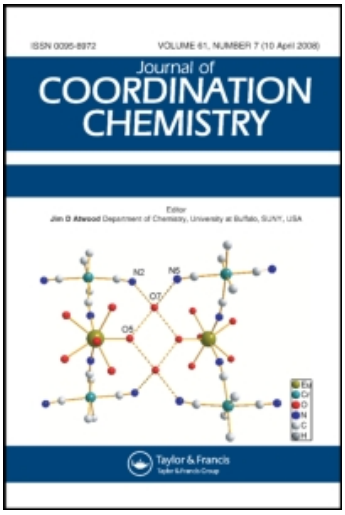


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# CYCLIC VOLTAMMETRIC STUDIES OF THE REDUCTION OF COPPER(II)-PEPTIDE COMPLEXES

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# CYCLIC VOLTAMMETRIC STUDIES OF THE REDUCTION OF COPPER(II)-PEPTIDE COMPLEXES

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Cyclic voltammetry is used to study the electrochemical reduction of copper(II) triglycine complexes and the electrochemical reduction of a ternary copper(II)-diglycine-(2,9-dimethyl-1,10-phenanthroline) complex, $Cu(H_{-1}G_2)dmp$. The reduction of the copper(II) triglycine complexes at a hanging mercury drop electrode occurs via two-electrons to form copper amalgam. Under some conditions the dissociation of the copper(II) triglycine complexes to $Cu^{2+}_{aq}$ is not sufficiently rapid to maintain diffusion control, and two reduction waves appear, one due to reduction of $Cu^{2+}_{aq}$ and the other, appearing at more negative values than the first, due to reduction of a residual copper(II) triglycine complex. The reduction of the ternary complex $Cu^{II}(H_{-1}G_2)dmp$ at a carbon paste electrode provides evidence of a transient copper(I) complex, $Cu^{I}(H_{-1}G_2)dmp$.



## INTRODUCTION

Electrochemical studies of copper(II,I) redox systems are often limited by the fact that the two-electron reduction of copper(II) is more favorable than the one-electron reduction. For example, the electrochemical reduction of $Cu^{2+}_{aq}$ preferentially occurs by two electrons to form copper metal. Complexation stabilizing copper(I) can give rise to a one-electron reduction of copper(II) which occurs more readily than the two-electron reduction. In this case polarography or cyclic voltammetry gives two successive one-electron waves. The occurrence of two one-electron reductions indicates that the copper(I) complex is stable towards disproportionation. In this study cyclic voltammetry is used to investigate the stability of copper(I) peptide complexes.

Peptide complexes of copper(II) occur with coordination by deprotonated peptide–nitrogen atoms which have been characterized in many studies.[1–5] The copper(II) complexes have been used as models for the interaction of the divalent metal with proteins. Peptide coordination stabilizes copper(III) as shown in many recent papers.[5–9] The copper(III) complexes are of interest because of the novelty of the +3 oxidation state and because of their possible role in biological redox processes.

In contrast with copper(II) and copper(III) there is very little evidence of deprotonated-peptide–nitrogen coordination in copper(I) complexes. Österberg[10] studied the interaction of copper(I) with triglycine using constant current electrolysis of a two-phase copper amalgam electrode to introduce the univalent metal ion. This study showed stoichiometric evidence of a binuclear complex of the formula $Cu^{I}Cu^{II}(H_{-3}G_3)_2)^{2-}$ in which three peptide hydrogens are ionized from two triglycine ($G_3^-$) ligands. Österberg concluded from these results that at least one of the peptide hydrogens was displaced by copper(I) coordination to a deprotonated peptide nitrogen. The copper(I) complexes of glycyl-L-histidine and L-histidyl-L-histidine have been studied via potentiometric titration by Kaden and Zuberbühler[11] and these authors concluded that for these peptide ligands there is no coordination of copper(I) by peptide nitrogens. The copper(I) complexes of acetyl-L-histidine and acetylhistamine were studied using proton NMR by Temussi and Vitagliano[12] and there was no evidence of copper(I)–peptide nitrogen coordination. Suguira has conducted proton NMR studies of copper(I) complexes of synthetic peptides containing sulfhydryl and imidazole groups, including *N*-mercaptoacetyl-L-histidine, 3-mercaptopropionyl-L-histidine, *N*-mercaptoacetylglycyl-L-histidine, and *N*-mercaptoacetyl-L-histidyl-L-histidine.[13,14] With one exception the NMR results show no evidence of copper(I)–peptide nitrogen coordination. In the case of *N*-mercaptoacetylhistidyl-L-histidine there was evidence of weak interaction between copper(I) and the two peptide nitrogens, which may have been weak

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coordination. Obviously, the bulk of the data in the literature indicates that coordination of a deprotonated peptide nitrogen to copper(I) does not occur readily. The report by Österberg of the species $Cu^{I}Cu^{II}(H_{-3}(G_3)_2)^{2-}$ is a notable exception to this. In the present study cyclic voltammetry of copper(II) triglycine complexes is used in order to investigate their possible copper(II,I) redox chemistry. However cyclic voltammetry gives no evidence of a copper(II,I) redox couple for the triglycine complexes.

