as tetraglycine as many as three deprotonated-

peptide nitrogens can be bound to copper depending upon the pH. Structure I shows the triply deprotonated form of copper(II) tetraglycine $Cu^{II}(H_{-3}G_4)^{2-}$. Freeman and co-workers have determined the crystal structure of this $d^9$ complex,[24] the analogous $d^8$ nickel(II) complex,[25] and many other metal-peptide complexes.[26-29] The kinetics and mechanisms of metal ion and proton transfer reactions for divalent copper and nickel oligopeptide complexes have been studied extensively.[30]



I

Studies in our laboratory of the autoxidation of Cu(II)-peptide complexes suggested that long-lived Cu(III)-peptide complexes are found in aqueous solution.[31,32] Our subsequent work[33] provided evidence for a readily accessible Cu(III) complex of tetraglycine, $Cu^{III}(H_{-3}G_4)^-$, which was prepared by chemical oxidation and characterized in aqueous solution. The low potential for the $Cu^{III,II}(H_{-3}G_4)^{-,2-}$ couple (0.63 V) is of special interest because it indicates that this type of Cu(III) complex is more stable thermodynamically than had been suspected. In the present work we have measured the electrode potentials for 40 $Cu^{III,II}$-peptide complexes (including peptide amides) in order to determine what factors lead to thermodynamic stabilization of Cu(III) relative to Cu(II). Cyclic voltammetry was used for these measurements and the reliability of the potentials was confirmed by redox equilibria with $IrCl_6^{2-}$, $IrCl_6^{3-}$ for five of the complexes. The potentials are very sensitive to the nature of the coordinating groups, spanning a range from more than 1.0 V to less than 0.5 V. The potentials decrease with an increase in the number of deprotonated-peptide and deprotonated-amide groups, but also are influenced by a number of other factors. It is possible to correlate the visible absorption spectra of the Cu(II) complexes with the $Cu^{III,II}$ potentials.

## Experimental Section

The peptides used to form the copper complexes are listed in Table I. The following abbreviations are used for the amino acid residues (L-isomers) of the peptides: glycyl, G; alanyl, A; β-alanyl, βA; valyl, V; leucyl, L; isoleucyl, I; phenylalanyl, F; histidyl, his; aspartyl, asp; and lysyl, lys. GGa is glycylglycylamide, etc. The source of each commercially available peptide is listed in Table I. The preparation of the *N*-formyl derivatives and the properties of their copper complexes are reported elsewhere.[34] The fully deprotonated complexes of $Cu^{II}(H_{-4}NfG_3a)^{2-}$, $Cu^{II}(H_{-4}NfGGAa)^{2-}$, $Cu^{II}(H_{-4}NfG_4)^{3-}$, and $Cu^{II}(H_{-4}NfG_5)^{3-}$ form above pH 11, while the complexes of the *N*-formyltripeptides form triply deprotonated species above pH 10.

Solutions of the copper(II) complexes ($5 \times 10^{-4}$ M) were prepared by the reaction of solutions of $Cu(ClO_4)_2$ with the peptides in 5 to 10% excess. The $pK_a$ values of the fully deprotonated forms of the complexes are listed in Table I. The pH was adjusted with 0.1 M NaOH to the value listed in the subgroup headings in Table I. The fully deprotonated forms of the complexes predominate at these pH values. The ionic strength was adjusted to 0.10 M with $NaClO_4$ (prepared from $Na_2CO_3$ and $HClO_4$). The wavelengths of the visible absorption maximum of many of the fully deprotonated copper(II)-peptide complexes are listed in Table I. Cyclic voltammetry was performed at 25.0 °C. using freshly prepared copper(II)-peptide solutions, with a three-electrode system consisting of a carbon paste working electrode, a platinum wire auxiliary electrode, and a saturated NaCl calomel reference electrode. Voltammograms were generated using a Bioanalytical Systems CV-1 instrument and recorded on a Hewlett-Packard HP7035B X-Y recorder. Preconditioning of the carbon paste electrode by cycling between the potential limits of the solvent was necessary to obtain quasi-reversible current-potential curves. The $E^0$ values were determined as the midpoint between the peak potentials with an accuracy of ±5 mV. The electrode potentials were obtained from an average of at least three separate cyclic voltammetric measurements and have a reproducibility of ±5 mV for most cases. All the $E^0$ values listed in Table I are given in terms of standard electrode potentials vs. NHE.

The redox-pH profiles were obtained over a pH range of 4.5–8.5 by the reaction of equimolar concentrations of copper(II) peptide and $IrCl_6^{2-}$ ($2.5 \times 10^{-4}$ M). The solutions of Cu(II) complexes were prepared by the reaction of equivalent amounts of peptide or peptide amide with $Cu(ClO_4)_2$ solutions. The pH was adjusted using 0.1 M NaOH and ionic strength was adjusted to 0.10 M with $NaClO_4$. Commercial sources of $Na_2IrCl_6$ contain varying amounts of $Ir^{III}Cl_6^{3-}$ and therefore pure $Na_2Ir^{IV}Cl_6$ was obtained by bubbling $Cl_2$ through the solutions which were then freeze-dried. The purity was checked spectrophotometrically and solutions were standardized with $Fe(CN)_6^{4-}$. Stock solutions of $IrCl_6^{2-}$ were freshly prepared before each set of experiments and were kept slightly acidic to avoid decomposition. Acetate buffer ($2 \times 10^{-3}$ M) was used for pH 4.5–6.0 and the reactants were unbuffered at higher pH. The pH was measured after the reaction and was corrected for ionic strength effects ($-\log [H^+] = pH - 0.11$). The redox equilibrium was measured by the disappearance of Ir(IV) 490 nm ($\epsilon$ 4075 $M^{-1}$ $cm^{-1}$)[35] and simultaneously by the appearance of Cu(III) at 365 nm. The measurement must be obtained soon after mixing, especially at high pH, where the rate of decomposition of the Ir(IV) and of the Cu(III) complexes becomes appreciable. At higher pH stopped-flow mixing was used. Uncomplexed peptide ligands and many common buffers must be avoided because they react with iridium. Concentrations of the $IrCl_6^{2-}$ were calculated from the absorbance measurements at 490 nm with corrections made for contributions from the Cu(III) species. Similarly, Cu(III) concentrations were calculated from the absorbance at 365 nm with corrections made for contributions from $IrCl_6^{2-}$. The absorbance due to the Cu(II) complex and $IrCl_6^{3-}$ was negligible at both wavelengths.

Visible measurements were obtained using a Cary 16, a Cary 14, and a Durrum stopped-flow spectrophotometer. A radiometer PHM 26 pH meter was used for pH measurements.

## Results

**Potentials Measured Using $IrCl_6^{2-}$.** In the oxidation of copper peptides by $IrCl_6^{2-}$ the offsetting of the redox equilibrium by acid-base equilibria permits very accurate determinations of $E^0$ values and permits the number of deprotonated-peptide (amide) or hydroxide groups in the Cu(III) complex to be assigned. The $E^0$ values from the iridium studies serve to calibrate those measured by cyclic voltammetry and to confirm those values even when the systems were not completely reversible electrochemically. In each case only one Cu(III) species was found, although there can be several Cu(II) species present in solution. The stopped-flow kinetics of Cu(III) formation by $IrCl_6^{2-}$ oxidation confirm this. All the Cu(III) complexes have strong absorption bands near 365

$$Cu^{II}(H_{-3}G_3a)^- + IrCl_6^{2-} \overset{K_{ox}}{\rightleftharpoons} Cu^{III}(H_{-3}G_3a) + IrCl_6^{3-} \quad (1)$$

$$\begin{bmatrix} \rightleftharpoons H^+ \\ Cu(H_{-2}G_3a) \\ \rightleftharpoons H^+ \\ Cu(H_{-1}G_3a)^+ \\ \rightleftharpoons H^+ \\ CuG_3a^{2+} \\ \rightleftharpoons H^+ \\ Cu^{2+} + HG_3a^+ \end{bmatrix}$$

