Characterization of Copper(III)−Tetrapeptide Complexes with Histidine as the Third Residue

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Received July 24, 1996[⊗]

Copper(III) complexes of $Gly_2HisGly$ and $Aib_2HisGly$ are characterized, where Gly is glycine, His is L-histidine, and Aib is α-aminoisobutyric acid. Their respective reduction potentials (V vs NHE) are 0.978 and 0.826. Both Cu(III) complexes undergo amine deprotonation with pK_a values of 8.79 and 8.81, respectively. The influence of the 5−5−6 membered chelate rings on the $E^{\circ\prime}$ and pK_a values is examined relative to the Cu(III)−tripeptide complexes without histidine that have 5−5−6, 5−6−5, 6−5−5, and 5−5−5 membered linked-consecutive rings. The presence of a six-membered ring in the third peptide residue causes a decrease of ∼1.0 pK_a unit relative to a 5−5−5 membered ring system. Imidazole coordination from histidine compared to carboxylate coordination causes an additional decrease of 1.3 pK_a units. Decompositions of $Cu^{III}(H_{-2}Gly_2HisGly)$ and $Cu^{III}(H_{-2}Aib_2HisGly)$ complexes are measured over the pH range 0.3−4.7. The kinetics and the reaction products show that abstraction of a proton at the α carbon of the histidyl residue is the rate-determining step. The initial decomposition product of $Gly_2HisGly$ is a tetrapeptide with an α-hydroxyhistidyl residue that dehydrates to give an alkene with an α,*β*-dehydrohistidyl residue. UV−vis spectral properties are reported for the alkene as well as the Cu(III) complexes of $Gly_2HisGly$ and $Aib_2HisGly$.

Introduction

In previous work[1] we have shown that tripeptide complexes of Cu(III) with L-histidine (His) as the third residue undergo very rapid oxidative decarboxylation reactions. The corresponding histamine complex is a factor of 10^5 slower to decompose. Both types of Cu(III) complexes undergo amine deprotonation at relatively low pH (pK_a values of 8.2−8.8) compared to many other tripeptide and tetrapeptide Cu(III) complexes with pK_a values of 11.0−12.6.[2]

In the present work, we examine the properties of two Cu(III)−tetrapeptide complexes ($Gly_2HisGly$ and $Aib_2HisGly$), where Gly is the glycyl residue and Aib is the α-aminoisobutyryl residue. Proposed structures of peptide complexes are given in Figure 1. Previous work[1] with a *τ*-methylimidazole derivative of Gly_2His showed that deprotonation occurs at the amine nitrogen and not at the pyrrole nitrogen. Dervan and coworkers[3] attached Gly_2His to a glycyl amine terminal of a DNA binding agent. They added Cu(II) and oxidizing agents to cause site-specific DNA cleavage. Thus, if a Cu(III) complex is formed, $Gly_2HisGly$ is the most appropriate peptide to use in order to understand the behavior of this system.

Coordination of an imidazole nitrogen forms six-membered rings; therefore, we also address the effect of 5−5−5 vs 6−5−5, 5−6−5, and 5−5−6 membered ring systems on the pK_a values of amine deprotonation for Cu(III)−peptide complexes.

Experimental Section

Reagents. $Gly_2HisGly$ and $Aib_2HisGly$ were synthesized in this laboratory by H. D. Lee. *β*-$AlaGly_2$ and Gly-*β*-AlaGly were obtained from BACHEM Bioscience Inc., and Gly_2-*β*-Ala was obtained from Vega. All solutions were prepared with double-deionized distilled water. Solid $NaClO_4$ was dissolved in water, filtered through 0.45 *μ*m Millipore filter paper, recrystallized, redissolved, and standardized gravimetrically. It was used to adjust the ionic strength of all solutions to $\mu = 1.0$ M. A stock solution of $Cu(ClO_4)_2$ was prepared from $CuCO_3$ and $HClO_4$. The solution was standardized with EDTA using murexide as the indicator. The peptides were dissolved in water, and $Cu(ClO_4)_2$ was added to give 5−10% excess of the peptide after formation of 1:1 complexes. The Cu(II)−peptide complex was formed by addition of NaOH to increase the pH of the solution.

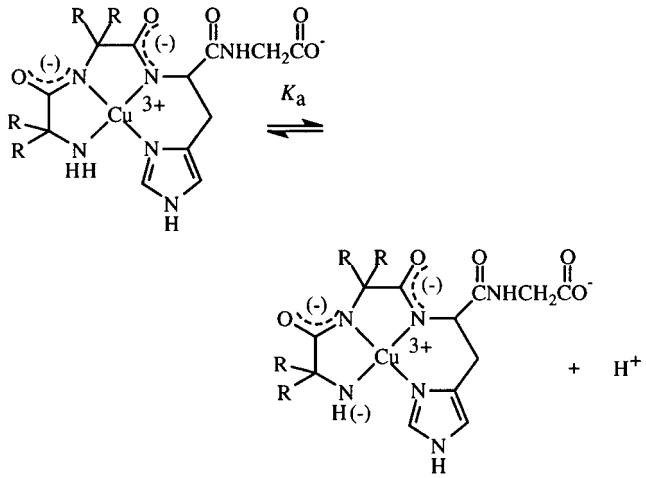

Figure 1. Proposed structures for $Cu^{III}(H_{-2}L)$, the doubly-deprotonated complex, and $Cu^{III}(H_{-3}L)^-$, the deprotonated amine complex: R = H, $Gly_2HisGly$; R = CH_3, $Aib_2HisGly$.

