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Reaction Entropies of Copper(III,II) Peptide and Nickel(III,II) Peptide Redox Couples and the Role of Axial Solvent Coordination

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The entropy differences, $S°_{II} - S°_{III}$, for several copper(III,II) peptide and nickel(III,II) peptide couples have been determined from cyclic voltammetric measurements as a function of temperature in a nonisothermal electrochemical cell. The entropy differences are of similar magnitude but are of opposite sign, indicating that changes in axial coordination by water occur upon reduction of copper(III) and nickel(III) peptide complexes according to the half cell reactions $Cu^{III}(peptide) + e^- + 2H_2O \rightleftarrows Cu^{II}(peptide)(H_2O)_2$ and $Ni^{III}(peptide)(H_2O)_2 + e^- \rightleftarrows Ni^{II}(peptide) + 2H_2O$. Similarly, decreases in the water content of mixed solvents lower the values of $E°$ for copper(III,II) couples and increase them for nickel(III,II) couples by changing the extent of axial coordination in the copper(II) and nickel(III) complexes. The results indicate the absence of axial solvent coordination of the nickel(II) and copper(III) peptide complexes in accord with a square-planar coordination for these d^8 electronic configurations. In dry acetonitrile, the reduction potential of the copper(III) complex of tri-α-aminoisobutyric acid is decreased to 0.12 V which is 0.54 V lower than in aqueous solution. Hence, in a hydrophobic protein environment, the copper(III) state might well be stabilized significantly.

Introduction

Entropy differences and other thermodynamic parameters for redox couples can be determined by measuring the temperature coefficients of electrode potentials for the couples of interest. The dependence of electrode potentials upon temperature can be studied in one of two cell configurations, differing essentially in the temperature of the reference electrode. If the reference electrode and the solution containing the redox couple of interest are always at the same temperature, the cell is said to be in an isothermal configuration. The cell is in a nonisothermal configuration if the reference electrode is held at a constant temperature while the temperature of the solution containing the redox couple of interest is varied.[1,2]

For isothermal cells, the temperature coefficients of electrode potentials give a direct measure of the $\Delta S°_{cell}$, the entropy change for the overall cell reaction (eq 1).[1] For nonisothermal

$$\left(\frac{dE°}{dT}\right)_{iso} = \frac{\Delta S°_{cell}}{nF} \quad (1)$$

cells, on the other hand, the temperature coefficient of the electrode potential yields the entropy change for the half cell reaction involving the redox couple of interest (eq 2, 3).[1,2] The

$$Ox + e^- \rightleftarrows Red \quad (2)$$

$$\left(\frac{dE°}{dT}\right)_{non} = \frac{S°_{Red} - S°_{Ox}}{nF} \quad (3)$$

isothermal configuration is somewhat impractical experimentally because of the slowness with which most reference electrodes come to equilibrium after the temperature is changed. In the nonisothermal configuration observed values of $(dE°/dT)_{non}$ contain contributions arising from temperature gradients across the liquid junction and along the working electrode. These contributions can be made to be sufficiently small that no serious error results in neglecting them in cases where $(dE°/dT)_{non}$ is at least 0.20 mV/deg.[2] Thus, the nonisothermal cell configuration provides a method of directly measuring entropy changes for half-cell reactions, $S°_{Red} - S°_{Ox}$.

Weaver and coworkers have conducted a study of the half-reaction entropies of a number of transition-metal redox couples using cyclic voltammetry in a nonisothermal electrochemical cell.[2] They found that for aquo and simple monodentate metal ion complexes the values of $S°_{Red} - S°_{Ox}$ were affected primarily by electrostatic factors and the hydrogen-bonding ability of the solvent with bound ligands, but were relatively insensitive to the nature of the metal ion.[2] However, values of $S°_{Red} - S°_{Ox}$ for redox couples containing chelating ligands were found to depend on electronic structures of the oxidized and reduced metal ions as well as their charge and the nature of the bound ligand.[2] Copper(III,II) and nickel(III,II) peptide complexes are interesting in that they provide the first examples of $S°_{Red} - S°_{Ox}$ for d^8, d^9 and d^7, d^8 transition-metal redox couples.