A recent publication by Seff and coworkers[15] reported the formation in solution and the crystal structure of a ternary complex formed between $Cu^{2+}$, diglycine ($G_2$), and 2,9-dimethyl-1,10-phenanthroline (dmp). These workers undertook the investigation of the ternary complex, $Cu(H_{-1}G_2)$dmp in an attempt to form a tetrahedral copper(II) complex involving deprotonated peptide–nitrogen coordination. These methyl groups on dmp will not allow the square planar arrangement of the four nitrogen donors (amino, deprotonated-peptide, and two phenanthroline nitrogens) around copper(II) in the bis ternary complex. The results of the crystallographic study show that $Cu(H_{-1}G_2)$dmp is indeed formed but that it has distorted square pyramidal geometry with coordination not only by the nitrogen donors but by the carboxylate group of diglycine. However, the nitrogen donors are not planar. Hence, as it is reduced, the $Cu^{II}(H_{-1}G_2)$dmp complex should be easily converted to the tetrahedral geometry which is known to stabilize copper(I) complexes,[16] by loss of carboxylate coordination and slight rearrangement of bond angles. This prompted us to investigate the possibility of a $Cu^{II,I}(H_{-1}G_2)dmp^{0,1-}$ redox couple. Our cyclic voltammetry investigations indicate that there is a reversible $Cu^{II,I}(H_{-1}G_2)dmp^{0,1-}$ couple ($E^0$ = 0.29 V *vs.* NHE) but that the $Cu^{I}(H_{-1}G_2)dmp^{-}$ complex is a short-lived species which is rapidly converted to the more stable $Cu^{I}(dmp)_2^{+}$.

## EXPERIMENTAL

### *Chemicals*

Copper(II) complexes were formed using a stock $Cu(ClO_4)_2$ solution prepared from the twice recrystallized salt and standardized by EDTA titration. Ligands used in this study are triglycine, diglycine, 2,9-dimethyl-1,10-phenanthroline and 2,9-dimethyl-4,7-diphenyl-1,10-phenanthrolinedisulfonate disodium salt ($Na_2$dpmp), all of which were obtained from Sigma Chemical Company. The purity of the peptides were checked by elemental analysis and liquid chromatography. The dmp and $Na_2$dpmp were used as received. HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) and sodium metaborate were used as buffers.

### *Cyclic Voltammetry of Copper(II) Trigylcine Complexes*

Solutions of copper(II) triglycine complexes for cyclic voltammetry were typically $1.28 \times 10^{-3}$ M in copper(II) with concentration of triglycine ranging from 0.01 to 0.37 M, pH ranging from 7.0 to 10.0 (buffered with HEPES from 7.0 to 8.0 and by borate and tryglycine from 8.0 to 10.0). All solutions were 0.1 M in $NaClO_4$. Cyclic voltammograms were performed using a Bioanalytical Systems Model CV-1A instrument with a hanging mercury drop electrode (HMDE) working electrode, platinum auxiliary electrode, and saturated calomel (SCE). The HMDE used is a Metrohm E410 model which allows for precise reproduction of drop size by use of a micrometer dial. The precision of measured potential values is ±0.005 volts. For experiments in which the effects of triglycine concentration and pH on the cyclic voltammograms were examined a voltage scan rate of 100 mV/s was used. Scan rates from 50 mV/s to 10 V/s were used to examine the effect of scan rate on the cyclic voltammograms. For scan rates of 100 mV/s or less a Hewlett–Packard 7035B X-Y recorder was used, while at scan rates greater than 100 mV/s a Tektronix 7313 oscilloscope was used. Cyclic voltammograms were recorded at room temperature (23 ± 1°C).

### *Cyclic Voltammetry of $Cu^{II}(H_{-1}G_2)$dmp and Spectral Investigation of $Cu^{II}(H_{-1}G_2)dpmp^{2-}$*

Cyclic voltammetry of $Cu^{II}(H_{-1}G_2)$dmp ([$Cu^{II}$] = $1.28 \times 10^{-3}$ M, $G_2$ and dmp in 10% excess over $Cu^{2+}$) was performed in buffered solution from pH 6.65 to 8.70 (HEPES buffer) in 0.10 M $NaClO_4$. The cyclic voltammetry apparatus and procedure was as described in the previous section except that a carbon paste working electrode was used rather than the HMDE. The mole ratio plot used to study the formation of $Cu(H_{-1}G_2)dpmp^{2-}$ from $Cu(H_{-1}G_2)$ and $dpmp^{2-}$ was obtained by adding aliquots of a 0.0207 M $dpmp^{2-}$ solution to a $3.20 \times 10^{-3}$ M $Cu(H_{-1}G_2)$ solution buffered at pH 8.01 with HEPES.