Methods. An Orion Model 601A pH meter equipped with a Corning combination electrode was used to measure pH. The electrode was calibrated by titration of standardized solutions of $HClO_4$ and NaOH to correct the meter response to $-\log [H^+]$ (=p$[H^+]$) values at 25.0 ± 0.1 °C and $\mu = 1.0$ M ($NaClO_4$). Reduction potentials were measured by Osteryoung square wave voltammetry (OSWV) as a function of p$[H^+]$ with a BAS-100 electrochemical analyzer. The working electrode was a planar glassy carbon electrode (3 mm diameter), the auxiliary electrode was platinum wire, and the reference electrode was a Vycor tip Ag/AgCl electrode stored in 3 M NaCl ($E^\circ = 0.194$ V vs

[⊗] Abstract published in *Advance ACS Abstracts,* June 15, 1997.

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S0020-1669(96)00871-3 CCC: $14.00

NHE). Solutions of the Cu(II)-peptide complexes, adjusted to the desired pH, were placed in the electrochemical cell. The Cu(II) complexes are stable above pH 5, whereas the Cu(III) complexes decompose quickly. The OSWV method uses differential current measurements which enable rejection of background currents observed for measurements at high pH values. Sufficiently rapid sampling times are used to avoid interference from the decomposition reactions of Cu(III).

A flow-through bulk electrolysis unit, reported previously,[4] was used for the preparation of Cu(III)−peptide complexes. The graphite column was 0.5 cm (i.d.) × 1.2 cm. Flow rates of 1−3 mL/min were used. Solutions of the Cu(II)−peptide complex were injected on the column at p[H^+] ~7. Electrolysis of the solvent during preparation lowers the p[H^+] of the solution to 4.5−5.5. Work by Xu and Margerum[5] shows that, in millimolar solutions, the $Cu^{II}(H_{-2}Gly_2HisGly)^-$ complex is 99% formed at p[H^+] 5.3. Solutions of the Cu(III)-peptide were mixed with solutions of perchloric or acetic acid immediately after preparation to adjust the p[H^+] to 0.30−4.7 for subsequent kinetic measurements. UV−vis spectra were collected on a Perkin-Elmer Lambda 9 UV−vis−NIR spectrometer interfaced to a Zenith 386/20 computer. Kinetic data were collected by monitoring the absorbance of solutions of Cu(III)−peptide complexes.

Separation of the decomposition products by HPLC was carried out on a Varian 5000 HPLC liquid chromatography system equipped with variable-wavelength UV detector or a Hewlett Packard 1050 diode array detector. A Whatman Partisil 10 strong cation exchange column (4.6 mm diameter × 250 mm) was used with a mobile phase of 0.05 M NaH_2PO_4, 0.1 M NaCl at p[H^+] 2.6 (flow rate = 1.0 mL/min, injection volume = 50 μL, and λ = 220 nm).

Numbers in parentheses next to measured values represent one standard deviation in the last digit reported.

Results and Discussion

Cu(III,II) Electrode Potentials and pK_a Values for Amine Deprotonation of Cu(III)−Peptide Complexes. Reduction potentials (E vs NHE) for the Cu(III) complexes of Gly_2HisGly, Aib_2HisGly, $Gly_2\beta$-Ala, Glyβ-AlaGly, and β-$AlaGly_2$ are measured. Figure 2 shows the experimental pH dependence of the electrode potentials for four complexes. Equations 1 and 2 give the reduction and amine deprotonation reactions of the

$$Cu^{III}(H_{-2}L) + e^- \rightleftarrows Cu^{II}(H_{-2}L)^- \qquad E^{\circ\prime} \qquad (1)$$

$$Cu^{III}(H_{-2}L) \rightleftarrows Cu^{III}(H_{-3}L)^- + H^+ \qquad K_a \qquad (2)$$

Cu(III) peptide complexes. Figure 1 shows the amine-deprotonated structure. Copper(II) complexes do not undergo amine deprotonation.[4] Therefore, as more of the amine-deprotonated species, $Cu^{III}(H_{-3}L)^-$, forms with increasing pH, the measured E values decrease in accord with eq 3. Least-squares fit of the

$$E = E^{\circ\prime} - \frac{RT}{nF}\ln\left(\frac{[H^+] + K_a}{[H^+]}\right) \qquad (3)$$

curves in Figure 2 give the $E^{\circ\prime}$ values (below p[H^+] 8) and the K_a values for each complex. The results are summarized in Table 1.

In general as the electrode potentials of Cu(III,II)−peptide complexes increase, the rates of self-decomposition of the Cu(III)−peptides increase.[2] The $E^{\circ\prime}$ value of 0.978 V for the $Cu^{III}(H_{-2}Gly_2HisGly)$ complex (with a coordinated imidazole group) is much higher than that of tetrapeptide complexes with a third deprotonated-N(peptide) group coordinated. For example, $Cu^{III}(H_{-3}Gly_4)^-$ has an $E^{\circ\prime}$ value of 0.63 V and it is relatively slow to decompose at p[H^+] 7, where its half-life is 6 h.[6] At p[H^+] 2 the Cu(III) complex of Gly_4 has a half-life of 11.6 min compared to a half-life of 1.9 min for $Cu^{III}(H_{-2}Gly_2$-HisGly). Although the tripeptide complex, $Cu^{III}(H_{-2}Gly_2His)$, has an $E^{\circ\prime}$ value of 0.94 V, it is much faster to decompose ($t_{1/2}$ = 5 ms at p[H^+] 7) than the corresponding tetrapeptide complex, $Cu^{III}(H_{-2}Gly_2HisGly)$, because it undergoes rapid oxidative decarboxylation.[1] We prepared the $Cu^{III}(H_{-2}Aib_2HisGly)$ complex with an $E^{\circ\prime}$ value of 0.826 V in order to have a longer lasting histidyl-containing tetrapeptide complex. At p[H^+] 2, its half-life is 37 min.