Copper(II) and nickel(II) promote ionization of peptide hydrogens upon complexation by oligopeptides. This has been shown by extensive potentiometric, spectrophotometric, kinetic, and crystallographic studies conducted by several groups.[3] Deprotonated peptide nitrogen coordination makes the trivalent oxidation states of copper and nickel easily accessible.[4,5] The electrode potentials of a large number of copper(III,II) peptide and nickel(III,II) peptide couples have been determined in this laboratory, and the values were found to be ligand dependent.[4,5] For both metals, an increase in the number of deprotonated peptide nitrogens coordinated to the metals causes the $E°$ values to decrease. The presence of alkyl side chains in the peptide also affects the values of $E°$, significantly lowering $E°$ in the case of copper(III,II) peptides and generally increasing $E°$ in the case of nickel(III,II) peptides.

The effect of alkyl groups on $E°$ values has been explained as a steric interference of axial solvation which is expected to be substantial for the d^9 copper(II) and d^7 nickel(III) complexes but not for the d^8 copper(III) and nickel(II) complexes.[5] The results of the present study support this explanation. The measured entropy differences, $S°_{II} - S°_{III}$, for the copper(III,II) peptide and nickel(III,II) peptide redox couples indicate that the reduction of copper(III) peptides is accompanied by capture of bulk water molecules ($S°_{II} - S°_{III} < 0$) while the reduction of nickel(III) peptides is accompanied by a release of coordinated water molecules ($S°_{II} - S°_{III} > 0$). The comparative behavior of the copper and nickel complexes is important because the complexes are of the same size and charge, and therefore factors other than solvent coordination which affect the entropies of the half-reactions should be similar.

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Table I. Nonisothermal Temperature Coefficients of Electrode Potentials and Entropy Differences for $Cu^{III,II}$(peptide) and $Ni^{III,II}$(peptide) Redox Couples at 0.10 and 1.0 M Ionic Strength

	μ = 0.10 M ($NaClO_4$)		μ = 1.0 M (KCl)	
$M^{III,II}L^a$	$dE°/dT$, mV/deg	$S°_{II} - S°_{III}$, cal/(deg mol)	$dE°/dT$, mV/deg	$S°_{II} - S°_{III}$, cal/(deg mol)
$Cu^{III,II}(H_{-3}G_4)^{1-,2-}$	−0.86 ± 0.02	−19.8 ± 0.5	−0.67 ± 0.01	−15.4 ± 0.2
$Cu^{III,II}(H_{-3}V_4)^{1-,2-}$	−0.69 ± 0.05	−16 ± 1	−0.65 ± 0.05	−15 ± 1
$Cu^{III,II}(H_{-3}G_3a)^{0,1-}$	−0.57 ± 0.03	−13.1 ± 0.7	−0.59 ± 0.03	−13.6 ± 0.7
$Cu^{III,II}(H_{-3}G_4a)^{0,1-}$	−0.71 ± 0.03	−16.4 ± 0.7	−0.62 ± 0.07	−14 ± 2
$Cu^{III,II}(H_{-2}Aib_3)^{0,1-}$	−0.73 ± 0.07	−17 ± 2	−0.57 ± 0.04	−13.1 ± 0.9
$Cu^{III,II}(H_{-2}A_3)^{0,1-}$			−0.58 ± 0.06	−13 ± 1
$Cu^{III,II}(H_{-2}DGEN)^{1+,0}$	−0.28 ± 0.04	−6.4 ± 0.9	−0.36 ± 0.04	−8.3 ± 0.9
$Ni^{III,II}(H_{-3}G_4)^{1-,2-}$	0.46 ± 0.06	11 ± 1	0.65 ± 0.07	15 ± 2
$Ni^{III,II}(H_{-3}G_3a)^{0,1-}$	0.39 ± 0.06	9 ± 1	0.48 ± 0.12	11 ± 3

[a] The notation M(H_{-x}-peptide) indicates the metal (M), the number of deprotonated peptide nitrogens (x) and the peptide ligand (G = glycyl, A = L-alanyl, V = L-valyl, Aib = α-aminoisobutyryl, a = amide, DGEN = diglycylethylenediamine).

In addition to the entropy measurements, we have studied the effect of varying the concentration of water by diluting the solutions of the metal peptides with other solvents. These results also indicate that axial water coordination is an important factor in determining $E°$ values for copper(III,II) peptide and nickel(III,II) peptide complexes.

Experimental Section

Tetraglycine, tetra-L-valine, and tri-L-alanine were obtained from Biosynthetika (Oberdorf, Switzerland). Tetraglycinamide hydrobromide and triglycinamide hydrochloride were obtained from Vega Fox Chemical Co. The tripeptide of α-aminoisobutyric acid and *N,N'*-diglycyl-1,2-diaminoethane (diglycylethylenediamine) were prepared in this laboratory by procedures reported in the literature.[6,7] The purity of all peptides was checked by elemental analysis and liquid chromatography.