Table I. Properties of Copper Peptide Complexes

| Copper-peptide | Ligand source | $Cu^{II}(H_{-x}L)$ | | $Cu^{III,II}L$ | |
|---|---|---|---|---|---|
| | | $pK_a$ | $\lambda_{max}$, nm | $\Delta$, mV[a] | $E^0$ (NHE), V[b] |
| Tripeptides,[c] pH 7.7 | | | | | |
| GGG | *j* | 6.72 ($x = 2$)[n] | 555 | 78 | 0.92 |
| AGG | *g* | 6.72 ($x = 2$)[o] | — | 79 | 0.89 |
| GAG | *g* | 6.50 ($x = 2$)[o] | — | 78 | 0.88 |
| GGA | *g* | 6.77 ($x = 2$)[o] | 551 | 74 | 0.88 |
| GAA | *g* | | 546 | 84 | 0.85 |
| AAA | *g* | | 543 | 82 | 0.81 |
| LGG | *h* | | — | 73 | 0.88 |
| GGL (pH 8.7) | *g* | 7.21 ($x = 2$)[o] | 545 | 73 | 0.87 |
| GLG | *h* | 6.43 ($x = 2$)[o] | — | 110 | 0.87 |
| LGL (pH 8.7) | *k* | | — | 75 | 0.83 |
| LLL (pH 8.7) | *g* | | — | 89 | 0.77 |
| VGG | *h* | | — | 88 | 0.88 |
| GGV | *h* | | 543 | 72 | 0.87 |
| lGG | *l* | | — | 85 | 0.88 |
| GGl | *g* | | 541 | 72 | 0.86 |
| GGF | *i* | | — | 74 | 0.89 |
| GGβA | *h* | 6.08 ($x = 2$)[p] | 555 | 76 | 0.94 |
| Dipeptide amides[d] | | | | | |
| GGa | *j* | 9.82 ($x = 2$, OH)[n] | 575 | 110 | 0.85 |
| GAa | *h* | | — | 120 | 0.82 |
| AAa | *h* | | — | 140 | 0.78 |
| Histidine-containing peptides,[c] pH 7.5 | | | | | |
| GGhis | *i* | ~4.9 ($x = 2$)[s] | 525 | 82 | 0.98 |
| GGhisG | *g* | | 526 | 73 | 1.02 |
| asp·ala·his·lys | *g* | | 531 | 99 | 0.96 |
| Tripeptide amides,[e] pH 9.5 | | | | | |
| $G_3a$ | *h* | 8.69 ($x = 3$)[n] | 517 | 72 | 0.64 |
| GGAa | *i* | | — | 82 | 0.60 |
| VGGa | *h* | | — | 80 | 0.61 |
| Higher order peptides and peptide amides,[e] pH 9.5 | | | | | |
| $G_4$ | *g* | 9.16 ($x = 3$)[q] | 515 | 85 | 0.63 |
| $AG_3$ | *g* | 9.4 ($x = 3$)[o] | — | 128 | 0.61 |
| $G_4a$ | *i* | | — | 84 | 0.68 |
| $G_5$ | *g* | 8.04 ($x = 3$)[r] | 510 | 80 | 0.66 |
| $G_4A$ | *g* | 8.13 ($x = 3$)[o] | — | 95 | 0.66 |
| $G_6$ | *g* | | — | 95 | 0.67 |
| $A_4$ | *g* | | — | 90 | 0.60 |
| $V_4$ | *g* | | 520 | 61 | 0.51 |
| *N*-Formyl tripeptides,[e] pH 10.0 | | | | | |
| $NfG_3$ | *m* | | 538 | 75 | 0.75 |
| $NfA_3$ | *m* | | 527 | 77 | 0.66 |
| *N*-Formyl peptides and peptide amides,[f] pH 11.5 | | | | | |
| $NfG_4$ | *m* | | 523 | 82 | 0.55 |
| $NfG_5$ | *m* | | — | 66 | 0.63 |
| $NfG_3a$ | *m* | | 507 | 83 | 0.49 |
| NfGGAa | *m* | | — | 75 | 0.45 |

[a] Potential peak separation indicating reversibility of electrode reaction. [b] Determined by cyclic voltammetry at 100 mV $s^{-1}$ with a carbon paste working electrode, $[CuL]_T = 5 \times 10^{-4}$ M, 25.0 °C, $\mu = 0.10$ M $NaClO_4$, $E^0$ vs. NHE, the pH values of $Cu^{II}L$ solutions used in the electrochemical study are listed with the group headings. [c] $E^0$ value for $Cu^{III}(H_{-2}L)^n + e \rightleftarrows Cu^{II}(H_{-2}L)^{n-1}$. [d] $E^0$ value for $Cu^{III}(H_{-2}L)(OH) + e \rightleftarrows Cu^{II}(H_{-2}L)(OH)^-$. [e] $E^0$ value for $Cu^{III}(H_{-3}L)^n + e \rightleftarrows Cu^{II}(H_{-3}L)^{n-1}$. [f] $E^0$ value for $Cu^{III}(H_{-4}L)^n + e \rightleftarrows Cu^{II}(H_{-4}L)^{n-1}$. [g] Biosynthetika. [h] Vega-Fox Chemical Co. [i] Cyclo Chemical Co. [j] Sigma Chemical Co. [k] Mann Research Laboratories. [l] Bachem Inc. [m] Reference 34. [n] T. F. Dougatti and E. J. Billo, *J. Inorg. Nucl. Chem.*, **37**, 1515 (1975). [o] A. E. Martell and R. M. Smith, "Critical Stability Constants", Vol 1, Plenum Press, New York, N.Y., 1974. [p] O. Yamauchi, Y. Nakao, and A. Nakahara, *Bull. Chem. Soc. Jpn.*, **46**, 2119 (1973). [q] A. Kaneda and A. E. Martell, *J. Coord. Chem.*, **4**, 137 (1975). [r] C. R. Hartzell and F. R. N. Gurd, *J. Biol. Chem.*, **244**, 147 (1969). [s] Estimated from $\beta_3 = 10^{-14.65}$ $M^3$ where three protons are lost simultaneously, G. F. Bryce, R. W. Roeske, and F. R. N. Gurd, *J. Biol. Chem.*, **240**, 3837 (1965).

nm and the experimentally determined molar absorptivities for several of these complexes are given in Table II.

The redox reaction and the offsetting acid-base equilibria are exemplified in eq 1 for the copper triglycinamide complex. Only the fully deprotonated species is directly oxidized to give the single Cu(III) complex. The chemical oxidation is reversible and pH dependent resulting in a redox-pH curve.[33] Knowing the formation and deprotonation constants for the copper(II) species, the value of $K_{ox}$ can be calculated by linear least-squares analysis of the redox-pH profiles. The experimental $K_{ox}$ values are reported in Table II for a series of copper peptides. The formation and deprotonation constants of the Cu(II) complexes of pentaglycine, triglycinamide, and tetraglycine have been determined (see Table I for references). The

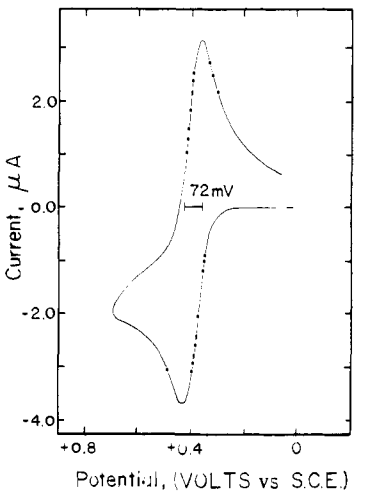



**Figure 1.** Cyclic voltammogram of $[Cu^{II}(H_{-3}G_3a)]^-$ in aqueous solution at a carbon paste electrode: $[CuG_3a]_T = 5 \times 10^{-3}$ M, pH 9.5, $\mu = 0.10$ M $NaClO_4$, 25 °C, scan rate = 100 mV $s^{-1}$. $E^0 = 0.64$ V vs. NHE.

