Stability of the Cu(II) Complexes of Tripeptides, Cu($H_{-2}L$), in Dynamic Aspects; L = Tripeptides Composed of Various Combinations of α-, β-, and γ-Amino-Acid Residues. Stopped-Flow Kinetic Studies on the Reaction of Cu($H_{-2}L$) with Cysteine

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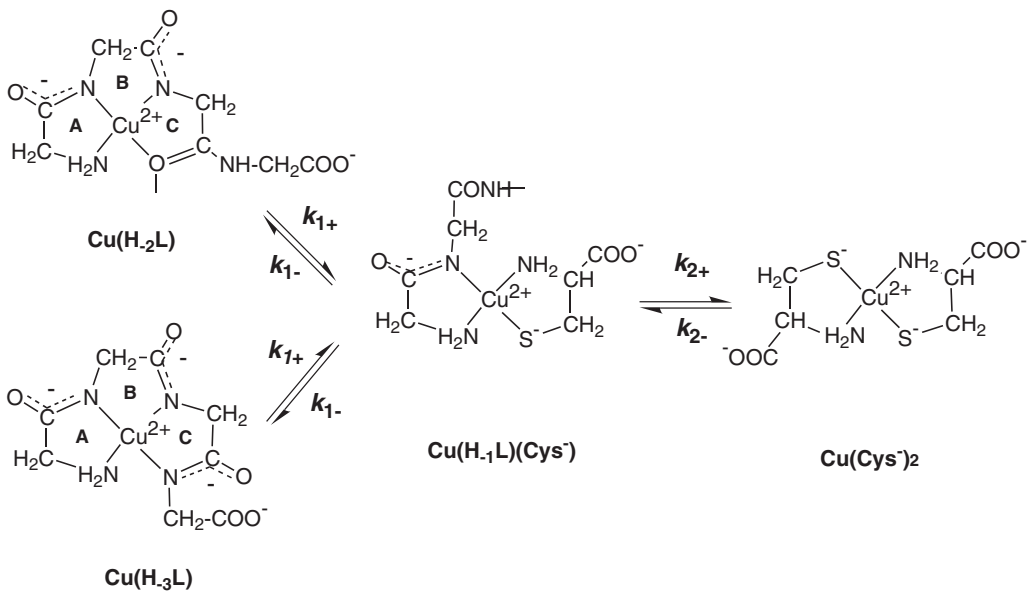
The stability of the fused chelate-ring structures of Cu($H_{-2}L$) concerning dynamic aspects was evaluated based on the rate constant (k_{1+}) for the formation of ternary complexes, Cu($H_{-1}L$)(Cys^-), from Cu($H_{-2}L$) and cysteine (Cys), where L denotes tripeptides composed of various combinations of α-, β-, and γ-amino-acid residues. The ligand-exchange reaction was examined by the stopped-flow spectrophotometric method. The Cu($H_{-2}L$) species, having 5–6–5- and 6–5–5-membered fused-chelate rings, were kinetically fairly stable; $k_{1+} = 2\text{–}3 \times 10^4\ M^{-1}\,s^{-1}$. Other Cu($H_{-2}L$) species, having 5–5–5-, 5–5–6-, 5–5–7-, 5–6–6-, and 6–5–6-membered rings, reacted rapidly with Cys to form the Cu($H_{-1}L$)(Cys^-) species; $k_{1+} \geqq 1 \times 10^5\ M^{-1}\,s^{-1}$. The Cu($H_{-1}L$)($Cys^-$) species, upon forming, successively reacted with Cys to afford a binary complex, Cu(Cys^-)$_2$. The Cu($H_{-1}L$)(Cys^-) species, with the β-Ala residue at the N-terminus, were kinetically labile to be rapidly transformed to Cu(Cys^-)$_2$.