Solutions of copper(II) and nickel(II) peptide complexes (1×10^{-3} M) were prepared by the reaction of $Cu(ClO_4)_2$ and $Ni(ClO_4)_2$ with 5–10% excess ligand. The pH of the solutions of copper(II) and nickel(II) peptide complexes were buffered at pH 9.5 and 8.5, respectively, with 0.016 M borate buffer. The ionic strength of aqueous solutions was controlled at 0.10 M with $NaClO_4$ and at 1.0 M with KCl. $NaClO_4$ was replaced by KCl at the higher ionic strength in order to avoid precipitation of $KClO_4$ in the saturated KCl salt bridge.

Cyclic voltammetry was performed by using freshly prepared solutions of copper(II) and nickel(II) peptide complexes with a three-electrode system consisting of a carbon-paste working electrode, a platinum-wire auxiliary electrode, and a saturated calomel reference electrode. The reference electrode was thermostated at 25.0 °C and was connected to the solution containing the working and auxiliary electrodes by a saturated KCl agar bridge. For a concentrated KCl salt bridge, the temperature coefficient of the thermal liquid junction potential, dE_{tj}/dT, is less than 0.020 mV/deg.[1,8-11] This is less than the magnitude of the experimental uncertainty in the values of $(dE°/dT)_{non}$ determined in this study. The working-electrode compartment was thermostated at variable temperatures, generally from 10 to 50 °C for the copper complexes and from 15 to 40 °C for the nickel complexes. The cyclic voltammograms for the nickel complexes were of poor quality above 40 °C because of the instability of the Ni(III) complexes at higher temperature. Difficulty also was encountered below 15 °C, perhaps because of a slow heterogeneous electron-transfer process.

For solutions which contained nonaqueous solvents, the entire electrochemical cell was thermostated at 25.0 °C, and a glassy carbon working electrode was employed. Ionic strengths of mixed-solvent solutions were maintained at 0.10 M with $NaClO_4$. The $Cu^{III}(H_{-2}Aib_3)$ was isolated as a pure solid,[6] and cyclic voltammograms were performed in acetonitrile (dried over molecular sieves for several days) using 0.10 M tetraethylammonium perchlorate (TEAP) as the supporting electrolyte. For these voltammograms, the ferrocene/ferrocenium couple (0.40 V vs. NHE) was used as the potential reference[12] and a coiled platinum wire served as the quasi-reference electrode. All cyclic voltammograms were generated by using a Bioanalytical Systems CV-1A instrument and were recorded on a Hewlett-Packard HP7035B X-Y recorder.

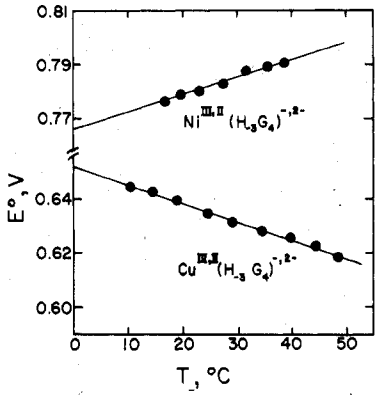

Figure 1. Dependence of $E°$ for $Cu^{III,II}(H_{-3}G_4)^{1-,2-}$ and $Ni^{III,II}(H_{-3}G_4)^{1-,2-}$ upon temperature at 1.0 M ionic strength.

Results and Discussion

The dependences of $E°$ on temperature for seven copper(III,II) peptide and two nickel(III,II) peptide couples were determined by cyclic voltammetry at ionic strengths of 0.1 and 1.0 M in a nonisothermal electrochemical cell configuration. The copper(III,II) and nickel(III,II) couples are quasi-reversible with separations of 65–100 mV between anodic and cathodic peaks in the cyclic voltammograms. The values of $E°$ were evaluated with a precision of ±5 mV at each temperature. The values of $(dE°/dT)_{non}$ for all of the redox couples were determined by a least-squares analysis of data such as shown in Figure 1 for the triply deprotonated tetraglycine complexes $Cu^{III,II}(H_{-3}G_4)^{1-,2-}$ and $Ni^{III,II}(H_{-3}G_4)^{1-,2-}$. The values of $S°_{II} - S°_{III}$ in Table I were calculated according to eq 3.