## RESULTS AND DISCUSSION

The cyclic voltammogram of a solution containing $1.41 \times 10^{-3}$ M copper(II), 0.10 M triglycine, 0.025 M borate buffer, and 0.10 M $NaClO_4$ at pH 9.08 is shown in Figure 1. Under these conditions the predominant copper(II) complexes are $Cu(H_{-1}G_3)_2^{2-}$ (64%) and $Cu(H_{-1}G_3)G_3^-$ (32%), while the minor constituent is $Cu(H_{-2}G_3)^-$ (4%).[17,18] In the following discussion the quasireversible wave at the less negative potentials in Figure 1 is referred to as "the first wave" and the apparent electrode potential of this wave, which is given by the mean of the cathodic and anodic peak potentials, is referred to as $E^{0\prime}$. The irreversible wave is hereafter referred to as "the second wave". The cathodic currents observed for the first and second waves are referred to as $i_1$ and $i_2$, respectively. The value of $E^{0\prime}$ and the ratio of $i_1/i_2$ are sensitive to changes in pH, triglycine concentration, and $v$, the rate of potential scan. The dependences of $E^{0\prime}$ and $i_1/i_2$ on pH, $[G_3^-]$, and $v$ were examined in order to determine the nature of the reduction of copper(II) in the triglycine system. The cyclic voltammetry behavior is summarized below:

1) At constant $v$ and $[G_3^-]$, $E^{0\prime}$ becomes more negative with increasing pH (Table I, Figure 2).

2) At constant $v$ and pH, $E^{0\prime}$ becomes more negative with increasing $[G_3^-]$ (Table I, Figure 3).

3) At constant pH and $[G_3^-]$, $E^{0\prime}$ is independent of $v$.

TABLE I
Dependence of the observed electrode potential for the reduction of copper(II) triglycine on triglycine concentration and pH.[a]

| $[G_3]$, M | pH | $E^{0\prime}$, V |
|---|---|---|
| 0.10 | 6.97 | −0.190 |
| 0.10 | 7.54 | −0.230 |
| 0.10 | 7.62 | −0.222 |
| 0.10 | 8.00 | −0.256 |
| 0.10 | 8.04 | −0.253 |
| 0.10 | 8.55 | −0.294 |
| 0.10 | 8.60 | −0.288 |
| 0.10 | 9.08 | −0.306 |
| 0.10 | 9.30 | −0.335 |
| 0.10 | 9.92 | −0.349 |
| 0.10 | 8.00 | −0.209 |
| 0.040 | 8.04 | −0.237 |
| 0.37 | 7.98 | −0.302 |

[a] pH < 8 buffered with 0.01 M HEPES; pH > 8 buffered with 0.025 M Borate.

4) At constant pH and $[G_3^-]$, $i_2/i_1$ increases with increasing $v$.

5) At constant $v$ and $[G_3^-]$ $i_2/i_1$ increases with increasing pH.

6) At constant $v$ and pH, $i_2/i_1$ decreases with increasing $[G_3^-]$.

7) At constant total copper(II) concentration, the sum of the scan rate-normalized peak currents, $(i_1/v^{1/2} + i_2/v^{1/2})$, is approximately constant and is independent of pH, $[G_3^-]$, and $v$.

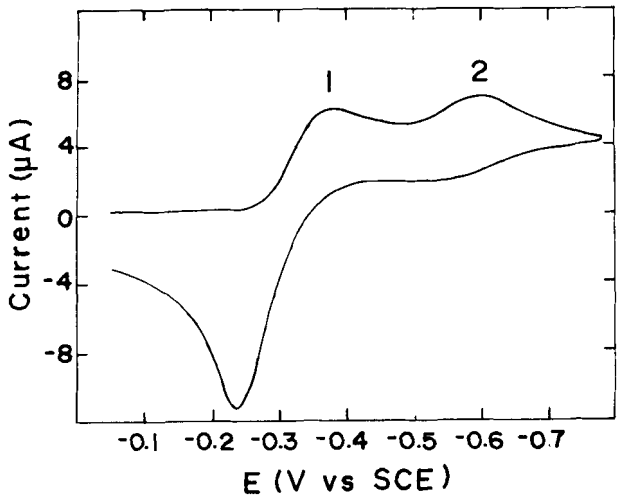


FIGURE 1 Cyclic voltammogram of a solution containing 1.41 millimolar $Cu^{2+}$, 0.10 M triglycine, 0.025 M borate buffer and 0.10 M $NaClO_4$ at pH 9.08. 0.100 V/s scan rate.