Naturally occurring peptides, condensation products of α-amino acid residues, can form stable Cu(II) complexes, abbreviated as Cu($H_{-i}L$); L = peptide and i = 1, 2, or 3, in which three amino-acid residues at the amino terminus condense to construct a 5–5–5-membered fused-chelate ring. Here, the chelate-rings are named **A**, **B**, and **C**, respectively, from the amino-terminus. The fused-chelate ring is primarily coplanar, and tightly wraps the metal ion, which can be delivered by aminothiols, such as cysteine (Cys). The delivery of Cu(II) from Cu($H_{-i}L$) begins by replacing the fourth-donor atom by the thiolate S.[1,2] The Cu(II) complex, Cu(H_{-2}GlyGlyGly), in which the fourth donor is an easily exchangeable carboxylate oxygen,[3] rapidly reacted with Cys to form a ternary complex, Cu(H_{-1}GlyGlyGly)(Cys^-) (Scheme 1). In contrast, Cu(H_{-3}-tetra-Gly), in which the fourth site is occupied by a deprotonated amide nitrogen,[4] was sluggish toward ligand-exchange. The kinetic stability of the fused-chelate structure for Cu($H_{-i}L$) is likely to depend on the coordination environment around the Cu(II).

We have been studying the transport of Cu(II) from oligopeptides to aminothiols, such as L-cysteine (Cys) and D-penicillamine (Pes), by using the stopped-flow techniques, and



Scheme 1. Ligand-exchange reaction of Cu($H_{-i}L$) with Cys; i = 2 or 3.

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have elucidated the relation between the coordination structure and the exchangeability of the Cu(II) in Cu(H$_{-i}$L).[2,5] The Cu(H$_{-i}$L) reacts with aminothiols (Rs) to primarily form a transient ternary complex, Cu(H$_{-1}$L)(Rs$^-$), which has been characterized by the stopped-flow spectrophotometric and ESR spectroscopic methods.[2] The Cu(H$_{-1}$L)(Rs$^-$) subsequently reacts with Rs, forming a binary Cu(Rs$^-$)$_2$ complex (Scheme 1).[2] Here, unless otherwise stated, the Cu in the complex denotes Cu(II). Since the reaction begins by the replacement of the fourth donor by the thiolate S$^-$, the affinity of the Cu(II) for the fourth donor atom is likely to determine the rate of ligand replacement. From static aspects, the stability of Cu(H$_{-i}$L) depends on the size of the fused-chelate ring.[7]

The magnitude of the rate constants is considered to be of great importance in elucidating the chemical form of Cu(II) in its transport process. In the reaction of Cu(H$_{-i}$L) with Rs, when k_{1+} is bigger by far than k_{2+}, the ternary Cu(H$_{-1}$L)(Rs$^-$) species is found as a major species. On the contrary, when k_{2+} is bigger than k_{1+}, the binary Cu(Cys$^-$)$_2$ could be a major species, instead of Cu(H$_{-1}$L)(Rs$^-$). In this paper, we report on the kinetic stability of the whole structure of a fused-chelate ring for Cu(H$_{-2}$L), in which the peptides L were composed of various combinations of α-, β-, and γ-amino acids. The kinetic stability was evaluated from the rate constant, k_{1+}, for the formation of Cu(H$_{-1}$L)(Cys$^-$) from Cu(H$_{-2}$L). The tripeptides used were as follows: composed of two Gly residues and one β-Ala residue, such as β-Ala-GlyGly, Gly-β-AlaGly, and GlyGly-β-Ala; one Gly residue and two β-Ala residues, such as β-AlaGly-β-Ala and Gly-β-Ala-β-Ala, and combinations of Gly, β-Ala, and γ-aminobutyric acid (γ-Aba) residues, such as Gly-β-Ala-γ-Aba and β-AlaGly-γ-Aba. Based on the kinetic data, the biological significances of the various types of Cu(H$_{-2}$L) species in the metal transport process were considered.