Sign of $S°_{II} - S°_{III}$. The most striking feature of the data in Table I and Figure 1 is the difference in sign of $S°_{II} - S°_{III}$ for the copper and nickel complexes. The negative values for the copper complexes and the positive values for the nickel complexes suggest changes in axial coordination in accord with eq 4 and 5, where L is the peptide ligand and x is the number

$$Cu^{III}(H_{-x}L) + 2H_2O + e^- \rightleftarrows Cu^{II}(H_{-x}L)(H_2O)_2 \quad (4)$$

$$Ni^{III}(H_{-x}L)(H_2O)_2 + e^- \rightleftarrows Ni^{II}(H_{-x}L) + 2H_2O \quad (5)$$

of deprotonated peptide nitrogens in the complex. Copper(III) peptide complexes[13] and nickel(II) peptide complexes with at

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least two deprotonated peptide nitrogens[3] exhibit behavior in solution which indicates that they are square planar. Hence it is reasonable that these complexes, with d^8 electronic configurations, would have little or no axial coordination. On the other hand, the copper(II) and nickel(III) complexes with d^9 and d^7 electronic configurations, respectively, are likely to have tetragonally distorted octahedral geometry in solution, and thus have a significant degree of axial coordination. Kinetic evidence has shown that axial coordination by carboxylate groups in the peptide side chains occurs in copper(II) peptide complexes.[14] Electron spin resonance studies of nickel(III) peptide complexes[15] show a tetragonal geometry with waters coordinated in the axial positions. Furthermore, nitrogen donors form quite strong axial adducts with nickel(III).

Half-reaction entropies for transition-metal redox couples are influenced by factors other than changes in solvent coordination, including changes in charge and ionic radius and shielding of the metal ion from the bulk solvent by the coordinated ligand.[2] These three factors are essentially the same for the copper(III,II) and nickel(III,II) couples. Hence, the *difference* in $S°_{II} - S°_{III}$ for copper and nickel should mainly reflect the difference in axial coordination according to eq 4 and 5. At 1.0 M ionic strength, where charge effects are at least partially minimized, the $S°_{II} - S°_{III}$ values for the copper and nickel complexes are, to a fair approximation, equal and opposite. This strongly suggests that the predominant contribution to the half-reaction entropies is the change in axial solvent coordination.

Magnitudes of $S°_{II} - S°_{III}$. The values of $S°_{II} - S°_{III}$ for all of the complexes in Table I are generally less negative at the higher ionic strength with the exception of that for $Cu^{III,II}(H_{-2}DGEN)^{1+,0}$ which is markedly more negative. This ionic strength dependence is consistent with the minimization of charge effects with increasing ionic strength. Highly charged species exert a stronger solvent-structuring influence than do species of lower charge. Except for $Cu^{III}(H_{-2}DGEN)^{+}$ the reduction of each of the copper(III) complexes in Table I proceeds with an increase in charge and therefore should contain a negative contribution to $S°_{II} - S°_{III}$. An increase in ionic strength is expected to decrease the influence of the charge on the complexes and therefore should make the values of $S°_{II} - S°_{III}$ less negative as is observed in Table I. The $S°_{II} - S°_{III}$ value for $Cu^{III,II}(H_{-2}DGEN)^{1+,0}$ becomes more negative with ionic strength as expected since in this case reduction proceeds with a decrease in charge.

The entropy change for the liberation of a single coordinated water molecule can be estimated by subtracting the average entropy contribution of a single molecule of water of hydration in a solid, 9.4 cal/(deg mol),[16] from the entropy of liquid water, 16.71 cal/(deg mol),[17] yielding 7.3 cal/(deg mol). It is interesting that the values of $S°_{II} - S°_{III}$, particularly at 1.0 M ionic strength where charge effects are minimal (Table I), are in good agreement with this estimate if the changes in water coordination for the copper(III,II) and nickel(III,II) couples are given by eq 4 and 5.

Enthalpy Changes for the $M^{III,II}$ Peptide Redox Couples. While the magnitudes and signs of the $S°_{II} - S°_{III}$ values in Table I can be used to infer the absence or presence of significant axial solvent coordination in each of the oxidation states of the copper(III,II) and nickel(III,II) couples, these values are rather invariant and therefore do not directly affect the values of $E°$ which vary with the peptide ligand. For example, the $E°$ value for $Cu^{III,II}(H_{-3}V_4)^{1-,2-}$ is 120 mV lower than that for $Cu^{III,II}(H_{-3}G_4)^{1-,2-}$ even though the $S°_{II} - S°_{III}$ values for these two couples are virtually identical. Hence alkyl side chains in the peptide alter the $E°$ values ($\Delta G°$) through an enthalpic effect rather than through an entropic effect.