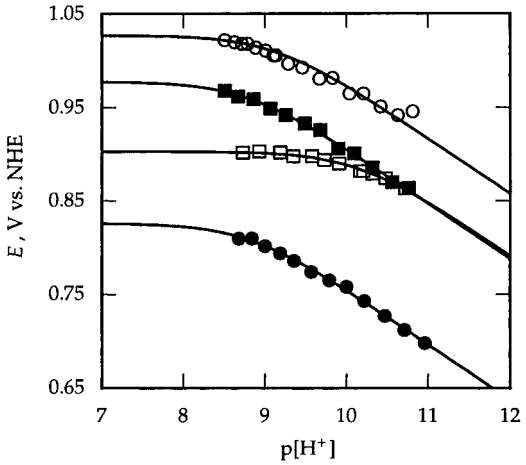

Figure 2. Dependence of the reduction potential (E) of Cu(III)−peptide complexes on p[H^+] at 25.0 °C and μ = 1.00 M ($NaClO_4$): (○) $Cu^{III}(H_{-2}Gly$-β-AlaGly); (■) $Cu^{III}(H_{-2}Gly_2HisGly)$; (□) Gly_2-β-Ala; (●) $Aib_2HisGly$. The solid lines are fit fits to eq 3.

Table 1. Copper(III) Peptide Reduction Potentials and Amine Deprotonation Values[a]

peptide	$E^{\circ\prime}$, V vs NHE[b]	amine deprotonation pK_a
$Gly_2HisGly$	0.978	8.79 ± 0.06
$Aib_2HisGly$	0.826	8.81 ± 0.05
$Gly_2\beta$-Ala	0.903	10.12 ± 0.02
β-$AlaGly_2$	1.023	9.94 ± 0.04
Glyβ-AlaGly	1.027	9.14 ± 0.05

[a] Conditions: 25.0 °C, μ = 1.0 M $NaClO_4$. [b] Precision: ±0.004 V.

Effect of 5−5−6, 5−6−5, 6−5−5, and 5−5−5 Membered Rings. Amine deprotonation of $Cu^{III}(H_{-2}L)$ complexes to give $Cu^{III}(H_{-3}L)^-$, where L is Gly_2HisGly or Aib_2HisGly (Figure 1), occurs at pH 8.8 (Table 1). These complexes have 5−5−6 membered chelates, whereas many tripeptide and tetrapeptide complexes with 5−5−5 membered chelates have pK_a values of 11.0−12.6 (Figure 3). We tested tripeptide complexes with a β-alanyl residue (which give six-membered chelate rings without imidazole coordination) to determine the effect of 5−5−6, 5−6−5, and 6−5−5 membered rings on the $E^{\circ\prime}$ and pK_a values (Table 1). The $E^{\circ\prime}$ values range from 0.903 to 1.027 V, only 0.023−0.147 V greater than the value of 0.88 V for the 5−5−5 chelate of Gly_2Ala.[7] However, the corresponding amine deprotonation pK_a values are 10.12 (5−5−6), 9.14 (5−6−5), and 9.94 (6−5−5) compared to a value of 11.1 for the Gly_2Ala complex.[7] It is not clear why the presence of six-membered rings permit easier deprotonation of the amine nitrogen of these Cu(III) complexes. However, we conclude that the 5−5−6 membered chelates in $Gly_2HisGly$ account for a decrease of

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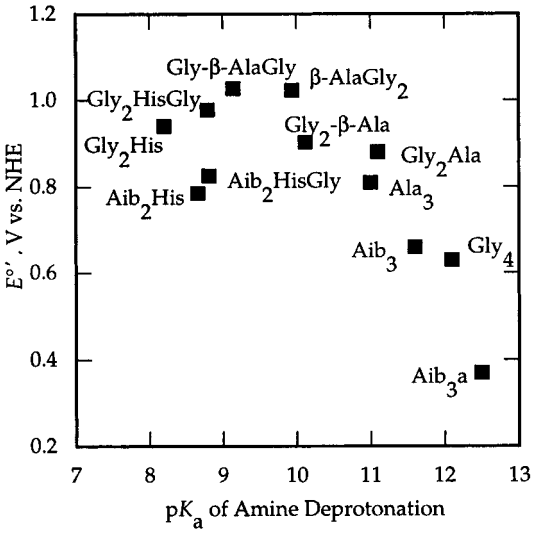

Figure 3. Reduction potentials vs pK_a values for amine deprotonation for tripeptide and tetrapeptide complexes of Cu(III).

1.0 of the total 2.3 pK_a unit decrease when compared to the 5−5−5 membered chelates in Gly_2Ala. The remainder of the effect must be attributed to imidazole coordination in contrast to carboxylate coordination. Figure 3 shows that, for peptides, where coordination of a third deprotonated-N(peptide) or deprotonated-N(amide) group occurs, the pK_a values are as large as 12.5. Unlike the $E°'$ values, the pK_a values are not very sensitive to α-alkyl groups but are very sensitive to linked-consecutive ring sizes and to the donor nature of the coordinated groups.