Experimental

Materials. The tripeptides, including glycylglycyl-γ-aminobutyric acid (GlyGly-γ-Aba), glycyl-β-alanyl-γ-aminobutyric acid (Gly-β-Ala-γ-Aba), β-alanylglycyl-γ-aminobutyric acid (β-AlaGly-γ-Aba), were prepared by the conventional solution technique.[8] Other peptides, including glycylglycylglycine (Gly-GlyGly), glycylglycyl-β-alanine (GlyGly-β-Ala), glycyl-β-alanylglycine (Gly-β-Ala-Gly), β-alanylglycylglycine (β-Ala-Gly-Gly), glycyl-β-alanyl-β-alanine (Gly-β-Ala-β-Ala), β-alanylglycyl-β-alanine (β-Ala-Gly-β-Ala), glycylglycyl-L-isoleucine (Gly-Gly-L-Ile), and penta-glycine (penta-Gly), were purchased from BACHEM Feinkemikalien AG. (Switzerland). They were pure, as checked by liquid-chromatography. Cu(II) perchlorate, Cu(ClO$_4$)$_2$·6H$_2$O, obtained from G. Frederick Smith Chem. Co. (Columbus, Oh), was used after recrystallization. L-Cysteine was a products of Sigma Chemical Co.(St. Louis, Mo). All other chemicals were of reagent grade and used without further purification.

Preparation of Sample Solutions. A stock solution of Cu(II), prepared by dissolving Cu(ClO$_4$)$_2$·6H$_2$O in 0.05 M (1 M = 1 mol dm^{-3}) borate buffer, was standardized by titration with 0.01 M EDTA, using murexide as an indicator.[9] Solutions of Cu(II) complexes were freshly prepared using aliquots of the standardized Cu(II) solution with a 3–5 mol% excess peptide to ensure complex formation. Solutions of Cys were freshly prepared just prior to spectroscopic measurements. The ionic strength (I) was maintained at 0.1 M NaClO$_4$ for absorption spectral measurements.

Spectrophotometric Measurement. The absorbance changes during the reaction were measured at 25 °C and analyzed on a computerized Union RA-401 stopped-flow spectrophotometer equipped with a 0.5 mm quartz cell. The solutions of the 5.00×10^{-4} M Cu(H$_{-2}$L) and four equivalents Cys were equilibrated at 25 °C. After equilibration for 20 min, the reaction was initiated by mixing both solutions under N_2 at 8 kg/cm^2.

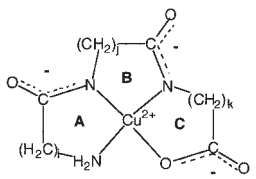
The absorption spectrum was prepared by a point-by-point plot of the absorbances against the wavelength at intervals of either 5 nm or 10 nm in the range from 250 to 700 nm.[2] The concentrations of the Cu(H$_{-2}$L), Cu(H$_{-1}$L)(Cys$^-$), and Cu(Cys$^-$)$_2$ species during the reaction were calculated from absorbance-time plots recorded at 265 nm, 330 nm, and 390 nm by a previously reported method.[2] The dead time (DT) of the instrument was 1.2 ms, as determined by the reaction of ascorbate with 2,4-dichlorophenolindophenol.[10]

Stopped-Flow Kinetic Measurements. Stopped-flow kinetic runs were carried out under pseudo first-order conditions using a large excess of Cys at pH 9.3 and 25 °C (I = 0.1 M NaClO$_4$). Solutions of 2.10×10^{-4} M Cu(H$_{-2}$L) and 15–65 equivalents CysH were equilibrated at 25 °C under N_2 before measurements. After equilibration for 20 min, the reaction was initiated by mixing both solutions at 8 kg/cm^2 under N_2, and subsequent absorbances at 330 nm and at 390 nm were recorded. Plots of the observed rate constant, k_{obsd}, against the concentration of Cys gave a straight line. The forward rate constant (k_+) and backward rate constant (k_-) were determined from the slope and the intercept on ordinate, respectively.[11,12]

Results and Discussion

Absorption Spectrum. The parameters of the absorption spectra for Cu(H$_{-2}$L) at pH 9.3 are given in Table 1. The complexes, except for the Gly-β-Ala-γ-Aba complex, exhibit the d–d transition absorption at 550–560 nm. This indicates that those complexes possess the (N,N$^-$,N$^-$,O$^-$) donor set,[13] forming fused-chelate rings from three amino-acid residues, and were abbreviated as Cu(H$_{-2}$L), in which the first and second amide groups were deprotonated. The Gly-β-Ala-γ-Aba complex, exhibiting a d–d absorption at 625 nm, has a 5,6-mem-