Table II. Thermodynamic Parameters for $Cu^{III,II}$(peptide) Couples vs. the Standard Hydrogen Couple at 1.0 M Ionic Strength (KCl)[a]

$M^{III,II}$(peptide)[b]	$\Delta G°$, kcal/mol	$\Delta H°$, kcal/mol	$\Delta S°$, cal/(deg mol)
$Cu^{III,II}(H_{-3}V_4)^{1-,2-}$	−11.9 ± 0.1	−22.6 ± 0.3	−36.1 ± 1.0
$Cu^{III,II}(H_{-3}G_4)^{1-,2-}$	−14.6 ± 0.1	−25.5 ± 0.1	−36.5 ± 0.2
$Cu^{III,II}(H_{-3}G_3a)^{0,1-}$	−14.6 ± 0.1	−24.9 ± 0.2	−34.7 ± 0.7
$Cu^{III,II}(H_{-2}Aib_3)^{0,1-}$	−14.9 ± 0.1	−25.1 ± 0.3	−34.2 ± 0.9
$Cu^{III,II}(H_{-3}G_4a)^{0,1-}$	−15.4 ± 0.1	−25.9 ± 0.6	−35.1 ± 2.0
$Cu^{III,II}(H_{-2}DGEN)^{1+,0}$	−18.5 ± 0.1	−27.3 ± 0.2	−29.4 ± 0.9
$Cu^{III,II}(H_{-2}A_3)^{0,1-}$	−18.7 ± 0.1	−28.9 ± 0.3	−34.1 ± 1.0

[a] Full cell reaction is $Cu^{III} + {}^1/_2H_2 \rightleftarrows Cu^{II} + H^+$. [b] For notation $Cu(H_{-x}$–peptide) see footnote to Table I.

Enthalpies and free energies, unlike entropies, are not absolute quantities and must always refer to a complete chemical reaction. The values of $\Delta G°$, $\Delta H°$, and $\Delta S°$ listed in Table II are for the redox reaction in eq 6 at 1.0 M ionic strength.

$$Cu^{III}(peptide) + {}^1/_2H_2 \rightleftarrows Cu^{II}(peptide) + H^+ \quad (6)$$

The overall entropy change for eq 6 is calculated by adding the entropy change $S°_{H^+} - {}^1/_2S°_{H_2}$ (−21.1 cal/(deg mol))[18] to the values of $S°_{II} - S°_{III}$ in Table I, and the corresponding enthalpy and free energy changes are then calculated from $\Delta G° = \Delta H° - T\Delta S° = -nFE°$. Table II clearly shows that the effect of ligand on the values of $\Delta G°$ for eq 6 is due to changes in enthalpy.

Comparison of $\Delta H°$ values for $Cu^{III}(H_{-2}A_3)$ vs. $Cu^{III}(H_{-2}Aib_3)$ and $Cu^{III}(H_{-3}G_4)^-$ vs. $Cu^{III}(H_{-3}V_4)^-$ permits an assignment of approximately 1 kcal increase in $\Delta H°$ for each alkyl substituent added to amino acid residues involved in chelate ring formation. Probably the major factor causing this effect is the weakening of the axial water coordination in the copper(II) complex, resulting in a decreased stability of copper(II) vs. copper(III). If this is the case, it is easy to see why alkyl side chains in the coordinated peptide, particularly bulky ones which hinder axial coordination, will cause the copper(III,II) $E°$ values to shift in favor of the copper(III) complexes. This behavior could be extremely important in proteins where blocking of axial water coordination to a copper ion coordinated to a peptide backbone should strongly favor the trivalent over the divalent oxidation state.