Copper(II)−polyamines with 5−6−5 membered linked-consecutive rings form significantly more stable complexes than is the case for corresponding 5−5−5 membered rings.[8] For tripeptides, the relative stabilities of the Cu(II) complexes are 5−6−5 ≃ 6−5−5 > 5−5−6 > 5−5−5.[9] For 5−5−5 membered peptide rings, the average bond distance for Cu(III) is 0.14 Å less than for Cu(II).[10] The smaller copper radius in its trivalent oxidation state can account for its preference for 5−5−5 membered peptide rings and the higher $E°'$ values for the 5−6−5 membered peptide rings. The presence of a six-membered ring has a large effect on the pK_a values of amine deprotonation. The added effect of histidine may be due to the ability of the imidazole group to accept electron density from copper(II) through π-back-bonding.

UV−Vis Properties. Solutions of the Cu(III) complexes were prepared by flow-through bulk electrolysis, and UV−vis spectra were collected immediately. The rate of decomposition of $Cu^{III}(H_{-2}Gly_2HisGly)$ is too rapid for its spectrum to be obtained before appreciable decomposition occurs. The $Cu^{III}(H_{-2}Aib_2HisGly)$ complex is slow enough in its decay that molar absorptivities could be determined. Its UV−vis spectrum is shown in Figure 4 along with the spectrum of $Cu^{II}(H_{-2}Aib_2HisGly)^-$. Spectral properties are summarized in Table 2.

Decomposition Kinetics of $Cu^{III}(H_{-2}Gly_2HisGly)$ and $Cu^{III}(H_{-2}Aib_2HisGly)$. p[H$^+$] 0.3−4.7. $Cu^{III}(H_{-2}Gly_2HisGly)$ and $Cu^{III}(H_{-2}Aib_2HisGly)$ are sufficiently stable to be prepared by flow-through bulk electrolysis for kinetic studies in the p[H$^+$] range 0.35−4.7. The loss in absorbance of Cu(III) was monitored at 402 nm for $Cu^{III}(H_{-2}Gly_2HisGly)$ and 425 nm for

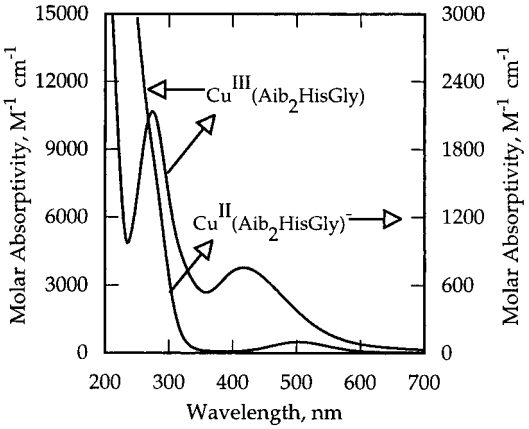

Figure 4. UV−vis spectra of $Cu^{III}(H_{-2}Aib_2HisGly)$ at p[H$^+$] = 2.0 and $Cu^{II}(H_{-2}Aib_2HisGly)$ at p[H$^+$] = 7.80.

Table 2. UV−Vis Spectral Properties of Cu(II)− and Cu(III)−Tetrapeptide Complexes

complex	λ_{max}, nm	ϵ, M^{-1} cm^{-1}
$Cu^{II}(H_{-2}Gly_2HisGly)^-$	528[a]	96.7
$Cu^{III}(H_{-2}Gly_2HisGly)$	402, 260	
$Cu^{II}(H_{-2}Aib_2HisGly)^-$	504[b]	99.2(5)
$Cu^{III}(H_{-2}Aib_2HisGly)$	416[c]	3.8(1) × 10^3
	274[c]	1.06(3) × 10^4

[a] Reference 5. [b] p[H$^+$] = 7.80. [c] p[H$^+$] = 2.0.

$Cu^{III}(H_{-2}Aib_2HisGly)$. The rate law for the decomposition reaction is given in eq 4, where L is $Gly_2HisGly$ or $Aib_2HisGly$

$$-\frac{d[Cu^{III}(H_{-2}L)]}{dt} = k_{obsd}[Cu^{III}(H_{-2}L)] \quad (4)$$

and the only form of Cu(III) present in solution is the doubly-deprotonated peptide complex. The method of initial rates was used for the analysis of data for the decomposition of $Cu^{III}(H_{-2}Gly_2HisGly)$ in the p[H$^+$] range 3.7−4.7.

Decomposition of $Cu^{III}(H_{-2}Gly_2HisGly)$ over the p[H$^+$] range 0.35−4.7 involves a self-decomposition path (eq 5), an acid

$$Cu^{III}(H_{-2}L) \xrightarrow{k_d} \text{intermediate} \quad (5)$$

$$Cu^{III}(H_{-2}L) + H^+ \xrightarrow{k_H} \text{intermediate} \quad (6)$$

$$Cu^{III}(H_{-2}L) + OH^- \xrightarrow{k_{OH}} \text{intermediate} \quad (7)$$

$$Cu^{III}(H_{-2}L) + \text{intermediate} \xrightarrow{\text{fast}} Cu^{II}(H_{-2}L)^- + \text{products} \quad (8)$$

$$k_{obsd} = 2(k_d + k_H[H^+] + k_{OH}[OH^-]) \quad (9)$$

path (eq 6) below p[H$^+$] 2, and a base bath (eq 7) above p[H$^+$] 4. These reactions generate an intermediate that reacts in a fast step (eq 8) to consume a second 1 equiv of Cu(III). The intermediates are either Cu^I−dehydropeptides or Cu^{II}−R$^\bullet$ species, where R$^\bullet$ is a carbon radical at the α position of the histidyl residue. Either intermediate reacts rapidly with a second Cu(III) peptide to give a two-electron oxidation product. The first-order rate constants as a function of p[H$^+$] for the decomposition of $Cu^{III}(H_{-2}Gly_2HisGly)$ were fit of the expression for k_{obsd} given in eq 9. The values of k_d, k_H, and k_{OH} determined from the fit of the data are 2.88(8) × 10^{-3} s^{-1}, 2.3(6) × 10^{-3} M^{-1} s^{-1}, and 3(1) × 10^6 M^{-1} s^{-1}, respectively. The rate of $Cu^{III}(H_{-2}Aib_2HisGly)$ decomposition is pH independent in the