Table 1. Parameters of Absorption Spectra for Cu(H$_{-2}$L) at pH 9.3



Cu(H$_{-2}$L)	i	j	k	λ_{max}/nm (ε/M^{-1} cm^{-1})
Cu(H$_{-2}$GlyGlyGly)	1	1	1	552 (152)
Cu(H$_{-2}$GlyGly-β-Ala)	1	1	2	541 (166)
Cu(H$_{-2}$GlyGly-γ-Aba)	1	1	3	565 (110)
Cu(H$_{-2}$Gly-β-AlaGly)	1	2	1	547 (156)
Cu(H$_{-2}\beta$-Ala-GlyGly)	2	1	1	548 (162)
Cu(H$_{-2}$Gly-β-Ala-β-Ala)	1	2	2	555 (131)
Cu(H$_{-2}\beta$-Ala-Gly-β-Ala)	2	1	2	555 (131)
Cu(H$_{-2}$Gly-β-Ala-γ-Aba)	1	2	3	625 (71.0)
Cu(H$_{-2}\beta$-Ala-Gly-γ-Aba)	2	1	3	565 (72.0)

bered fused-chelate structure, $Cu(H_{-1}L)$, with the (N,N^-,O^-) donor set. In any complex, the fourth site is occupied by an exchangeable oxygen from either carboxylate or water.

The absorption spectra of transients at various stages of the reactions with $Cu(H_{-2}Gly\text{-}\beta\text{-}AlaGly)$ and $Cu(H_{-2}\beta\text{-}AlaGly\text{-}Gly)$ are shown in Figs. 1 and 2. The feature for the spectral changes appeared to be different from each other, depending on the position of the β-Ala residue in the peptide chains. $Cu(H_{-2}Gly\text{-}\beta\text{-}AlaGly)$ reacted with Cys to initially afford a ternary complex, $Cu(H_{-1}Gly\text{-}\beta\text{-}AlaGly)(Cys^-)$, which exhibited a spectrum with the $S^- \rightarrow Cu(II)$ charge transfer (LMCT) absorption at 335 nm and a d–d transition band at 535 nm. Subsequently, it reacted with one molar Cys, yielding a binary complex, $Cu(Cys^-)_2$, with LMCT absorptions at 332 nm and 390 nm and a d–d band at 520 nm. In a reaction with $Cu(H_{-2}\beta\text{-}AlaGlyGly)$, the spectrum at the beginning of the observation was assignable to $Cu(Cys^-)_2$. The ternary complex, $Cu(H_{-1}\beta\text{-}AlaGlyGly)(Cys^-)$, could not be detected, because a six-membered chelate-ring **A** in $Cu(H_{-1}\beta\text{-}AlaGly\text{-}Gly)(Cys^-)$ would be rapidly opened by Cys.

Time-Dependent Distribution of the Cu(II) Complexes. The time-dependent distribution of the transients, including $Cu(H_{-1}L)(Cys^-)$ and $Cu(Cys^-)_2$, as well as $Cu(H_{-2}L)$, were calculated by a previously reported method.[2] The distributions curves for the reactions with $Cu(H_{-2}Gly\text{-}\beta\text{-}AlaGly)$ and $Cu(H_{-2}\beta\text{-}AlaGlyGly)$ are shown in Figs. 3 and 4. The distribution curve for the $Cu(H_{-2}Gly\text{-}\beta\text{-}AlaGly)$/CysH system clearly visualized a sequence of the following reactions: $Cu(H_{-2}L) \rightarrow Cu(H_{-1}L)(Cys^-) \rightarrow Cu(Cys^-)_2 \rightarrow Cu(I)Z$, where Z denotes unidentified ligands. The rate of the formation of $Cu(H_{-1}Gly\text{-}\beta\text{-}$