Variation of Solvent Composition. In order to simulate the lower activity of water which is likely to be encountered in a protein environment and to test the validity of eq 4 and 5, we chose to measure the $E°$ values of the copper(III,II) and nickel(III,II) couples as a function of solvent composition. From eq 4 and 5 we predicted that increasing the percentage of a nonaqueous solvent with poor solvating ability in a mixture with water should drive $E°$ upward for the nickel(III,II) couple and downward for the copper(III,II) couple. The change in liquid junction potential which accompanies the changing solvent composition precludes the evaluation of absolute electrode potential values. However, the difference in $E°$ values for a copper(III,II) and a nickel(III,II) couple with the same ligand as a function of solvent composition can be determined reliably and will not be affected by the changing liquid junction potential. Table III gives the apparent $E°$

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Table III. Dependence of E° for $Cu^{III,II}(H_{-3}G_3a)^{0,1-}$ and $Ni^{III,II}(H_{-3}G_3a)^{0,1-}$ upon Solvent Composition at 25 °C and 0.10 M Ionic Strength ($NaClO_4$)[a]

solvent	E°_{Cu}, V	E°_{Ni}, V	ΔE°, V	λ_{max}-(Cu^{II}), nm	λ_{max}-(Ni^{II}), nm
water	0.635	0.830	0.195	517	408
50% acetonitrile	0.582	0.830	0.248	508	
50% acetone	0.578	0.842	0.264	508	
50% methanol	0.621	0.830	0.209		
25% 2-propanol	0.628	0.853	0.225	512	408
50% 2-propanol	0.608	0.850	0.242	506	
67% 2-propanol	0.594	0.854	0.260		
80% 2-propanol	0.580	0.856	0.276	503	410

[a] The values of E° ignore changes in liquid junction potential which are likely to arise when the solvent composition is changed—see text.

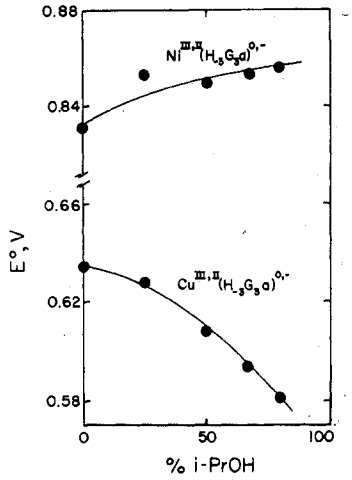

Figure 2. Dependence of E° for $Cu^{III,II}(H_{-3}G_3a)^{0,1-}$ and $Ni^{III,II}(H_{-3}G_3a)^{0,1-}$ upon solvent composition (percent by volume of *i*-PrOH) at 25 °C and 0.10 M ionic strength.

values (ignoring problems with liquid junction potential) for the $Cu^{III,II}(H_{-3}G_3a)^{0,1-}$ couple and the $Ni^{III,II}(H_{-3}G_3a)^{0,1-}$ couple in acetonitrile, acetone, methanol, and 2-propanol. The difference $\Delta E^\circ = E^\circ_{Ni} - E^\circ_{Cu}$ for the mixed-solvent systems is always greater than the corresponding difference in water and increases with increasing concentration of 2-propanol. The increasing value of ΔE° in Table III is a very good indication that the electrode potential of $Ni^{III,II}(H_{-3}G_3a)^{0,1-}$ increases with decreasing water concentration while that of $Cu^{III,II}(H_{-3}G_3a)^{0,1-}$ decreases. The qualitative behavior in Figure 2 also suggests that this is true although the magnitudes of the E° values plotted are certain to be influenced by the changing liquid junction potential.

Included in Table III are the wavelengths of the absorption maxima for the copper(II) and nickel(II) complexes of triglycinamide. The value of λ_{max} for $Cu^{II}(H_{-3}G_3a)^-$ decreases with increasing concentration of 2-propanol. An explanation of these observations consistent with the cyclic voltammetry results is that there is greater average tetragonal distortion of the complex as the concentration of water decreases. It is tetragonal distortion, or the inhibition of axial coordination, which favors copper(III) and thereby lowers E°. There is no significant trend in λ_{max} for the square-planar $Ni^{II}(H_{-3}G_3a)^-$ presumably because no further tetragonal distortion is possible.

The decreasing value of E° for $Cu^{III,II}(H_{-3}G_3a)^{0,1-}$ with increasing 2-propanol concentration suggests that in the total absence of water the electrode potentials of copper(III,II) couples might be drastically lowered. For a test of this prediction the oxidizing ability of $Cu^{III}(H_{-2}Aib_3)$ was examined in dry acetonitrile. In this solvent, $Cu^{III}(H_{-2}Aib_3)$ did not