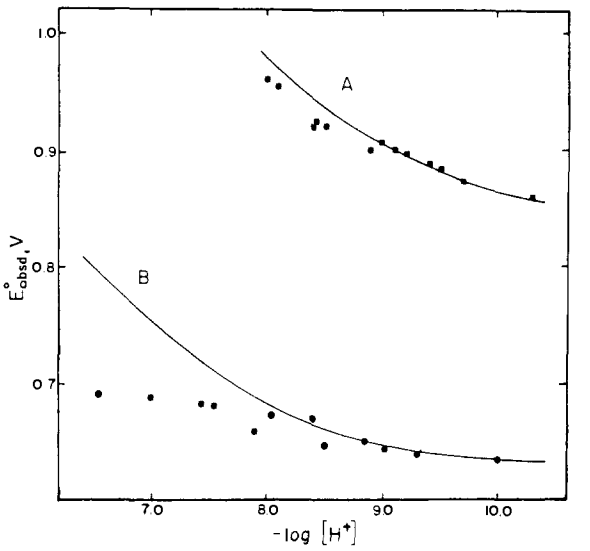



**Figure 2.** $E^0_{obsd}$ vs. $-\log [H^+]$ for $Cu^{III,II}$ couples: $[CuL]_T = 1 \times 10^{-3}$ M, $\mu = 0.10$ M $NaClO_4$, 25 °C; A, copper diglycinamide, solid line calculated from eq 3 and $E^0 = 0.85$ V; B, copper triglycinamide, solid line calculated from eq 4 and $E^0 = 0.635$ V.

**Table II.** Standard Electrode Potentials of $Cu^{III,II}$–Peptide Complexes[a]

| Cu(III) Peptide | Redox-pH $\epsilon_{365}$, $M^{-1}$ $cm^{-1}$ | Redox-pH $K_{ox}$[b] | Redox-pH $E^0$, V | Cyclic voltammetry[c] $E^0$, V |
|---|---|---|---|---|
| $Cu(H_{-3}G_6)^-$ | 7580 ± 90 | $5.2 \times 10^3$ | 0.672 | 0.67 |
| $Cu(H_{-3}G_5)^-$ | 7670 ± 300 | $1.5 \times 10^4$ | 0.646 | 0.66 |
| $Cu(H_{-3}G_3a)$ | 7500 ± 200 | $2.3 \times 10^4$ | 0.635 | 0.64 |
| $Cu(H_{-3}G_4)^-$ | 7120 ± 200 | $2.7 \times 10^4$ | 0.631 | 0.63 |
| $Cu(H_{-2}AAa)$-(OH) | 5700 ± 500 | 77 | 0.78 | 0.78 |

[a] 25 °C, $\mu = 0.10$ M $NaClO_4$, $E^0$ vs. NHE. [b] $K_{ox}$ from the $IrCl_6^{2-}$ reaction determined from relationships similar to eq 1 with a pH range of 4.5–8.5. [c] Carbon paste working electrode, 100 mV $s^{-1}$ scan rate.

hexaglycine constants were estimated using the constants for pentaglycine and similarly the constants for di-L-alaninamide were estimated from those of diglycinamide. Using these values of $K_{ox}$ and a value of 0.892 V for the $E^0$ of the $Ir^{IV,III}Cl_6^{2-,3-}$ couple under our conditions,[33] the electrode potentials of the $Cu^{III,II}$ couples can be calculated. For triglycinamide a value of 0.635 V was determined for the electrode potential given in eq 2. Values of $E^0$ for five copper peptides determined by this method are listed in Table II.

$$Cu^{III}(H_{-3}G_3a) + e \rightleftarrows Cu^{II}(H_{-3}G_3a)^- \quad (2)$$

The redox-pH analysis for determining standard electrode potentials of the $Cu^{III,II}$ couples suffers from several disadvantages. The formation and deprotonation constants must be well known and stopped-flow techniques are necessary at higher pH in order to perform the analysis. Since equilibrium constants have been determined for only a limited number of copper(II)–peptide complexes, an extensive survey of ligand effects on $Cu^{III,II}$ potentials is difficult by the iridium redox method. Therefore, this method was used to confirm the $E^0$ values obtained using cyclic voltammetry.

**Potentials Measured by Cyclic Voltammetry.** Figure 1 shows the current-voltage response obtained from applying cyclic voltammetry to the copper triglycinamide complex. The initial solution contains predominantly the $Cu^{II}(H_{-3}G_3a)^-$ complex which generates the oxidation wave (negative current). The Cu(III) complex which is formed generates the reduction wave (positive current) as the potential is reversed. This voltammogram is representative of all the complexes. The separation of the anodic and cathodic peaks was 70–90 mV in all but a few cases and peak current ratios were near unity. Slight variations of peak potential separation with different scan rates were observed. These features are indicative of quasi-reversible electrochemical behavior and, therefore, the midpoint between the oxidation peak and the reduction peak should be a reasonable estimate of the electrode potential for the $Cu^{III,II}$–peptide couples. A comparison of the electrode potentials obtained from the redox-pH equilibria and from cyclic voltammetry is given in Table II. The results indicate that the values obtained under our conditions by cyclic voltammetry are reliable electrode potentials even when the systems analyzed were not completely reversible electrochemically.

Table I lists the standard electrode potentials (vs. NHE) determined for a series of $Cu^{III,II}$ couples with dipeptide amides, tripeptides, and tripeptide amides, as well as with higher-order peptides, derivatized-peptides, and derivatized-peptide amides. The peak potential separations are listed as an indication of the reversibility of the electrochemical process. The theoretical separation for a completely reversible system is 59.5 mV for a one-electron exchange.[36]

**pH Effects on $E^0$ Values Determined by Cyclic Voltammetry.** The midpoint potentials as well as the magnitude of the current response measured by cyclic voltammetry can vary with pH. The degree of the variation depends on the extent and nature of the Cu(II) acid-base equilibria. Two general cases are shown in Figure 2. The variation of potential for the copper diglycinamide complex is shown in curve A. The solid line is calculated on the basis of rapid equilibria in eq 3. Above pH

$$\begin{array}{ccc} Cu^{II}(H_{-2}GGa)(OH)^- & \underset{E^0}{\rightleftarrows} & Cu^{III}(H_{-2}GGa)(OH) + e^- \quad (3) \\ H^+ \updownarrow K_a = 10^{-9.5} & & \\ Cu(H_{-2}GGa) & & \\ H^+ \updownarrow K_a = 10^{-8.01} & & \\ Cu(H_{-1}GGa)^+ & & \end{array}$$

9 the acid-base equilibrium depends on the rapid exchange of a proton between a coordinated hydroxide group and coordinated water and the experimental points follow the calculated curve. On the other hand the exchange of a proton at a peptide nitrogen is known to be slower[30,37-41] so that below pH 9 the

scan time competes with the equilibration time. This second case exists in the acid-base equilibrium for copper triglycinamide as given in eq 4. The potential variation with pH is shown in curve B of Figure 2. The solid line is calculated on the basis of rapid equilibria in eq 4. However, at the lower pH the

$$\begin{array}{ccc} Cu^{II}(H_{-3}G_3a)^- & \underset{E^0}{\rightleftharpoons} & Cu^{III}(H_{-3}G_3a) + e^- \qquad (4) \\ H^+ \updownarrow K_a = 10^{-8.09} & & \\ Cu(H_{-2}G_3a) & & \\ H^+ \updownarrow K_a = 10^{-6.99} & & \\ Cu(H_{-1}G_3a)^+ & & \end{array}$$

cyclic voltammetry scan rate is faster than the acid-base equilibria and the electrode potential is nearly constant with pH. As the potentials remain constant the peak current response decreases with decreasing pH because less of the oxidizable form is present.