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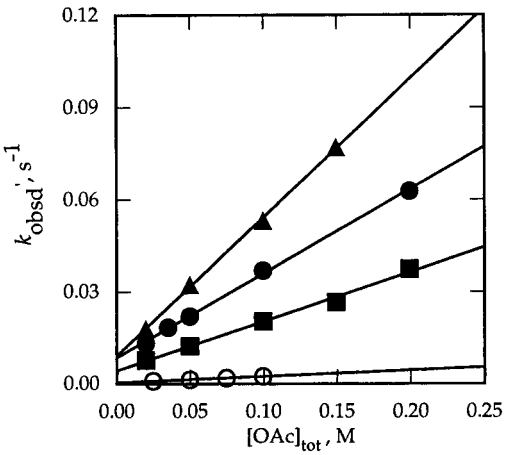

Figure 5. Dependence of the first-order rate constants for the decomposition of Cu(III)−tetrapeptide complexes on $[OAc]_{tot}$: $Cu^{III}(H_{-2}Gly_2HisGly)$ at $p[H^+]$ 3.89 (■), 4.39 (●), and 4.70 (▲); $Cu^{III}(H_{-2}Aib_2HisGly)$ at $p[H^+] = 4.22$ (○).

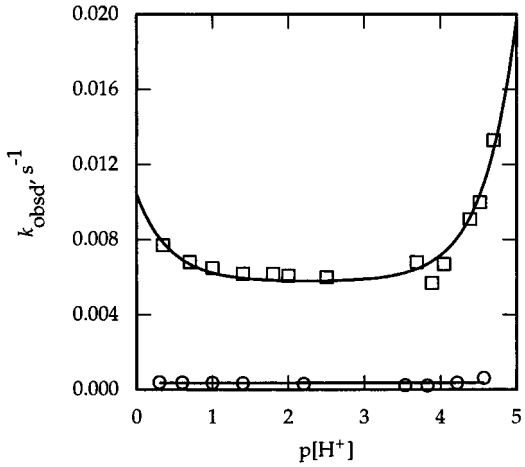

Figure 6. Dependence of the first-order rate constant on $p[H^+]$ for $Cu^{III}(H_{-2}Gly_2HisGly)$ (□) and $Cu^{III}(H_{-2}Aib_2HisGly)$ (○).

$p[H^+]$ range 0.30−4.3. The value of k_d for $Cu^{III}(H_{-2}Aib_2HisGly)$ is $1.8(6) \times 10^{-4}$ s^{-1}, where $k_{obsd} = 2k_d$.

Decomposition kinetics in the presence of acetic acid-acetate buffer vary with the acetate ion concentration (eq 10) as shown

$$Cu^{III}(H_{-2}L) + OAc^- \xrightarrow{k_{OAc}} \text{products} \qquad (10)$$

in Figure 5. Rate constants in the presence of buffer (k_{obsd}') are defined in eq 11, where k_{obsd} is the observed rate constant

$$k_{obsd}' = k_{obsd} + 2k_{OAc}\left(\frac{K_a^{HOAc}[OAc]_{tot}}{K_a^{HOAc} + [H^+]}\right) \qquad (11)$$

in the absence of buffer (eq 9), $K_a^{HOAc} = 10^{-4.55} = [H^+][OAc^-]/[HOAc]$,[11] and $[OAc]_{tot} = [OAc^-] + [HOAc]$. The values of k_{OAc} for $Cu^{III}(H_{-2}Gly_2HisGly)$ and $Cu^{III}(H_{-2}Aib_2HisGly)$ are 0.37(3) M^{-1} s^{-1} and 0.063(4) M^{-1} s^{-1}, respectively, at 25.0 ± 0.1 °C and $\mu = 1.0$ M ($NaClO_4$). Figure 6 shows a plot of k_{obsd} (corrected for the OAc^- contribution) for the decomposition of $Cu^{III}(H_{-2}Gly_2HisGly)$ and $Cu^{III}(H_{-2}Aib_2HisGly)$ as a function of $p[H^+]$.

The rate of decomposition of $Cu^{III}(H_{-2}Gly_2HisGly)$ increases in the presence of acetate ion as well as with an increase in the hydroxide ion concentration. The second-order rate constants

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Table 3. Summary of Constants for the Brønsted Relationship

B	p[b]	q[c]	pK_a^{HB}	k_B, $M^{-1}s^{-1}$
H_2O	3	2	−1.74[d]	5.19×10^{-3} [e]
CH_3COO^-	1	2	4.55[f]	0.37
OH^-	2	3	15.39[g]	3×10^6

[a] Conditions: 25.0 °C, $\mu = 1.0$ M ($NaClO_4$). [b] p is the number of equivalent proton sites on HB. [c] q is the number of equivalent basic sites on B^-. [d] −log(55.5). [e] $k_B = k_d/55.5$. [f] Reference 11. [g] $K_w/55.5$, M.