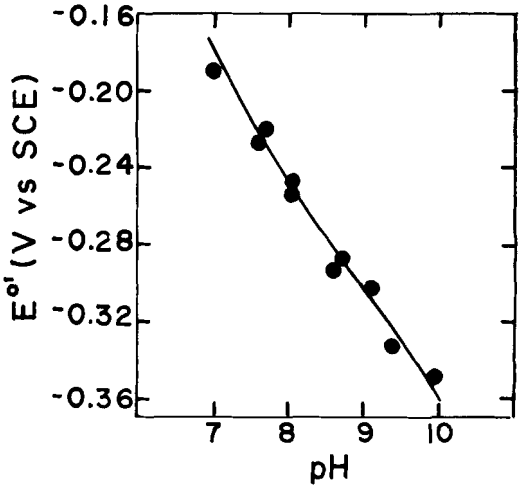


FIGURE 2 Dependence of $E^{0\prime}$ for the reduction of copper(II) triglycine upon pH. The solid line represents values of $E^{0\prime}$ calculated from Eq. (5).

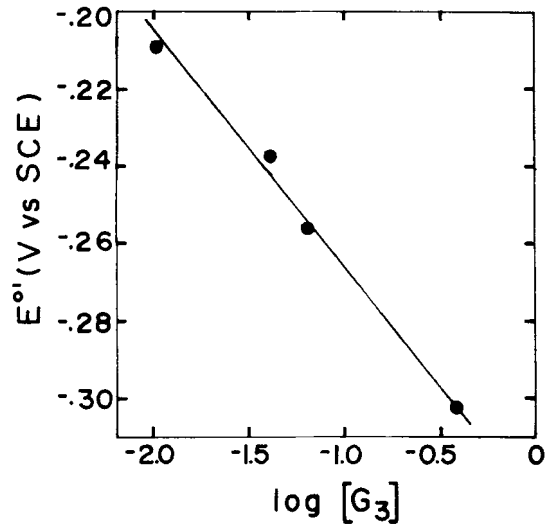


FIGURE 3 Dependence of $E^{0'}$ for the reduction of copper(II) triglycine upon triglycine concentration.

The occurrence of two reduction waves in Figure 1 suggests the possibility that the reduction of copper-(II) occurs via two one-electron steps. However, the cyclic voltammetry behavior is not completely consistent with this explanation. Increases in $i_2$ when conditions are changed occur with decreases in $i_1$. This behavior would not be expected if $i_1$ were due to copper(II.I) reduction and $i_2$ were due to copper-(I.0) reduction. Hence, it is unlikely that the mixed valence binuclear complex $Cu^{I}Cu^{II}(H_{-3}(G_3)_2)^{2-}$, proposed by Österberg is involved in the electrode reduction. The data in Table II show that $E^{0'}$ and $i_2/i_1$ have no significant dependence on the total copper(II) concentration. This indicates that only monomeric species are involved in the electrode reactions. This result clearly rules out involvement of $Cu^{I}Cu^{II}(H_{-3}(G_3)_2)^{2-}$.

TABLE II
Summary of the effect of copper(II) concentration on the cyclic voltammetry behavior for the reduction of copper(II) triglycine.[a]

| $10^3[Cu^{II}]_T$, M | $E^{0'}$, V[b] | $i_2/i_1$ |
|---|---|---|
| 0.640 | −0.204 | ~0.1 |
| 1.90 | −0.205 | ~0.1 |
| 3.14 | −0.202 | ~0.1 |
| 4.05 | −0.196 | ~0.1 |
| 4.96 | −0.201 | ~0.1 |
| 6.15 | −0.196 | ~0.1 |

[a]Scan rate = 100 mV/s. $[G_3^-]_T$ = 0.05 M. pH = 7.60. 0.01 M HEPES. 0.10 M $NaClO_4$.
[b]SCE.

A more plausible explanation for the cyclic voltammetric behavior of the copper(II) triglycine system is that the first wave is a two-electron reduction of $Cu_{aq}^{2+}$ preceded by an equilibrium which is not instantaneously established. The second irreversible wave then is due to direct reduction to copper(0) of a copper(II) complex which is slowly converted to $Cu_{aq}^{2+}$. The species which account for 99% or more of the total copper(II) from pH 7 to pH 10 in 0.1 M triglycine (the conditions used for data in Figure 2) are $Cu(H_{-1}G_3)_2^{2-}$, $Cu(H_{-1}G_3)G_3^-$, and $Cu(H_{-2}G_3)^-$. Hence, the equilibria in Eqs. (1)–(3) must precede reduction of $Cu^{2+}$ (Eq. (4)).