The electrode potentials reported in Table I are believed to be an accurate measurement of the redox reaction involving species of $Cu(H_{-2}L)$ for tripeptides, $Cu(H_{-3}L)$ for *N*-formyl tripeptides, tripeptide amides, tetrapeptides, and higher peptides, $Cu(H_{-2}L)OH$ for dipeptide amides, and $Cu(H_{-4}L)$ for *N*-formyl tetrapeptides and *N*-formyl tripeptide amides. These values were determined by obtaining current-potential curves at a pH where the fully deprotonated copper(II) species is predominant (95-99%). Where this was not possible, as in the case of the dipeptide amides, the potential-pH profiles were fit to the appropriate equilibria in order to determine the value of $E^0$.

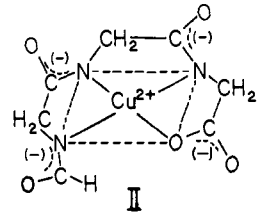
## Discussion

In general the values of the electrode potentials in Table I indicate that deprotonated-peptide bonding stabilizes the trivalent oxidation state of copper. The peptide complexes have very strong in-plane ligand donors. The overall thermodynamic stability of the trivalent complex results from the ability of these ligands to conform to the geometrical preference of the $d^8$ Cu(III) complex with the consequent ligand field stabilization. The iridium-pH study and the electrochemical-pH study indicate that only the fully deprotonated form of the complex is present as the trivalent complex. The structures of the Cu(III) complexes should be similar to those of the $d^8$ Ni(II)-peptide complexes. Crystal structures have been determined for both $Cu^{II}G_4$[24] and $Ni^{II}G_4$.[25] Although they are both four-coordinate, the copper(II) complex is described as an extremely flattened tetrahedron, while the nickel(II) complex has been described as truly square planar. In the transition from $d^9$ to $d^8$ for the divalent peptide complexes, the metal and the four nitrogens become slightly more coplanar. The metal-nitrogen bond distances are shorter for the nickel complex (Ni-N(peptide) bonds average 1.85 Å) than for the copper complex (Cu-N(peptide) bonds average 1.93 Å). Shorter metal-nitrogen bonds for Ni(II) compared to Cu(II) are a unique feature of peptide complexes and can be attributed to the relative gain in ligand field stabilization of the $d^8$ over the $d^9$ electronic configuration. In addition the N-M-N bond angles are a little closer to 90° for the nickel complex, averaging 85.7°, compared to those of copper, which average 83.4°. The relative effects should be even larger in going from Cu(II) to Cu(III) because the gain in ligand field stabilization due to change in electronic configuration from $d^9$ to $d^8$ is further increased due to the change from a divalent to a trivalent metal ion.[42] This in turn would be expected to give smaller copper-nitrogen bond distances and, hence, bond angles averaging even close to 90°. These general features should be applicable to the other peptide ligands.

The data in Table I also show that a greater than 500 mV variation in electrode potentials of the $Cu^{III,II}$ couples is observed upon changing the nature of the peptide ligands. The values of $E^0$ are affected by changes in the coordinating groups, the number and type of C-substituents of the individual amino acid residues, and axial coordination in the divalent complex. With the broad family of complexes described here and the corresponding wide range of potentials, the observed $E^0$ values can be related to the individual contributions of these effects.

**Effect of Coordinating Groups on $E^0$.** The deprotonated peptide nitrogen is higher in the spectrochemical series and is a stronger $\sigma$ donor than either the amine or the carboxylate group.[43] Its strong electron-donor properties should help to stabilize the higher oxidation state of copper. This effect is evidenced in the comparison of coordinating groups and electrode potentials found in Table I. The triply deprotonated complex of $Cu(H_{-3}G_3a)^-$ has four nitrogens bound to the metal, including an amine, two deprotonated-peptide nitrogens, and a deprotonated amide. The electrode potential is lowered by 280 mV for $[Cu^{III,II}(H_{-3}G_3a)]^{0,-}$ (0.64 V) compared to the triglycine complex, $[Cu^{III,II}(H_{-2}GGG)]^{0,-}$ (0.92 V), which has an amine, two deprotonated-peptide nitrogens, and a carboxylate group. The tripeptide amides, tetrapeptides, and higher order peptides and peptide amides have similar coordination to that of triglycinamide and as a group they have lower electrode potentials that the corresponding doubly deprotonated tripeptide complexes.

Further stabilization of the trivalent state of copper is possible by formylation of the amine end of the peptides. At high pH the resulting ligands have additional deprotonation with binding of the *N*-formyl amine group to copper, providing properties of donor strength similar to that expected for addition of another deprotonated-peptide nitrogen bond. A fully deprotonated Cu(II) complex of *N*-formyltriglycine (structure II) forms with a $pK_a$ of 9.2, accompanied by a blue shift in the



II

visible absorption maximum, when compared to the corresponding triglycine complex. A similar spectral shift occurs in the copper(II)-*N*-formyltriglycinamide complex and the fourth proton deionizes at a $pK_a$ value of 10.1. The direction and magnitude of the spectral shift for the derivatized tripeptides and tripeptide amides are in agreement with that predicted on the basis of the replacement of an amine nitrogen by a deprotonated-peptide nitrogen in a copper(II) complex.[43]

The electrode potential of the $Cu^{III,II}$ couple of *N*-formyltriglycine is 150 mV lower than the triglycine couple. The other tripeptides and tripeptide amides show similar decreases in electrode potential upon *N*-formylation. Accessibility of the Cu(III) state in aqueous solution is increased by these coordinating effects to a point where the electrode potential of $[Cu^{III,II}(H_{-4}N\text{-formylG}_3a)]^{-,2-}$ is only 0.49 V.

When a water molecule occupies one of the equatorial coordination positions in a Cu(II)-peptide complex, it loses a proton upon oxidation to give a Cu(III)-OH bond, as determined by the redox-pH study of copper di-L-alaninamide and by the electrochemical-pH study of the dipeptide amide complexes. The electrode potential of $[Cu^{III,II}(H_{-2}GGa)(OH)]^{0,-}$ (0.85 V) is 70 mV lower than that of the carboxylate bound triglycine complex and 180 mV higher than the potential of the triglycinamide complex with the deprotonated-amide

group in place of the hydroxide group. Hydroxide ion forms more stable complexes with trivalent compared to divalent metal ions.[44-46] The magnitude of the electrode potentials measured for the copper dipeptide amide complexes indicates that hydroxide ion also is effective at stabilizing Cu(III). In fact in strong base in the absence of peptide bonding it is possible to oxidize $Cu^{II}(OH)_4^{2-}$ with hypochlorite ion to give trivalent copper.[47]

**Effect of C-Substituents on $E^0$.** The electron-donating alkyl substituents affect the electron-donor properties of bound nitrogens and help to stabilize the trivalent oxidation state of copper. The presence of a single C-substituent has a much smaller effect than changing the type of donor group, but the effect is additive and becomes substantial if C-substitution occurs on all three of the bound chelate rings. The potentials for the $Cu^{III,II}$ couples of the tripeptides where alanyl replaces glycyl residues decrease in the following manner: GGG (0.92 V), GGA (0.88 V), GAA (0.85 V), and AAA (0.81 V). Similar lowering of electrode potentials by C-substitution is observed for the tripeptide amides and tetrapeptides. The comparison of the tetraglycylalanine complex with the pentaglycine complex indicates that this effect is sensitive only to substitution of residues involved in chelate ring formation.