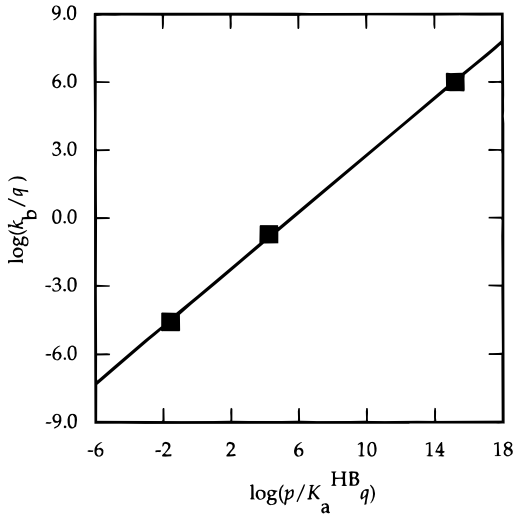

Figure 7. Brønsted plot for the decomposition reaction of $Cu^{III}(H_{-2}Gly_2HisGly) + B^-$, where $B^- = H_2O$, OAc^-, and OH^-, p is the number of equivalent proton sites on HB, and q is the number of equivalent basic sites on B^-. Slope (β) = 0.63 ± 0.01.

(k_B) increase with increasing base strength and follow a Brønsted relationship (eq 12),[12] where p is the number of equivalent

$$\log(k_B/q) = \log(G_B) + \beta \log(p/qK_a^{HB}) \qquad (12)$$

proton sites on HB, q is the number of equivalent basic sites on B^-, G_B is a constant, and β is the Brønsted coefficient. Table 3 summarizes the values of p, q, k_B, and pK_a^{HB}. Figure 7 is a Brønsted plot for $Cu^{III}(H_{-2}Gly_2HisGly)$ and gives a β value of 0.62(1). This represents a relatively large degree of proton transfer in the rate-determining step. Assistance of general bases in the decomposition of $Cu^{III}(H_{-2}Gly_2HisGly)$ shows that proton abstraction is a rate-determining step. A similar Brønsted correlation has been reported for the decomposition reaction of the copper(III)−glycylglycylhistamine complex, $Cu^{III}(H_{-2}Gly_2Ha)^+$, where $\beta = 0.59$. Thus, the decomposition of the tetrapeptide complex is analogous to the histamine complex where proton abstraction is also the rate-determining step.[1]

The value of k_{OAc} for $Cu^{III}(H_{-2}Aib_2HisGly)$ is smaller than the value of k_{OAc} for $Cu^{III}(H_{-2}Gly_2HisGly)$ by a factor of 5.9. A k_{OH} value was not resolved for $Cu^{III}(H_{-2}Aib_2HisGly)$, although evidence for a hydroxide path exists above $p[H^+]$ 4.3.

p[H⁺] 6.0−10.3. Flow-through bulk electrolysis could not be used for kinetic studies of $Cu^{III}(H_{-2}Gly_2HisGly)$ in the $p[H^+]$ range 6.0−10.3. The Cu(III) complexes are less stable at high pH and require stopped-flow methods to study their rates of decomposition. Several minutes are required to load the stopped-flow drive syringes with reactant solutions before pushes and data collection can begin. During this time, much of the Cu(III) decays and rate measurements were not possible. For this reason, kinetic measurements of the decomposition of

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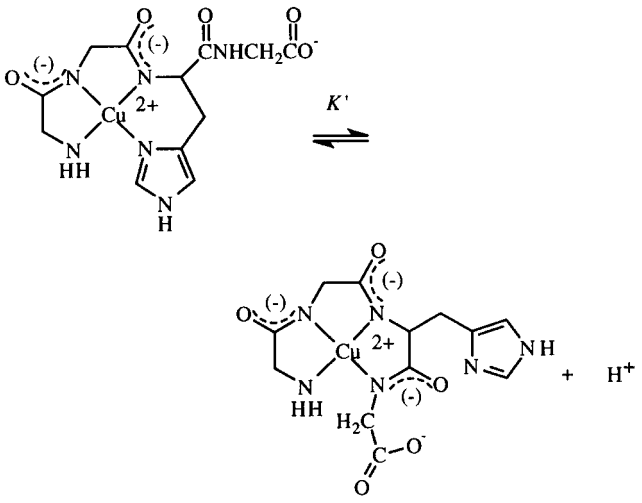


Figure 8. Proposed structures of $Cu^{II}(H_{-2}Gly_2HisGly(Im))^-$ and $Cu^{II}(H_{-2}Gly_2HisGly(N))^{2-}$, where $K_a = 10^{-11.94}$.[5]

$Cu^{III}(H_{-2}Gly_2HisGly)$ in the p[H^+] range 6.0−10.3 were not attempted with this method of preparation.