$$Cu(H_{-1}G_3)_2^{2-} + 2H^+ \rightleftharpoons Cu^{2+} + 2G_3^- \quad (1)$$

$$Cu(H_{-1}G_3)G_3^- + H^+ \rightleftharpoons Cu^{2+} + 2G_3^- \quad (2)$$

$$Cu(H_{-2}G_3)^- + 2H^+ \rightleftharpoons Cu^{2+} + G_3^- \quad (3)$$

$$Cu_{aq}^{2+} + 2e^- \overset{HMDE}{\rightleftharpoons} Cu^0(Hg) \quad (4)$$

Based on the above model, the predicted dependence of $E^{0'}$ on hydrogen ion concentration is given by Eq. (5) where $\beta'_1$, $\beta'_2$, and $\beta'_3$ are the

$$E^{0'} = E^0 - \frac{0.0592}{2} \log\left(\frac{[H^+]^2 + \beta'_1[H^+][G_3]^2 + \beta'_2[G_3]^2 + \beta'_3[G_3]}{[H^+]^2}\right) \quad (5)$$

the conditional cumulative formation constants for $Cu(H_{-1}G_3)_2^{2-}$, $Cu(H_{-1}G_3)G_3$, and $Cu(H_{-2}G_3)$ which are defined in Eq. (6)–(9).

$$K_H = \frac{[HG_3^+]}{[H^+][G_3^-]} \quad (6)$$

$$\beta'_1 = \frac{\beta_1}{(1 + K_K[H^+])^2} = \frac{[Cu(H_{-1}G_3)_2^{2-}][H^+]^2}{[Cu^{2+}][G_3^-]^2(1 + K_H[H^+])^2} \quad (7)$$

$$\beta'_2 = \frac{\beta_2}{(1 + K_H[H^+])^2} = \frac{[Cu(H_{-1}G_3)G_3^-][H^+]}{[Cu^{2+}][G_3^-]^2(1 + K_H[H^+])^2} \quad (8)$$

$$\beta'_3 = \frac{\beta_3}{1 + K_H[H^+]} = \frac{[Cu(H_{-2}G_3)^-][H^+]^2}{[Cu^{2+}][G_3^-](1 + K_H[H^+])} \quad (9)$$

The equilibrium constants $K_H$, $\beta_1$, $\beta_2$, and $\beta_3$ ($\mu$ = 0.1, $T$ = 25°C) are $10^{7.87}$, $10^{-4.56}$, $10^{4.14}$, and $10^{-6.80}$, respectively.[12,13] The $E^0$ value for Eq. (4) is 0.277 V *vs.* NHE.[19] The relationship given in Eq. (5) is strictly valid only if the rate of the electrode reaction is

diffusion controlled and the equilibria preceding the electrode reaction are established instantaneously. If the equilibria are not established instantaneously the observed current, $i_1$, is not solely diffusion controlled, but becomes at least partially controlled by the kinetics of the reactions preceding the electrode reaction. Under these conditions $i_1$ is less than the diffusion-limited value and the second wave appears, corresponding to the direct reduction of some undissociated copper(II) complex. The total diffusion current then is given by $i_1 + i_2$. For cyclic voltammograms in which two waves appear, that is, for $i_2 > 0$, the equilibria in Eqs. (1)–(3) are not established rapidly and hence, Eq. 5 does not rigorously apply. However, it has been shown that if the kinetic current is at least one-half the total diffusion current, the potential at which the kinetic wave appears is within ten millivolts (for a two-electron reduction) of the potential where the purely diffusion-limited value would appear.[20,21] For the cyclic voltammograms used to measure $E^{0'}$ as a function of pH, the quantity $i_1/(i_1 + i_2)$ was never less than 0.5. Indeed for all the data below pH 9.0 (and at 0.10 M $G_3^-$), $i_1/(i_1 + i_2)$ was nearly unity. Hence, Eq. (5), for practical purposes, is an adequate relationship to predict the dependence of $E^{0'}$ on hydrogen ion concentration. The solid curve in Figure 2 represents the values of $E^{0'}$ calculated from Eq. (5), which are good agreement with the experimental values of $E^{0'}$. This strongly supports the model given in Eqs. (1)–(4).

The dependence of $E^{0'}$ upon triglycine concentration (Figure 3) is also consistent with the scheme in Eqs. (1)–(4). The cyclic voltammograms for these data give $i_1/(i_1 + i_2)$ values near unity so that Eq. (5) is approximately valid. The slope in Figure 3 of −58 mV per decade indicates the overall loss of one ligand per electron change in the electrode reaction. Over the range of triglycine concentration 0.01 to 0.37 molar (Figure 3) the bis complexes, $Cu(H_{-1}G_3)_2^-$ and $Cu(H_{-1}G_3)G_3^-$, are the predominant forms of copper(II) in solution. Hence the dependence of $E^{0'}$ on triglycine concentration suggests the loss of two peptide ligands occur during a two-electron reduction.