When the electrode potentials of complexes with C-substitutents from leucyl, valyl, phenylalanyl, and isoleucyl are compared, the lowering of potentials is even larger than with the alanyl residues. The potential of the LLL complex is 0.77 V compared to a value of 0.81 V for the AAA complex. Since the electron donor ability of these substituents is nearly the same,[48] this additional stability can be attributed to steric effects. Bulky substituents decrease the N-C-C bond angle[49] and would consequently favor the smaller metal ion radius. The further possibility that bulky C-substituents interfere with axial solvation is more likely in the divalent complexes than in the more square-planar trivalent complex. The overall contribution of the C-substituents to the stabilization of the trivalent complexes appears to be a combination of electron-donor ability and steric effects.

**Effect of Histidine Coordination on $E^0$.** Histidine is an important amino acid residue in metal–pepetide binding with both a peptide nitrogen and an imidazole nitrogen available for bonding. Ligands with histidine as the third amino acid residue have an amine nitrogen, two deprotonated-peptide nitrogens, and an imidazole nitrogen bound to copper at physiological pH. Similar bonding is proposed for the first coordination of copper to serum albumin (human, bovine, and rat), where histidine is also the third amino acid residue.[50-53] Since the imidazole group is higher in the spectrochemical series than the carboxylate group,[43] it was surprising to find that the electrode potentials of the histidine-containing peptides were higher than for the tripeptide complexes. The electrode potentials for GGhis, GGhisG, and asp·ala·his·lys (first four residues of human serum albumin) are 0.98, 1.02, and 0.96 V, respectively.

The two distinct features contributing to the higher electrode potential are the relative changes in (1) cumulative ring strain and (2) $\pi$ back-bonding. In the first case the fully deprotonated copper GGhis complex has a 5,5,6-membered ring system. This type of ring system gives a more stable copper(II) complex than a 5,5,5-membered ring system as seen in the polyamine,[54] as well as the tripeptide, complexes.[55] This additional stability is attributed to a reduction in cumulative ring strain. Such stabilization apparently does not occur for the trivalent complex to the same extent as for the divalent complex, because the 5,5,5-membered complex of GGA (0.88 V) has a potential 60 mV lower than the 5,5,6-membered complex of GG$\beta$A (0.94 V). A similar increase in potential is expected for the histidine-containing complexes. In the second case $\pi$ back-bonding can occur in addition to $\sigma$ bonding between the imidazole nitrogen of histidine and copper(II) as discussed by Freeman[56] and evidenced by EPR hyperfine splitting data.[57] However, back-bonding from the more highly charged trivalent metal ion would be expected to be less important and the net result is a larger energy difference between the two oxidation states.

**Effect of Axial Coordination on $E^0$.** It has been shown from other studies[58] that while there is no axial coordination of the carboxylate group in the copper complex of GGhis, the terminal carboxylate does bond axially in $[Cu^{II}(H_{-2}GGhisG)]^{2-}$. In the copper(II) complex of asp·ala·his·lys the side chain carboxylate of aspartyl bonds axially in addition to the terminal carboxylate of the peptide. Since the Cu(III) state is expected to be square planar with little or no axial coordination, the presence of these carboxylate groups would be expected to increase the energy difference between the two oxidation states. This can account for the electrode potential of the GGhisG complex being 40 mV higher than that of the GGhis complex. In the case of asp·ala·his·lys the stabilizing effect of C-substituents (~80 mV) apparently overcomes the effect of the two axially coordinated carboxylates in the Cu(II) form and results in a slightly lower potential of 0.96 V.

**Additivity of Individual Contributions to $E^0$.** The previous discussions indicate that the redox characteristics of copper peptide complexes are affected by several different factors. Table III lists five individual ligand effects which lower the value of $E^0$ and three individual ligand effects that increase the value of $E^0$. The relative magnitude of these effects was determined using the triglycine complex as a reference point and the individual contributions to the value of $E^0$ are listed in Table III. Electrode potentials of the copper complexes can be estimated on the basis of the sum of these individual contributions to $E^0$ and the calculated values are plotted against the experimental values in Figure 3. The resulting correlation is excellent over a wide range of potentials (0.45-1.02 V) and allows the prediction of electrode potentials on the basis of the nature of the complex.

The electrode potentials for the complexes of $NfG_4$ and $NfG_5$ have a large deviation from the calculated values. We could find no spectral evidence for the axial addition of a hydroxide group, which would have accounted for the deviation. The higher potentials may be attributed to the high charge density (five negative charges) of the fully deprotonated complex. The contraction of equilibrium bond distances upon oxidation of the metal would accentuate repulsive interactions of these groups. The $NfG_5$ complex has the additional possibility of axial coordination of the carboxylate group to Cu(II).

**The Effect of Crystal Field Stabilization Energy on $E^0$.** We have observed a correlation between the electrode potentials and the visible absorption maxima of the Cu(II) complexes (Figure 4). The reason for this relationship is not obvious, since the electrode potentials are the differences in ground state energies of Cu(III) and Cu(II) whereas the spectral properties are differences in the ground state and excited states of Cu(II). Nevertheless, the two properties can be correlated in terms of ligand field effects. Changes in donor groups or C-substituents change the crystal field stabilization energy (CFSE) and such stabilization will influence the energy difference between the divalent and trivalent oxidation states. An indication of the CFSE can be obtained from the energy of the d-d transitions. The visible spectra of the copper(II)-peptide complexes show a single absorption band which envelopes three d–d transitions. If the relative positions and intensities of the three transitions remain constant, the position of the maximum for the band envelope is a measure of the equatorial bond strength and indicative of the CFSE. For this series of copper complexes the absorption maximum varies between 575 and 508 nm, while the shape of the band remains the same. A plot of $\bar{\nu}_{max}$ for the

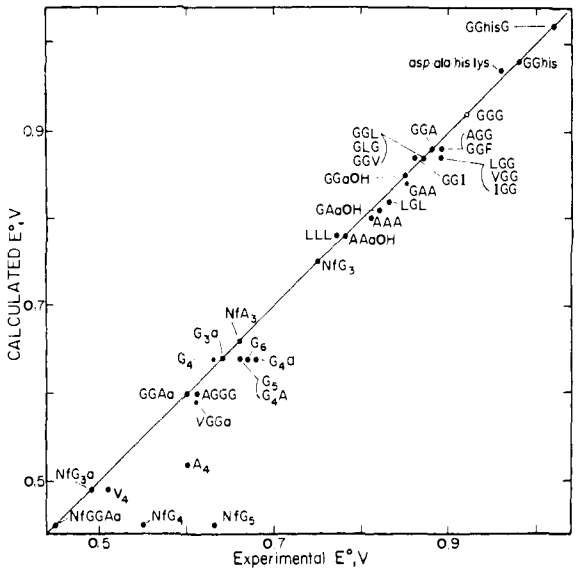



**Figure 3.** Relationship between values of $E^0$ calculated on the basis of the additivity of ligand effects listed in Table III and the experimental $E^0$ values.

Cu(II) complexes vs. the values of $E^0$ for many of the peptides is shown in Figure 4. The correlation indicates that an inverse relationship exists between the CFSE of the divalent complex and $E^0$. This suggests that the factors which increase the CFSE in the copper(II) complexes cause an even greater increase in the CFSE of the Cu(III) complexes. The two sources of this gain in stability are (1) the estimated increase in the CFSE for square-planar complexes from $12.28Dq$ to $24.56Dq$ for the change from $d^9$ to $d^8$ electronic configurations[59] and (2) the increase in the value of $Dq$ by 40–80% for the change from a divalent to a trivalent ion.[43] Figure 5 shows a simplified energy-level diagram for the oxidation of a copper(II) complex. The CFSE of the divalent complex is expressed as $\Delta$ and for the trivalent complex the CFSE is expressed as $x\Delta$. The relationship between $\Delta$ and $E^0$ can be expressed by eq 5.

$$E^0 = (1 - x)\Delta + E' \tag{5}$$

If the inverse correlation between $\bar{\nu}_{max}$ and $E^0$ is due to the gain in CFSE from both the change in electronic configuration (a twofold increase in CFSE) and the increased charge of the metal ion (a 1.4–1.8 increase in $Dq$), the value of $x$ should be between 2.8 and 3.6. (Since $\bar{\nu}_{max}$ is related to $\Delta$, the value of $x$ can be determined from Figure 4.)