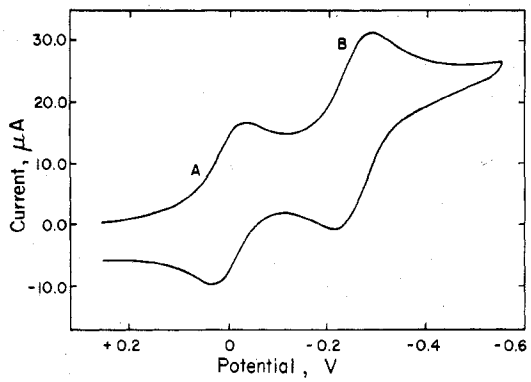

Figure 3. Cyclic voltammogram of 6×10^{-4} M ferrocene (A) and 1.1×10^{-3} M $Cu^{III}(H_{-2}Aib_3)$ (B) in 0.10 M TEAP–acetonitrile solution. Potential is vs. the ferrocene/ferrocenium couple (0.40 V vs. NHE).

oxidize ferrocene. However, upon addition of water the oxidation did take place. These results indicate that the E° value of $Cu^{III,II}(H_{-2}Aib_3)^{0,1-}$ in dry acetonitrile is lower than the ferrocene/ferrocenium value (0.40 V vs. NHE), which is considered to be independent of solvent.[12] The addition of water shifts the copper(III,II) E° value to greater than 0.40 V and thus causes the oxidation of ferrocene to occur. Figure 3 shows a cyclic voltammogram of a solution containing ferrocene and $Cu^{III}(H_{-2}Aib_3)$ in acetonitrile (0.10 M TEAP, 25 °C). Identification of the couples was accomplished by first performing a cyclic voltammogram with a solution of $Cu^{III}(H_{-2}Aib_3)$ in the absence of ferrocene. The displacement of the $Cu^{III,II}(H_{-2}Aib_3)^{0,1-}$ couple from the ferrocene/ferrocenium couple is −0.28 V. This corresponds to 0.12 V vs. NHE for the $Cu^{III,II}(H_{-2}Aib_3)^{0,1-}$ E° value. Hence, changing the solvent from water to acetonitrile causes the E° value of $Cu^{III,II}(H_{-2}Aib_3)^{0,1-}$ to change from 0.66 to 0.12 V vs. NHE, a shift of 0.54 V. Addition of water to the acetonitrile solution caused the wave separation between the copper(III,II) and the ferrocene/ferrocenium couples to decrease, indicating an increase in the $Cu^{III,II}(H_{-2}Aib_3)^{0,1-}$ E° value. These results underscore the importance of axial water coordination in determining the thermodynamic stability of copper(III) peptide complexes.

The extraordinarily large decrease in E° value for the $Cu^{III,II}(H_{-2}Aib_3)^{0,1-}$ couple upon changing the solvent from water to acetonitrile could have extremely important biological implications. If a copper ion were coordinated to the peptide backbone of a protein and were located in the hydrophobic interior where the activity of water is low, the copper(III,II) redox potential could be much lower than in any of the simple peptide complexes which we have studied. It is then quite possible that the biological redox activity of copper proteins could involve the trivalent oxidation state.

The large E° solvent shift also suggests that variations of the environment of a copper ion in a protein, from a totally hydrophobic environment to one in which there is a large degree of hydrophilic interaction, should give rise to a wide range of redox potentials for copper(III,II) couples. Thus the secondary structure of a protein containing a copper(III,II) couple could very selectively dictate its redox potential. Furthermore, secondary structural changes by a given protein could cause the copper(III,II) E° value to vary greatly. Hence, the expected features of copper(III,II) couples in a protein environment suggest that this redox system could be important in biological processes.

Conclusions

The E° values of copper(III,II) peptide and nickel(III,II) peptide couples are temperature dependent. The values of dE°/dT in a nonisothermal electrochemical cell are negative

for copper couples and positive for nickel couples. The values of $dE°/dT$ allow evaluation of the entropy difference between oxidation states, $S°_{II} - S°_{III}$, which are negative for the copper couples and positive for the nickel couples. The values of $S°_{II} - S°_{III}$ correspond to a gain of two water molecules for the reduction of copper(III) peptide complexes and a loss of two water molecules for the reduction of nickel(III) peptide complexes. In both cases the d^8 electronic configuration (copper(III) and nickel(II)) behaves as though it has no axial coordination of water, while the d^9 (copper(II)) and d^7 (nickel(III)) electronic configurations behave as if two water molecules are coordinated axially. This explains why alkyl side chains in the peptide ligand, especially bulky ones which significantly hinder axial coordination, will cause $E°$ to shift to lower values for copper(III,II) and to higher values for nickel(III,II). In the case of copper, this behavior could be important in biological systems. If a copper complex with peptide nitrogen coordination were located in a hydrophobic protein environment where axial coordination would be difficult, the copper(III) state might be much more easily accessible than has been realized. Indeed, this prediction is supported by our results which show that $E°$ values for the $Cu^{III,II}(H_{-3}G_3a)^{0,1-}$ couple decrease as the concentration of water is lowered by addition of nonaqueous solvents. In the total absence of water, the $E°$ value for $Cu^{III,II}(H_{-2}Aib_3)^{0,1-}$ is 0.12 V vs. NHE, which is 0.54 V lower than in aqueous solution.