In situ chemical oxidation with $IrCl_6^{2-}$ (eq 13) has been used previously[1] to prepare Cu(III) complexes that decompose

$$IrCl_6^{2-} + Cu^{II}P \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} IrCl_6^{3-} + Cu^{III}P \qquad (13)$$

rapidly. The electron-transfer reaction in eq 13 is very rapid.[13] The Cu(III) complex is generated within a stopped-flow system by mixing a buffered solution of the $Cu^{II}P$ complex in the p[H^+] range 6.0−10.3 and a solution of $IrCl_6^{2-}$, where P represents the peptide. Due to the high reduction potentials of the Cu(III) complexes, a large excess of $Cu^{II}P$ was used to force the reaction in eq 10 to at least 97% completion. Consumption of all of the $IrCl_6^{2-}$ prevents oxidation of decomposition products by unreacted $IrCl_6^{2-}$. The reduction potential of $Ir^{IV/III}Cl_6^{2-/3-}$ is 0.892 V vs NHE.[14]

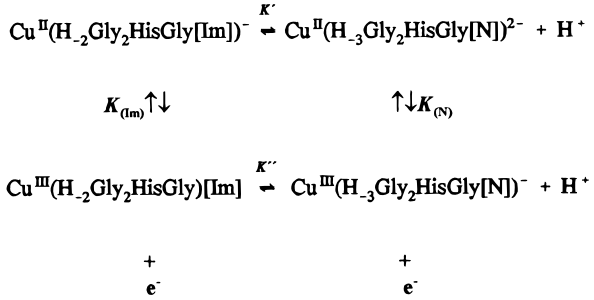
Above p[H^+] 6 the decomposition kinetics of the tetrapeptide complexes are not first-order due to the decay of two Cu(III) complexes that are not in rapid equilibrium. Xu and Margerum[5] have shown that the doubly-deprotonated Cu(II) complex of $Gly_2HisGly$ undergoes a change from coordination by the imidazole nitrogen of the histidine residue ($Cu^{II}(H_{-2}Gly_2HisGly[Im])^-$) to coordination by a third deprotonated peptide nitrogen ($Cu^{II}(H_{-3}Gly_2HisGly[N])^{2-}$) as shown in eq 14, where $K' = 10^{-11.94}$. The proposed structures of $Cu^{II}(H_{-2}Gly_2HisGly[Im])^-$ and $Cu^{II}(H_{-3}Gly_2HisGly[N])^{2-}$ are shown in Figure 8.

$$Cu^{II}(H_{-2}Gly_2HisGly[Im])^- \overset{K'}{\rightleftharpoons} Cu^{II}(H_{-3}Gly_2HisGly[N])^{2-} + H^+ \qquad (14)$$

The thermodynamic cycle in Scheme 1 was used to determine if formation of $Cu^{III}(H_{-3}Gly_2HisGly[N])^-$ is favored. An $E^{o\prime}$ value of 0.63 V vs NHE reported[14,4] for $Cu^{III}(H_{-3}Gly_4)^-$ is used as an estimate for that of $Cu^{III/II}(H_{-3}Gly_2HisGly[N])^{-,2-}$ since both complexes have the same donor groups to copper. A value of K'' of $10^{-6.04}$ is calculated, where $K_{(Im)} = 10^{16.5}$ and $K_{(N)} = 10^{10.6}$.

A thermodynamic cycle can also be constructed for the Aib_2-HisGly system. The equilibrium constant defined in eq 14 for the $Aib_2HisGly$ system, $10^{-11.77}$, is estimated from constants

Scheme 1



reported for $Gly_2HisGly$ ($pK'_{Gly_2HisGly} = 11.94$),[5] $Gly_2AibGly$ ($pK'_{Gly_2AibGly} = 8.11$),[15] and Aib_3Gly ($pK'_{Aib_3Gly} = 7.94$),[15] where $pK'_{Aib_2HisGly} = pK'_{Gly_2HisGly} + pK'_{Aib_3Gly} - pK'_{Gly_2AibGly}$. A reduction potential for $Cu^{III}(H_{-3}Aib_2HisGly[N])^{2-}$ of 0.47 V vs NHE is estimated from the reduction potential of $Cu^{III}(H_{-3}Gly_4)^-$ and the fact that each methyl group lowers the reduction potential by 0.04 V.[14] Therefore, K'' is $10^{-5.75}$, where $K_{(Im)} = 10^{13.96}$, $K_{(N)} = 10^{7.94}$, and $K' = 10^{-11.77}$.

The complex behavior of decomposition kinetics for Cu(III)−tetrapeptide complexes above pH 5 is due to the presence of both $Cu^{III}(H_{-2}L[Im])$ and $Cu^{III}(H_{-3}L[N])^-$ complexes. The rate of loss of Cu(III) becomes much faster as the pH increases. However, substitution reactions of Cu(III) are slow; therefore, interconversion between the two forms of Cu(III) goes through Cu(II). This can occur by electron-transfer reactions since both forms of Cu(II) are known to exist in solution and a large excess of the Cu(II)−peptide is present. The decomposition of $Cu^{III}(H_{-3}Gly_2HisGly[N])^-$ is expected to be slower than that of $Cu^{III}(H_{-2}Gly_2HisGly[Im])$ on the basis of the rate of decay of $Cu^{III}(H_{-3}Gly_4)^-$.[6] Therefore, the observed decay of Cu(III) is due to the interconversion between the two forms followed by decay of $Cu^{III}(H_{-3}Gly_2HisGly[Im])^-$. Rate constants were not resolved due to the complexity of the kinetic data.