The magnitude of $10Dq$ can be estimated from $\bar{\nu}_{max}$ and used to calculate the value of $\Delta$. The order of increasing energy for the 3d orbitals of Cu(II) peptides is considered $yz \sim xz < xy < z^2 < x^2 - y^2$ with the corresponding transitions of $A_{1g} \leftarrow B_{1g} < B_{2g} \leftarrow B_{1g} < E_{1g} \leftarrow B_{1g}$.[60,61] The transition of interest is $B_{2g} \leftarrow B_{1g}$ since its energy approximates $10Dq$. However, it is only one of the transitions contributing to the visible band of these complexes and $\bar{\nu}_{max}$ more closely approximates the energy of the $A_{1g} \leftarrow B_{1g}$ transition.[62] Since the $B_{2g} \leftarrow B_{1g}$ transition is at lower energy than the $A_{1g} \leftarrow B_{1g}$ transition, we can estimate from circular dichroism data[61] that $0.9\,\bar{\nu}_{max}$ approximates $10Dq$. With the CFSE for the $d^9$ Cu(II) complex estimated to be $12.28Dq$, the value of $\Delta$ is related to $\bar{\nu}_{max}$ by eq 6. In addition the value of $E^0$ can be expressed in terms of $\mu m^{-1}$ relative to the ionization of hydrogen (eq 7),[63] rather than in volts vs. NHE. Analysis of the best straight line through the experimental points gives a value of 2.8 for $x$. This is in reasonable agreement with the previously estimated value of $3.2 \pm 0.4$ for $x$. The analysis assumes that $E'$ is independent of ligand effects over the limited energy range of these Cu(II) complexes.

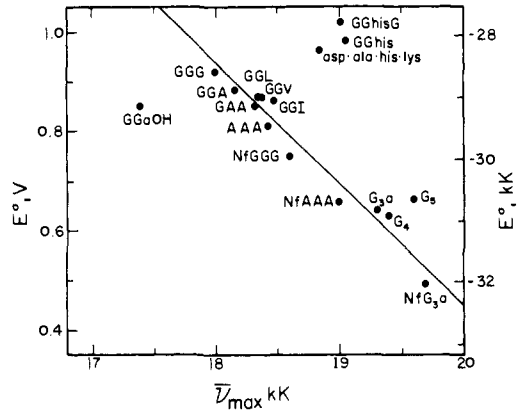



**Figure 4.** Electrode potentials (V vs. NHE) as a function of the copper(II)-peptide d–d absorption maxima (kK). Right-hand ordinate gives $E^0$ in terms of kK (eq 7). ($1\ kK = 1 \times 10^{-1}\ \mu m^{-1}$.)

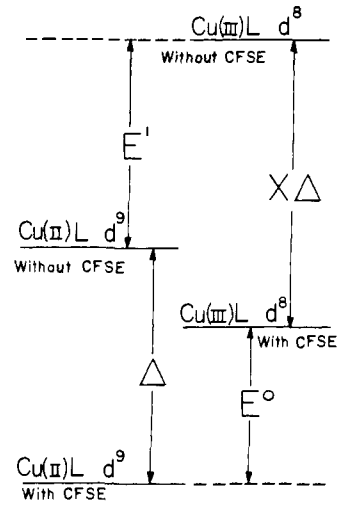



**Figure 5.** Relative energy-level diagram of $Cu^{III,II}$-peptide couples. Cu(II) CFSE is given by $\Delta$ and Cu(III) CFSE is $x\Delta$.

$$\Delta = \left(\frac{12.28}{10}\right)(0.9)\nu_{max} \tag{6}$$

$$(\tfrac{1}{2}H_2)_{gas} + H_2O_{aq} \rightleftarrows H_3O^+_{aq} + e^-_{vacuo} \qquad -3.6\ \mu m^{-1} \tag{7}$$

The correlation that results from this simplified model based on ligand field effects indicates that the relative gain in the CFSE for the change from $d^9$ Cu(II) to $d^8$ Cu(III) is an important factor in the overall thermodynamic stability of the Cu(III)-peptide complexes. The plot in Figure 4 indicates that the correlation fits for ligand effects involving the number of deprotonated-peptide groups or the number of C-substituents. The $E^0$ values of the complexes with equatorially bound hydroxide and imidazole groups do not correlate with the copper(II) visible absorption maxima, which is expected on the basis of our previous discussions.

**Cu(III) Formation at Physiological pH.** Some of these Cu(III) peptides have half-lives of several hours at neutral pH and the complexes are of special interest for biological redox reactions. The experimental values of $E^0$ were determined at pH values where the copper(II) complex was highly deprotonated. In order to determine the accessibility of the Cu(III) complexes at physiological pH, the electrode potentials were calculated from the Cu(II) species present in solution at pH 7.4 and the values for the effective electrode potentials are given in Table IV. At this pH the four-electron reduction of oxygen to water provides 0.79 V of oxidizing power. Table IV

Table III. Individual Contributions to the Electrode Potentials of $Cu^{III,II}$–Peptide Couples[a]

| | $\Delta E^0$, V |
|---|---|
| A. Ligand Effects Which Decrease the Value of $E^0$ | |
| (1) Replacement of an equatorially bound carboxylate group by a deprotonated-peptide group | −0.28 |
| (2) Replacement of an amine group by a deprotonated *N*-formylamine group | −0.15 |
| (3) Replacement of an equatorially bound carboxylate group by a hydroxide group | −0.07 |
| (4) Leucyl, valyl, isoleucyl C-substituents (per substituent) | −0.05 |
| (5) Alanyl or phenylalanyl C-substituent (per substituent) | −0.04 |
| B. Ligand Effects Which Increase the Value of $E^0$ | |
| (1) A 5,5,6-membered ring system | +0.06 |
| (2) Replacement of an equatorially bound carboxylate group by an imidazole group | +0.04 |
| (3) Axially coordinated carboxylate groups in the Cu(II) complex | +0.04 |

[a] Relative to $[Cu^{III,II}(H_{-2}GGG)]^{0,-}$, $E^0 = 0.92$ V.

Table IV. Effective Electrode Potentials for $Cu^{III,II}$ Peptide Couples at pH 7.4

| Cu(III) peptide | $E^0$ | $E_{eff}$, pH 7.4 |
|---|---|---|
| $Cu(H_{-3}G_5)^-$ | 0.66 | 0.71 |
| $Cu(H_{-3}G_3a)$ | 0.64 | 0.72 |
| $Cu(H_{-3}G_4)^-$ | 0.63 | 0.74 |
| $Cu(H_{-2}LLL)$ | 0.77 | ~0.79 |
| $Cu(H_{-2}AAA)$ | 0.81 | 0.81 |
| $Cu(H_{-2}GAG)$ | 0.88 | 0.88 |
| $Cu(H_{-2}G_3)$ | 0.92 | 0.92 |
| $Cu(H_{-2}GGhis)$ | 0.98 | 0.98 |

indicates that the doubly deprotonated copper(II)-tripeptide complexes are thermodynamically capable of conversion to Cu(III) by $O_2$ only when bulky C-substituents are present. Although the triply deprotonated Cu(II) complexes are present in only small amounts at 7.4, their effective potentials predict ready conversion to Cu(III) by $O_2$ even in the absence of C-substituents. Although the bonding of histidine is physiologically important, the coordination of the imidazole group in GGhis does not result in a very accessible Cu(III) complex. On the other hand the effect of coordinated amine groups on the value of $E^0$ can be calculated from Table III and an unsubstituted complex containing two amine groups and two deprotonated-peptide groups coordinated to copper is estimated to have an effective electrode potential of about 0.78 V.