The second wave in Figure 2 is apparently due to the direct reduction of the complex which undergoes dissociation to $Cu^{2+}$ at the slowest rate. Thus, when the scan rate is increased, the magnitude of $i_2/i_1$ increases because as the time scale of the experiment is reduced, less $Cu_{aq}^{2+}$ is formed and more of the undissociated complex remains. The dissociation of copper(II) complexes with deprotonated peptide–nitrogen coordination is catalyzed by acid.[3] Therefore it is reasonable that $i_2/i_1$ should increase with increasing pH due to slower dissociation to $Cu_{aq}^{2+}$ as the concentration of hydrogen ion decreases. The reason for the decreases in $i_2/i_1$ with increasing triglycine concentration is not immediately obvious. Two general explanations can be given but neither are conclusive. Since the overall dissociation to $Cu_{aq}^{2+}$ is likely to involve proton transfer to a depronated peptide group as the rate-determining step, triglycine could act as an acid and thus accelerate the proton transfer via general acid catalysis. Another possible explanation is that the kinetics of dissociation to $Cu^{2+}$ is limited by parallel proton transfer reactions of a deprotonated mono complex and a deprotonated bis complex which are in equilibrium, with the bis complex protonating at a faster rate than the mono complex. Thus as the concentration of triglycine is increased from levels where the mono complex prevails to levels where the bis complex prevails, the overall rate of dissociation to $Cu_{aq}^{2+}$ increases, causing the ratio $i_2/i_1$ to decrease.

The results of the cyclic voltammetry study of the reduction of copper(II) triglycine is consistent with a two-electron reduction in which no copper(I)–peptide is involved. The binuclear species, $Cu^{I}Cu^{II}(H_{-3}(G_3)_2)^{2-}$, reported by Osterberg is definitely not involved in the reduction of copper(II) triglycine complexes at a mercury electrode. However, due to the differences in the experimental approaches used in the present study and in Österberg's study, the sets of results are not necessarily contradictory. The present results indicate that dilute (millimolar) solutions of copper(II) triglycine complexes, including $Cu(H_{-1}G_3)_2^{2-}$, $Cu(H_{-1}G_3)G_3^-$, and $Cu(H_{-2}G_3)^-$ do not readily undergo one-electron reduction to the copper(I) state. Österberg's results show that after electrolyzing a copper amalgam electrode with a constant current and allowing the system to equilibrate for long periods of time (as long as twelve hours), a copper(I) complex is formed and that the results of varying solution parameters suggest a species of the formula $Cu^{I}Cu^{II}H_{-3}(G_3)_2^{2-}$. The results of the present study are significant in that, even though they are not necessarily inconsistent with the existence of the binuclear mixed valence complex, they strongly indicate that it is not readily formed by the reduction of copper(II) triglycine in dilute solution.

### *Cyclic Voltammetry of $Cu^{II}(H_{-1}G_2)$dmp*

Figure 4 shows cyclic voltammograms (carbon paste working electrode) of $Cu^{II}(H_{-1}G_2)$dmp, buffered at pH 7.70 with HEPES buffer, at scan rates of 0.50,