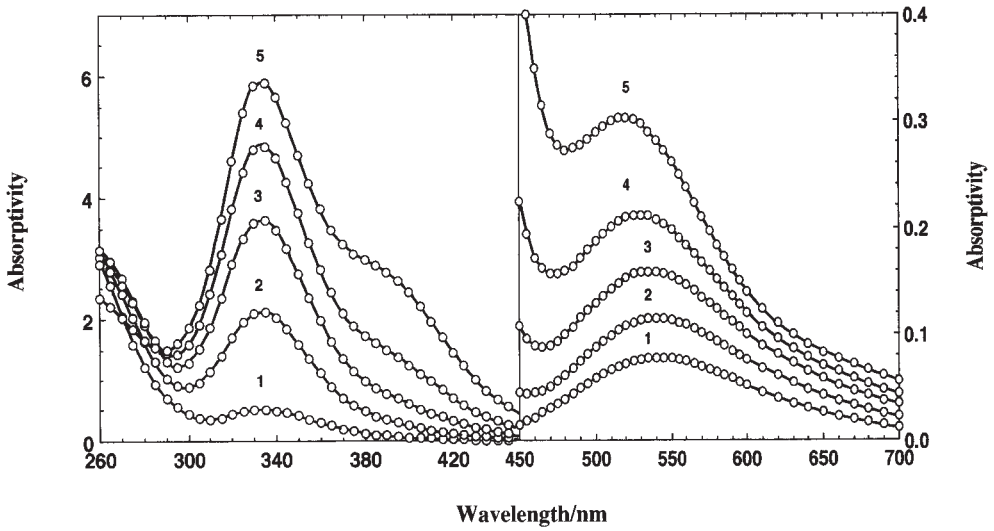


Fig. 1. Time-dependent spectra on addition of $Cu(H_{-2}Gly\text{-}\beta\text{-}Ala\text{-}Gly)$ to Cys at pH 9.3. (1) dead time, (2) 10 ms, (3) 30 ms, (4) 75 ms, and (5) 350 ms. $[Cu(II)] = 5.11 \times 10^{-4}$ M and $[Cys] = 1.95 \times 10^{-3}$ M (3.81 equiv.), 0.01 M borate buffer ($I = 0.1$ M $NaClO_4$), $T = 25$ °C.