## Conclusions

Since the calculated gain in the CFSE in the change of divalent $d^9$ to trivalent $d^8$ copper is experimentally approximated for a series of copper–peptide complexes, similar behavior should be applicable to other metal–peptide redox systems. If the gain in the CFSE is the predominant contributor to the stability of the higher oxidation state of the nickel complexes, we would predict that the $Ni^{III,II}$–peptide couples would have smaller changes in their electrode potential with changes in the nature of the ligand than is observed for the copper peptides. There is relatively little gain in the CFSE calculated for the change from divalent $d^8$ to trivalent $d^7$ for nickel–peptide complexes. Experimentally determined values of the electrode potential for a series of $Ni^{III,II}$ peptide couples confirms that the $E^0$ values are much less sensitive to changes in the ligands.[64,65]

The electron donor ability of the deprotonated-peptide group is the most important ligand effect involved in the stabilization of these trivalent copper complexes. It seems unlikely that a copper complex without a deprotonated-peptide group would be thermodynamically capable of oxidation by $O_2$ at physiological pH. The relative importance of an effect such as the microenvironment around the copper sites in proteins or enzymes cannot be anticipated from this study. However, the changes in the nature of the ligand (Table III) for these model complexes indicate how the value of $E^0$ could be effected.

Cu(III) is proposed to be part of the active site of galactose oxidase[66,67] and an attractive two-electron Cu(III) to Cu(I) redox reaction is invoked. The nature of the binding site of the single copper atom in this enzyme is uncertain, and other explanations of the nature of the enzyme have been proposed which do not involve the trivalent state of copper.[68,69] Trivalent copper also has been suggested as a possibility in tyrosinase.[67] While our study does not show that Cu(III) is present in enzymes, it does indicate that the trivalent state of copper is a reasonable oxidation state for biological redox reactions when the copper is coordinated to deprotonated-peptide groups.

**Acknowledgment.** This investigation was supported by Public Health Service Grant No. GM19775 from the National Institute of General Medical Sciences.

## References and Notes

(1) R. Scholder and U. Vaelskow, *Z. Anorg. Chem.*, **266**, 256 (1951).
(2) J. J. Bour and J. J. Steggerda, *Chem. Commun.*, 85 (1967).
(3) J. J. Bour, P. J. Birker, and J. J. Steggerda, *Inorg. Chem.*, **10**, 1202 (1971).
(4) D. C. Olson and J. Vasilekskis, *Inorg. Chem.* **10**, 463 (1971).
(5) D. Meyerstein, *Inorg. Chem.*, **10**, 638, 2244 (1971).
(6) A. Levitzki, M. Anbar, and A. Berger, *Biochemistry*, **6**, 3757 (1967).
(7) H. Dobbie and W. O. Kermack, *Biochem. J.*, **59**, 246, 257 (1955).
(8) A. R. Manyak, C. B. Murphy, and A. E. Martell, *Arch. Biochem. Biophys.*, **59**, 373 (1955).
(9) C. B. Murphy and A. E. Martell, *J. Biol. Chem.*, **226**, 37 (1957).
(10) W. L. Koltun, M. Fried, and F. R. N. Gurd, *J. Am. Chem. Soc.*, **82**, 233 (1960).
(11) W. L. Koltun, R. H. Roth, and F. R. N. Gurd, *J. Biol. Chem.*, **238**, 124 (1963).
(12) G. F. Bryce, J. M. H. Pinkerton, L. K. Steinraug, and F. R. N. Gurd, *J. Biol. Chem.*, **240**, 3829 (1965).
(13) S. P. Dalta and B. R. Rabin, *Trans. Faraday Soc.*, **52**, 1123 (1956).
(14) B. R. Rabin, *Trans. Faraday Soc.*, **52**, 1130 (1956).
(15) S. P. Dalta, R. Leberman, and B. R. Rabin, *Trans. Faraday Soc.*, **55**, 2141 (1959).
(16) M. K. Kim and A. E. Martell, *Biochemistry*, **3**, 1169 (1964).
(17) M. K. Kim and A. E. Martell, *J. Am. Chem. Soc.*, **88**, 914 (1966).
(18) M. K. Kim and A. E. Martell, *J. Am. Chem. Soc.*, **91**, 872 (1969).
(19) K. E. Falk, H. C. Freeman, T. Jansson, B. G. Malmström, and T. Vänngård, *J. Am. Chem. Soc.*, **89**, 6071 (1967).
(20) D. C. Gould and H. S. Mason, *Biochemistry*, **6**, 801 (1967).
(21) T. H. Crawford and J. O. Dalton, *Arch. Biochem. Biophys.*, **131**, 123 (1969).
(22) G. F. Bryce and F. R. N. Gurd, *J. Biol. Chem.*, **241**, **1439** (1966).
(23) M. Sheinblatt, In "Proceedings of the Second International Conference on Magnetic Resonance in Biological Systems", A. Ehrenberg, B. G. Malmström, and T. Vänngård, Ed., Pergamon Press, New York, N.Y., 1967, p 41.
(24) H. C. Freeman, J. M. Guss, and R. L. Sinclair, *Chem. Commun.*, 485 (1968).
(25) H. C. Freeman and M. R. Taylor, *Acta Crystallogr.*, **18**, 939 (1965).
(26) H. C. Freeman in "The Biochemistry of Copper", J. Peisach, P. Alsen, and W. E. Blumberg, Ed., Academic Press, New York, N.Y., 1966.
(27) H. C. Freeman, *Adv. Protein Chem.*, **22**, 257 (1967).
(28) M. T. Burnet, H. C. Freeman, D. A. Buckingham, I. N. Hsu, and D. van der Helm, *Chem. Commun.*, 367 (1970).
(29) H. C. Freeman in "Inorganic Biochemistry", G. I. Eichhorn, Ed., Elsevier, New York, N.Y., 1973, pp 121–166.
(30) D. W. Margerum and G. R. Dukes in "Metal Ions in Biological Systems", Vol. I, H. Siegel, Ed., Marcel Dekker, New York, N.Y., 1974, Chapter 5, pp 157–212.
(31) G. L. Burce, E. B. Paniago, and D. W. Margerum, *J. Chem. Soc., Chem. Commun.*, 261 (1975).
(32) G. L. Burce, Ph.D. Thesis, Purdue University, 1975.
(33) D. W. Margerum, K. L. Chellappa, F. P. Bossu, and G. L. Burce, *J. Am. Chem. Soc.*, **97**, 6894 (1975).
(34) T. A. Neubecker, S. T. Kirksey, K. L. Chellappa, and D. W. Margerum, to be submitted for publication.
(35) R. N. Thorneley and A. G. Sykes, *Chem. Commun.*, 331 (1969).
(36) E. R. Brown and R. F. Large in "Techniques in Chemistry," Vol. I, Part IIA, A. Weissberger and B. Rossiter, Ed., Wiley-Interscience, New York, N.Y., 1971, p 440.
(37) G. K. Pagenkopf and D. W. Margerum, *J. Am. Chem. Soc.*, **90**, 501