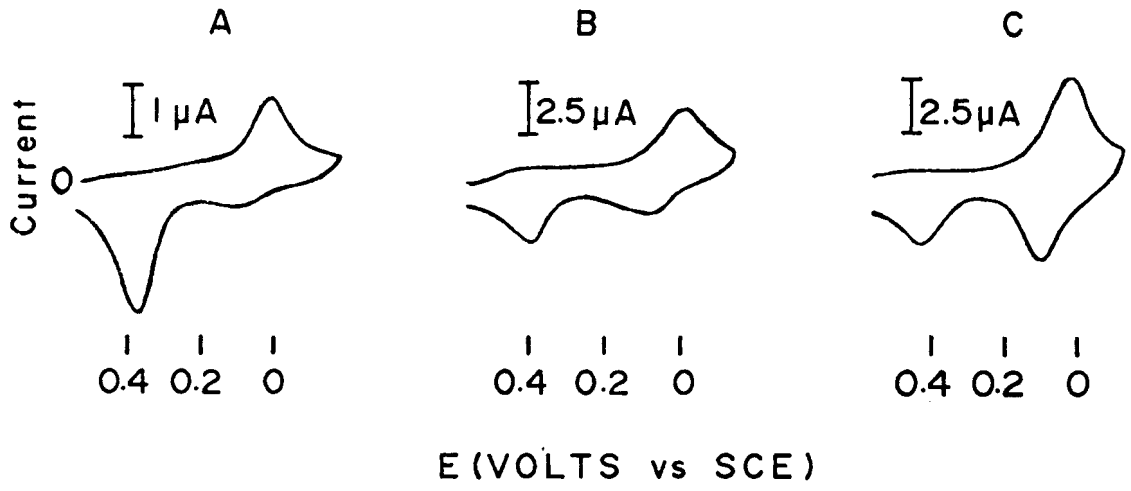


FIGURE 4 Cyclic voltammograms of $1.28 \times 10^{-3}$ M $Cu^{II}(H_{-1}G_2)dmp$ at potential scan rates of A: 0.5; B: 2.5; C: 5.0 V/s. pH = 7.70, 0.05 M HEPES buffer.

2.5, and 5.0 V/s. These cyclic voltammograms are consistent with the mechanism given in Eqs. (10)–(12). The redox couple at 0.05 V in Figure 4 *vs.* SCE

$$Cu^{II}(H_{-1}G_2)dmp + e^- \overset{E_1^0}{\rightleftharpoons} Cu^{I}(H_{-1}G_2)dmp^- \quad (10)$$

$$Cu^{I}(H_{-1}G_2)dmp^- + Cu^{II}(H_{-1}G_2)dmp + H^+ \xrightarrow{k_2} Cu^{I}(dmp)_2^+ + Cu^{II}(H_{-1}G_2) + G_2 \quad (11)$$

$$Cu^{I}(dmp)_2^+ \rightleftharpoons Cu^{II}(dmp)_2^{2+} + e \quad (12)$$

corresponds to Eq. (10), while the anodic wave at 0.4 V *vs.* SCE corresponds to the oxidation of $Cu^{I}(dmp)_2^+$ (Eq. (12)). As the scan rate is increased from 0.5 V/s to 5.0 V/s the normalized anodic current at 0.05 V, $i_{a1}/v^{1/2}$, increases relative to $i_{a2}/v^{1/2}$, the normalized anodic current at 0.40 V. The chemical step (Eq. (11)) interposed between the electrochemical steps accounts for the scan rate dependences of the two anodic waves. At a scan rate of 0.5 V/s the chemical step removes the $Cu^{I}(H_{-1}G_2)$-dmp before it can be reoxidized to $Cu^{II}(H_{-1}G_2)dmp$. Thus, the only anodic current observed at 0.5 V/s scan rate is due to the oxidation of $Cu^{I}(dmp)_2^+$. As the scan rate is increased, the chemical step is not sufficiently rapid to remove $Cu^{I}(H_{-1}G_2)dmp$ before it can be reoxidized to $Cu^{II}(H_{-1}G_2)dmp$, causing $i_{a1}/v^{1/2}$ to increase while $i_{a2}/v^{1/2}$ decreases.

The potential of the $Cu^{II,I}(dmp)_2^{2+,+}$ couple, $E_2^{0\prime}$, is 0.37 V *vs.* SCE. Hence, the assignment of the anodic current peak in the vicinity of 0.40 V *vs.* SCE (Figure 4) as being due to the oxidation of $Cu^{I}$-$(dmp)_2^+$ is reasonable. The pH dependence of $E_1^{0\prime}$ was examined at a scan rate of 10 V/s to show whether or not the deprotonated peptide–nitrogen bound to copper(II) in $Cu^{II}(H_{-1}G_2)dmp$ becomes protonated upon reduction. The data for the pH dependence of $E_1^{0\prime}$ are given in Table III. If protonation of the deprotonated peptide nitrogen is involved in the $E_1^{0\prime}$ electrochemical step, the potential should decrease by 60 mV for every unit decrease in pH. Hence the lack of an appreciable dependence of $E_1^{0\prime}$ on acidity suggests that protonation of $Cu^{II}$-$(H_{-1}G_2)dmp$ does not occur upon reduction and that the assignment in Eq. (10) is correct.

TABLE III
Dependence of the observed electrode potential for the proposed $Cu^{II,I}(H_{-1}G_2)dmp^{0,1-}$ couple upon pH.[a]

| pH | $E_1^{0\prime}$, V[b] |
|---|---|
| 6.65 | 0.056 |
| 7.68 | 0.048 |
| 8.70 | 0.043 |

[a]Scan rate = 10 V/s. 0.02 M HEPES buffer 0.01 M $NaClO_4$.
[b]SCE.