Decomposition Products. $Cu^{III}(H_{-2}Gly_2HisGly)$ and $Cu^{III}(H_{-2}Aib_2HisGly)$ have absorption bands centered at 361 (p[H^+] = 2.0) and 363 nm (p[H^+] = 4.5), respectively. Copper(III)−tripeptide complexes with histidine as the third residue give decomposition products with conjugated double bonds (Aib_2-NHCH=CH−Im, where Im is imidazole).[1] The tripeptide decomposition products without copper coordination have a UV band at 261 nm. Copper(III)−tetrapeptide decomposition products, shown in Figure 9, with a double bond between the α and β carbons of the third residue (X_2-α,β-dehydro-HisGly, where X is Gly or Aib) are more conjugated than the tripeptide-decomposition products; therefore, bands are observed at longer wavelengths. The alkene (X_2-α,β-dehydro-HisGly) is formed by dehydration of X_2-α-OH-HisGly. (In related work,[16] an X-ray crystal structure was determined for the Ni(II) complex of Gly_2-α-hydroxyl-histamine). This type of dehydration reaction was observed for decomposition products of Cu(III)−tripeptide complexes with L-histidine as the third residue. In studies of the tripeptide complexes, the Aib_2-α-OH-Ha and Aib_2-α,β-dehydro-Ha products were isolated by HPLC and identified by ^{1}H NMR and mass spectrometry.[1] However, tetrapeptide decomposition products are not as stable and were not isolated. Figure 10 shows the loss of Cu(III), $k_{obsd} = 5.9(1) \times 10^{-3}$ s^{-1}, to form Gly_2-α-OH-HisGly followed by its dehydration to give the alkene (Gly_2-α,β-dehydro-HisGly) monitored at 361 nm

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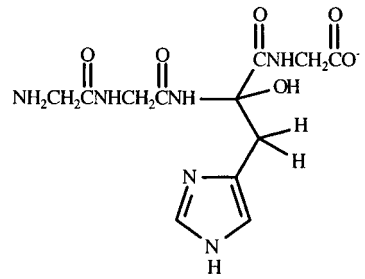
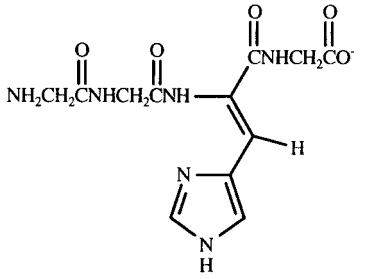


glycylglycyl-α-hydroxyhistidylglycine



glycylglycyl-α,β-dehydrohistidylglycine

Figure 9. $Cu^{III}(H_{-2}Gly_2HisGly)$ decomposition products.

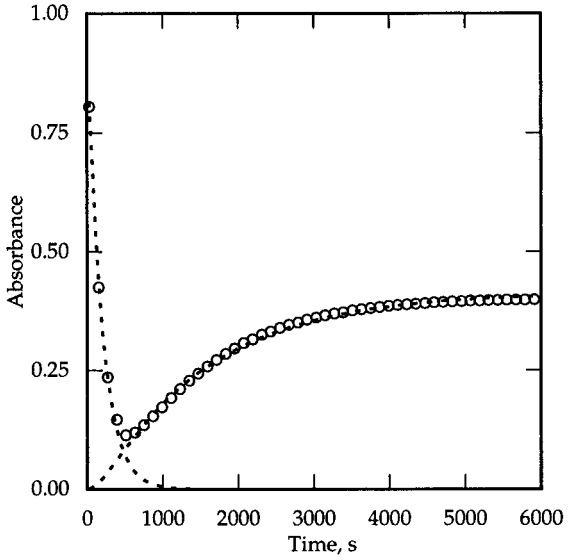


Figure 10. Absorbance at 361 nm vs time for a 0.5 mM solution of $Cu^{III}(H_{-2}Gly_2HisGly)$ at $p[H^+] = 2.00$, $T = 25.0 \pm 0.1$ °C, and path length = 1.0 cm. The decrease in absorbance is the loss of Cu(III), $k_{obsd} = 5.9 \times 10^{-3}$ s^{-1}. The increase in absorbance is peptide−olefin formation without Cu(II) complexation, $k_{obsd} = 6.9 \times 10^{-4}$ s^{-1}.

($p[H^+] = 2.0$). Products are not bound to Cu(II) at this pH. The first-order rate constant for the formation of the alkene is $6.9(1) \times 10^{-4}$ s^{-1}. Both the Cu(III) complex and the alkene have an appreciable absorbance at 361 nm, and the minimum in absorbance values in Figure 10 at 510 s is due to formation of Gly_2-α-OH-HisGly that does not absorb at 361 nm. The ϵ_{361} value for the alkene at $p[H^+] = 2.0$ is estimated to be 2000−2700 M^{-1} cm^{-1}, by assuming 100% oxidation of Cu(II) to Cu(III) by flow-through bulk electrolysis and a 30−40% yield of the alkene product. Separations of decomposition products by HPLC for both systems show recovery of the original peptide (% Gly_2HisGly not determined; % Aib_2HisGly is 57.2% at $p[H^+] = 1.90$, 61.8% at $p[H^+] = 3.52$, and 61.5% at $p[H^+] = 4.57$). The mechanism given in eqs 5−8 predicts 50% recovery. The additional 10% recovery that is observed is due to further Cu(III) oxidation of some decomposition products. Several products in addition to the alkene were observed by HPLC but were not identified.

Acidic solutions of Aib_2-α,β-dehydro-HisGly were monitored over a period of many days by HPLC separations with the diode-array detector. The original alkene disappeared in 4 days to give an unidentified intermediate with an absorption shoulder at lower wavelength. Aib_2amide was identified as a product that grew in slowly over a period of 9 days. During this period Aib_2 was not detected.

Acknowledgment. This work was supported by National Science Foundation Grants CHE-9024291 and 9622683. We are grateful for the syntheses of the tetrapeptides by Hsiupu D. Lee. M.R.M. is thankful for the Lilly Fellowship provided by Eli Lilly & Co.

IC9608713