(1968).
(38) G. K. Pagenkopf and D. W. Margerum, *J. Am. Chem. Soc.*, **90**, 6963 (1968).
(39) H. Hauer, G. R. Dukes, and D. W. Margerum, *J. Am. Chem. Soc.*, **95**, 3515 (1973).
(40) J. C. Cooper, L. F. Wong, D. L. Venezky, and D. W. Margerum, *J. Am. Chem. Soc.*, **96**, 7560 (1974).
(41) C. E. Bannister, D. W. Margerum, J. M. T. Raycheba, and L. F. Wong, *Faraday Symp. Chem. Soc.*, **No. 10**, 78 (1975).
(42) H. L. Schläfer and G. Gliemann, "Basic Principles of Ligand Field Theory," Wiley-Interscience, New York, N.Y., 1969, p 78.
(43) E. J. Billo, *Inorg. Nucl. Chem. Lett.*, **10**, 613 (1974).
(44) R. Skochdopole and S. Chaberek, *J. Inorg. Nucl. Chem.*, **11**, 222 (1959).
(45) J. Shankar and B. C. DeSouza, *Aust. J. Chem.*, **16**, 1119 (1963).
(46) L. H. Sutcliffe and J. R. Weber, *Trans. Faraday Soc.*, **52**, 1225 (1956).
(47) E. T. Gray, Jr., R. W. Taylor, and D. W. Margerum, to be submitted for publication.
(48) D. H. McDaniel and H. C. Brown, *J. Org. Chem.*, **23**, 420 (1958).
(49) S. J. Leach, G. Nemethy, and H. Scheraga, *Biopolymers*, **4**, 369 (1966).
(50) T. Peters, Jr., and F. A. Blumenstock, *J. Biol. Chem.*, **242**, 1574 (1967).
(51) E. Breslow, *J. Biol. Chem.*, **239**, 3252 (1964).
(52) W. T. Shearer, R. A. Bradshaw, F R. N. Gurd, and T. Peters, Jr., *J. Biol. Chem.*, **242**, 5451 (1967).
(53) R. A. Bradshaw, W. T. Shearer, and F. R. N. Gurd, *J. Biol. Chem.*, **243**, 3817 (1968).
(54) D. C. Weatherburn, E. J. Billo, J. P. Jones, and D. W. Margerum, *Inorg. Chem.*, **9**, 1557 (1970).
(55) O. Yamauchi, Y. Nakao, and A. Nakahara, *Bull. Chem. Soc. Jpn.*, **46**, 2119 (1973).
(56) H. C. Freeman, ref 29, Chapter 4, p 145.
(57) F. Akhtar, D. M. L. Goodgame, M. Goodgame, G. W. Rayner-Canham, and A. C. Skapski, *Chem. Commun.*, 1389 (1968).
(58) L. F. Wong and D. W. Margerum, submitted for publication.
(59) F. Basolo and R. G. Pearson, "Mechanisms of Inorganic Reactions", 2nd ed, Wiley, New York, N.Y., 1967, p 70.
(60) J. M. Tsanguras and R. B. Martin, *J. Am. Chem. Soc.*, **92**, 4255 (1970).
(61) G. F. Bryce, *J. Phys. Chem.*, **70**, 3549 (1966).
(62) I. M. Procter, B. J. Hathaway, and P. Nichols, *J. Chem. Soc. A*, 1678 (1968).
(63) C. K. Jørgensen, "Oxidation Numbers and Oxidation States", Springer Verlag, Berlin, 1969, p 57.
(64) F. P. Bossu and D. W. Margerum, *J. Am. Chem. Soc.*, **98**, 4003 (1976).
(65) F. P. Bossu and D. W. Margerum, *Inorg. Chem.*, in press.
(66) G. A. Hamilton, R. D. Libby, and C. R. Hartzell, *Biochem. Biophys. Res. Commun.*, **55**, 333 (1973).
(67) G. R. Dyrkacz, R. D. Libby, and G. A. Hamilton, *J. Am. Chem. Soc.*, **98**, 626 (1976).
(68) R. P. Bereman, R. S. Giordano, D. J. Kosman, M. J. Ettinger, and R. E. Weiner, American Chemical Society Meeting, New York, N.Y., 1976.
(69) L. Cleveland, R. E. Coffman, P. Coon, and L. Davis, *Biochemistry*, 14, 1108 (1975).

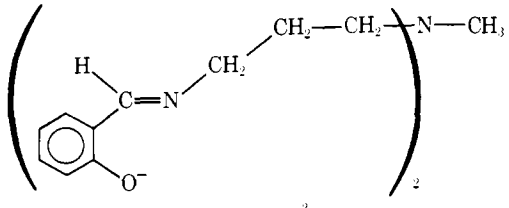
# Ambidentate Nature of the Imidazole Ring System

**Benjamin S. Tovrog and Russell S. Drago***

*Contribution from the William A. Noyes Laboratory, University of Illinois, Urbana, Illinois 61801.* *Received June 3, 1976*

**Abstract:** In this article, we present additional support for our earlier claim that the imidazole ring system can bind through the amine nitrogen. The isotropic shifts and the line widths of *N*-methylimidazole coordinated to Co(SMDPT) are consistently interpreted in terms of coordination at this site.

Our earlier report on the ambidentate nature of imidazole[1] caused great surprise[2] because the amine nitrogen was supposed to be like that in pyrrole, which is claimed not to be basic. In this article, we shall present further evidence for the ambidentate nature of this donor. In view of the limited solubility of the metal complexes used in the earlier work and the potential complications from the ability of imidazole to hydrogen bond, we decided that an even more convincing case could be made for the ambidentate nature of this ring system by examining the NMR spectra of *N*-methylimidazole (*N*-MeIm) coordinated to the complexes M(SMDPT), where M is Ni(II) and Co(II) and $SMDPT^{2-}$ is shown below.



$SMDPT^{2-}$

We report not only the isotropic shifts of the complexes, but the line widths of the resonances as well. In either the very fast or stopped exchange regions, the line width is often related to the distance of the observed nucleus from the metal center. This data (along with full details of the experimental procedure) shows a different mode of bonding for *N*-MeIm to the cobalt(II) and nickel(II) complexes of SMDPT.

## Experimental Section

M(SMDPT), M = Ni(II) and Co(II), were prepared by reported procedures.[3] *N*-Methylimidazole was obtained from Aldrich Chemical Co. and fractionally distilled over $CaH_2$ at 10-mm pressure, with only the middle fraction retained. Samples for NMR were prepared in volumetric flasks in an inert atmosphere dry box with dry deoxygenated $CDCl_3$ as solvent utilizing tetramethylsilane ($Me_4Si$) as an internal standard. NMR tubes were equipped with tight-fitting caps and wrapped with Parafilm to exclude oxygen. The proton NMR spectra were measured either with a JOELCO-C-60H high resolution NMR spectrometer equipped with a JES-VT-2 temperature controller, or a Varian Associates HA-100 NMR spectrometer operating in the field sweep mode equipped with a temperature control unit. In the HA-100 spectra, $Me_4Si$ was utilized as the lock signal. The probe temperatures were measured to ±1.0 °C using either a copper–constantan thermocouple or by measuring the NMR spectrum of methanol. Solution magnetic moments were determined by the Evans method, monitoring the $Me_4Si$ signal. Electron spin resonance spectra were collected on a Varian Model E-9 spectrometer equipped with a Hewlett-Packard frequency counter. Cooling was provided by cold nitrogen gas. The field was calibrated using a Varian weak pitch sample.

## Results and Discussion

**Nature of the Adducts Formed.** Co(SMDPT) (**1**) and Ni(SMDPT) (**2**) are five-coordinate high-spin $d^7$ and $d^8$ complexes, respectively. The magnetic moment of **1** is 4.28 $\mu_B$ in $CH_2Cl_2$ solution and 4.29 $\mu_B$ in the solid. Though the structure of **1** is not known, **2** has been examined and found to possess a distorted trigonal bipyramidal structure.[4,5] The similarity in spectral properties between **1** and **2** suggests similar structures. Infrared spectra are virtually identical, and their paramagnetic NMR spectra are also very similar. In **2**, all the aromatic ring protons are split into two equally intense resonances,[5] due to different orientations of the *N*-$CH_3$ group with respect to each phenyl ring.[6] The observation of a similar