*Stability of the $Cu^{II}(H_{-1}G_2)dpmp^{2-}$ Complex*

In the investigation of $Cu^{II}(H_{-1}G_2)$dmp by Seff and coworkers, no attempt was made to determine the formation constant of the ternary complex from $Cu(H_{-1}G_2)$ and dmp. The mechanism in Eqs. (10)–(12) assumes that the ternary complex is the major copper(II) complex when $G_2$ and dmp are added in 10% excess. We felt that at least a lower limit of the formation constant for addition of dmp to $Cu^{II}(H_{-1}G_2)$ was necessary to show that $Cu^{II}(H_{-1}G_2)$dmp is a reasonably strong complex. Unfortunately dmp is only sparingly soluble in water, making it difficult to vary its concentration in excess over that of $Cu(H_{-1}G_2)$. For this reason we chose to study the solution chemistry of the ternary complex $Cu^{II}(H_{-1}G_2)dpmp^{2-}$ where $dpmp^{2-}$ is 2,9-dimethyl-4,7-diphenyl-1,10-phenanthrolinedisulfonate. The dpmp ligand is extremely soluble in water. The electrode potentials of $Cu^{II,I}(dmp)_2^{2+,+}$ and $Cu^{II,I}(dpmp)_2^{2-,3-}$ are 0.615 and 0.620 V *vs.* NHE, respectively. Hence the phenyl groups in the 4 and 7 position have virtually no effect on the electrode potential. It is not unreasonable then, that dmp and dpmp should form ternary complexes with $Cu(H_{-1}G_2)$ with similar formation constants.

At pH 8.0 the apparent stability constant, $[Cu^{II}(H_{-1}G_2)]/[Cu^{2+}][G_2]$ is $3.2 \times 10^9$ M$^{-1}$.[17] The complex has a maximum in the visible spectrum at 635 nm ($\varepsilon$ = 82 cm$^{-1}$ M$^{-1}$). Addition of $dpmp^{2-}$ to $Cu^{II}(H_{-1}G_2)$, buffered at pH 8.01 ($\mu$ = 0.10 M $NaClO_4$), up to a 1 : 1 ratio results in a linear increase in absorbance at 635 nm with no wavelength shift in the absorbance maximum. Addition of $dpmp^{2-}$ in excess of a 1 : 1 $[dpmp^{2-}]_T/[Cu^{II}(H_{-1}G_2)]_T$ ratio results in no further change in absorbance. Figure 5 shows the absorbance at 635 nm (corrected for dilution due to addition of $dpmp^{2-}$ solution) as a function of $[dpmp^{2-}]_T/[Cu^{II}(H_{-1}G_2)]_T$. There is virtually no curvature in the plot at the endpoint, indicating quite a strong complex with a formation constant of at least $10^5$ M$^{-1}$. This strongly suggests that $Cu(H_{-1}G_2)$dmp is also a rather strong ternary complex and that it is the major copper(II) species in solution when $G_2$ and dmp are added to copper(II) in 10% excess.

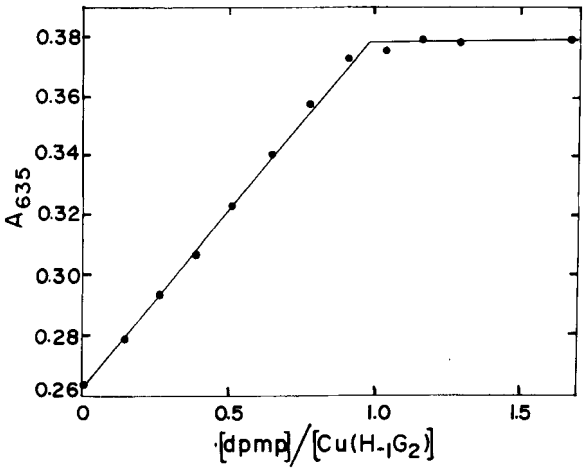


FIGURE 5 Mole-ratio plot for the addition of $dpmp^{2-}$ to $Cu(H_{-1}G_2)$. pH = 8.01, 0.025 M HEPES, $[Cu]_T = 3.2 \times 10^{-3}$ M.

The proposed mechanism (Eqs. (10)–(12)) for the electrochemical reduction of $Cu^{II}(H_{-1}G_2)$dmp could be very important for at least two reasons:

1) It is a very rare example of bonding of a deprotonated peptide group to copper(I).

2) It suggests that a nonplanar copper(II) complex which contains coordination by at least two "soft" nitrogen donor atoms in addition to a deprotonated peptide group, can be reversibly reduced to copper(I) without loss of deprotonated peptide coordination.

Thus, the results of this study have possible biological implications in view of the common occurrence of peptide and "soft" imidazole donors in proteins. The electrode potential for the proposed $Cu^{II,I}(H_{-1}G_2)$-$dmp^{0,1-}$ couple is 0.29 V *vs.* NHE. This potential is within the range found for the copper(II,I) couples in blue copper proteins[22] and is remarkably high in light of the fact that even at much more negative potentials, there is no evidence of a reversible copper(II,I) couple for the triglycine system.

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