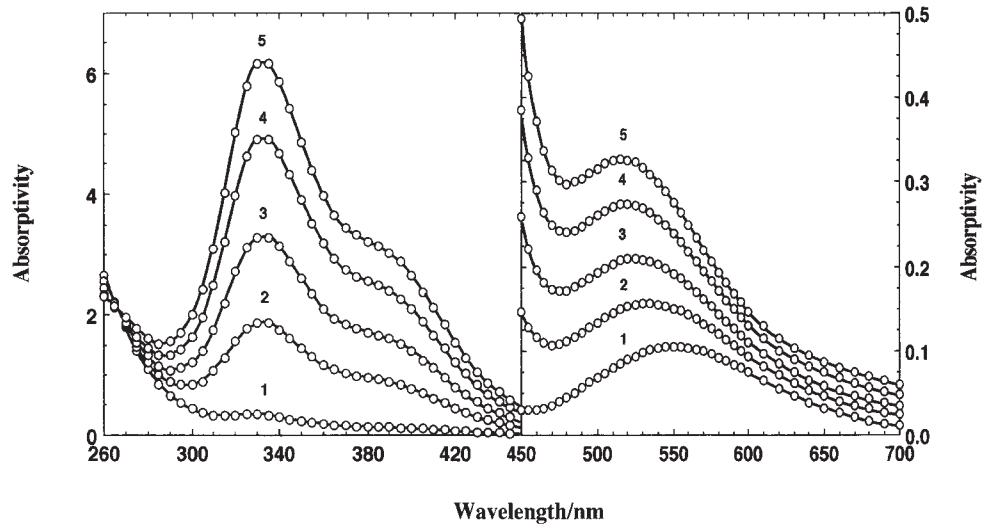


Fig. 2. Time-dependent spectra on addition of $Cu(H_{-2}\beta\text{-}Ala\text{-}Gly\text{-}Gly)$ to Cys at pH 9.3. (1) dead time, (2) 10 ms, (3) 25 ms, (4) 60 ms, and (5) 250 ms. $[Cu(II)] = 5.18 \times 10^{-4}$ M and $[Cys] = 1.94 \times 10^{-3}$ M (3.74 equiv.), 0.01 M borate buffer ($I = 0.1$ M $NaClO_4$), $T = 25$ °C.

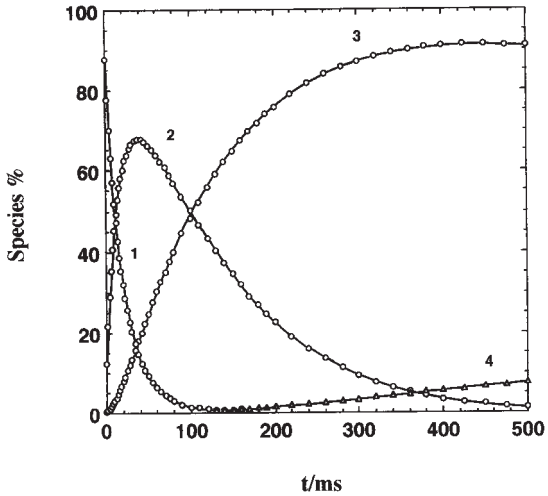


Fig. 3. Time-dependent distribution of the copper species in the reaction of Cu(H$_{-2}$Gly-β-Ala-Gly) with Cys at pH 9.3. (1) Cu(H$_{-2}$L), (2) Cu(H$_{-1}$L)(Cys$^-$), (3) Cu(Cys$^-$)$_2$, and (4) Cu(I)Z. Condition same as under Fig. 1.

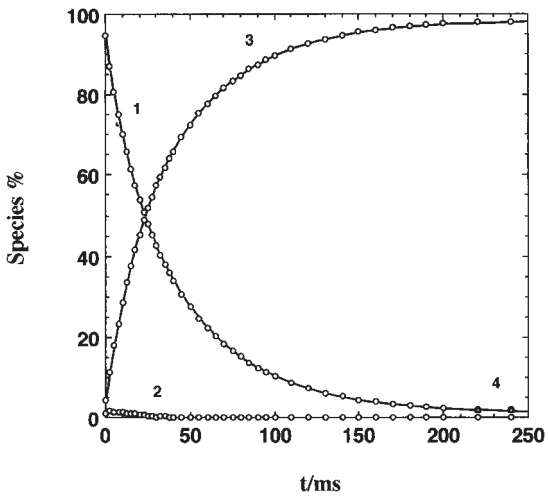


Fig. 4. Time-dependent distribution of the copper species in the reaction of Cu(H$_{-2}$$\beta$-Ala-GlyGly) with Cys at pH 9.3. (1) Cu(H$_{-2}$L), (2) Cu(H$_{-1}$L)(Cys$^-$), (3) Cu(Cys$^-$)$_2$, and (4) Cu(I)Z. Condition same as under Fig. 2.

AlaGly)(Cys$^-$) was significantly slow. On the other hand, the distribution curve for the Cu(H$_{-2}$$\beta$-AlaGlyGly)/CysH system indicated a sequence of the following reactions: Cu(H$_{-2}$L)$\rightarrow$ Cu(Cys$^-$)$_2$$\rightarrow$Cu(I)Z. Because the k_{2+} value was probably several orders of magnitude bigger than k_{1+}, the ternary complex upon forming spontaneously changed to Cu(Cys$^-$)$_2$. This is the reason why the Cu(H$_{-1}$$\beta$-AlaGlyGly)(Cys$^-$) species is not detected.