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Registry No. G_4, 637-84-3; V_4, 64577-64-6; G_3a, 35790-47-7; G_4a, 35790-48-8; Aib_3, 50348-89-5; A_3, 5874-90-8; DGEN, 5663-60-5; Ni, 7440-02-0; Cu, 7440-50-8.

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Proton-Transfer and Nucleophilic Displacement Reactions of the Triply Deprotonated Tetraglycine Complex of Copper(II)

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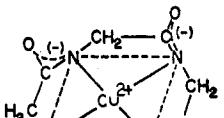
General-acid catalysis in the reaction of $Cu^{II}(H_{-3}G_4)^{2-}$ to form $Cu^{II}(H_{-2}G_4)^-$ indicates direct proton transfer to the terminal deprotonated peptide nitrogen of the triply deprotonated tetraglycine (G_4) complex. The rate constants (25.0 °C) increase with acid strength, reaching a value of 1.6×10^8 M^{-1} s^{-1} for H_3O^+. These constants are an order of magnitude greater than for the corresponding reactions with the triglycine complex, $Cu^{II}(H_{-2}G_3)^-$, and the glycylglycylhistidine complex, $Cu^{II}(H_{-2}Ghis)^-$. The H_2O rate constant is 16 s^{-1} for $Cu^{II}(H_{-3}G_4)^{2-}$, compared to a value of 0.12 s^{-1} for the triglycine complex. On the other hand, nucleophilic attack by triethylenetetramine is more than 4 orders of magnitude slower for the tetraglycine complex, with rate constants (M^{-1} s^{-1}) for Htrien$^+$ and trien equal to 71 and 4.9×10^2, respectively. The more protonated forms of trien are also reactive as acids with rate constants (M^{-1} s^{-1}) of 62 for H_2trien^{2+} and 3.7×10^2 for H_3trien^{3+} in their reactions with $Cu^{II}(H_{-3}G_4)^{2-}$.

Introduction

Copper(II) reacts with tetraglycine (G_4) in basic solution to form a complex in which three peptide hydrogens are ionized[1] to give $Cu(H_{-3}G_4)^{2-}$ (structure I). The crystal structure



I

of $Na_2Cu(H_{-3}G_4)\cdot 10H_2O$ has been determined,[2] and all four nitrogen atoms (one amine and three deprotonated peptide nitrogens) are bound to copper in a nearly square-planar arrangement. The carboxylate group is not coordinated. Potentiometric titrations,[1] the infrared spectrum,[3] and the visible spectrum[4] indicate that the same groups are coordinated to the copper(II) in solution. In the present study the kinetics of the reactions of $Cu(H_{-3}G_4)^{2-}$ with acids (eq 1) and with triethylenetetramine (trien) (eq 2) are examined.

$$Cu(H_{-3}G_4)^{2-} + HB \rightarrow Cu(H_{-2}G_4)^- + B^- \quad (1)$$

$$Cu(H_{-3}G_4)^{2-} + trien \rightarrow Cu(trien)^{2+} + G_4^- + 3OH^- \quad (2)$$

Two kinetic pathways have been observed for proton-transfer reactions of metal peptide complexes. The first of these is the outside protonation pathway[5,6] in which rapid protonation of the peptide oxygen occurs. The rapid protonation preequilibrium is followed by breaking of the metal–peptide nitrogen bond as the rate-determining step. The outside protonation pathway is accelerated by hydrogen ion but not by general acids. The second kinetic pathway for proton-transfer reactions of metal peptide complexes is direct, or inside, protonation of the deprotonated peptide nitrogen, which occurs simultaneously with metal–nitrogen bond breaking.[7] This pathway is accelerated by both hydrogen ion and general acids.[6] The outside protonation pathway contributes to the H_3O^+ rate constant only when the inside protonation is slow due to slow metal–peptide nitrogen bond rupture[5] or when metal–peptide nitrogen bond rupture is otherwise restricted.[8]

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