Kinetics of the Ligand-Exchange. The ligand-exchange of Cu(H$_{-2}$L) with Cys primarily consists of two sequential reactions, (1) and (2), as indicated by the species distribution curve.

$$\mathrm{Cu(H_{-2}L) + Cys^- \underset{k_{1-}}{\overset{k_{1+}}{\rightleftarrows}} Cu(H_{-1}L)(Cys^-) + H^+} \quad (1)$$

$$\mathrm{Cu(H_{-1}L)(Cys^-) + Cys^- + H^+ \underset{k_{2-}}{\overset{k_{2+}}{\rightleftarrows}} Cu(Cys^-)_2 + L.} \quad (2)$$

The first is the formation of Cu(H$_{-1}$L)(Cys$^-$) from Cu(H$_{-2}$L), and the second is the conversion of Cu(H$_{-1}$L)(Cys$^-$) to Cu(Cys$^-$)$_2$. This can also be certified kinetically in the reaction of Cu(H$_{-2}$Gly-β-AlaGly) with Cys. The rate plot consisted of a linear combination of two exponential functions, as $y = A_o + A_1 \exp(-k'_1 t) + A_2 \exp(-k'_2 t)$, where k'_1 and k'_2 denoted the rate constants for the first and second steps. The terms y and t are variables, and represent the absorbance, i.e., the concentration, and time, respectively. On the contrary, the rate plot for the Cu(H$_{-2}$$\beta$-AlaGly-Gly)/CysH system was linear as $y = A_o + A_1 \exp(-k't)$, apparently indicating a one-step reaction, i.e., $k_1 < k_2$. The rate plots for the Cu(H$_{-2}$Gly-β-AlaGly)/CysH and Cu(H$_{-2}$$\beta$-AlaGlyGly)/CysH reaction systems are shown in Figs. 5 and 6.

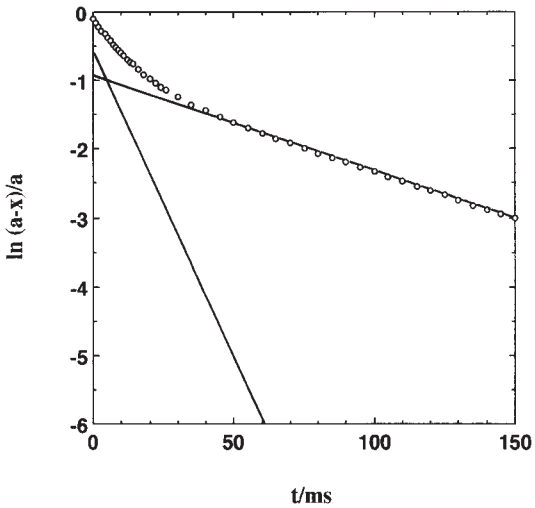


Fig. 5. First-order rate plot for the reactions of Cu(H$_{-2}$Gly-β-Ala-Gly) with Cys at pH 9.3. [Cu(II)] = 1.03×10^{-4} M, [CysH] = 2.93×10^{-3} M (28.4 equiv.).

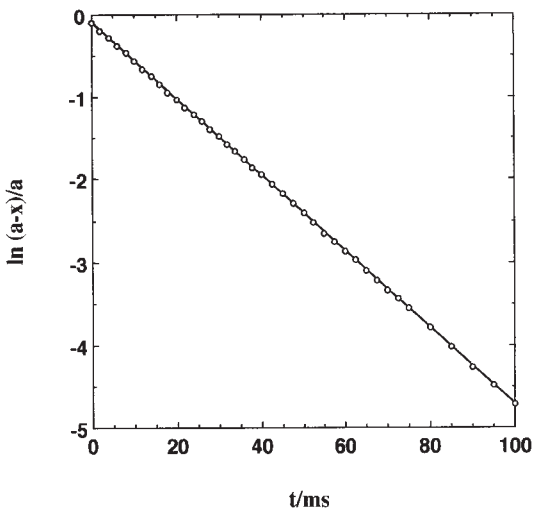


Fig. 6. First-order rate plot for the reactions of Cu(H$_{-2}$$\beta$-Ala-Gly-Gly) with Cys at pH 9.3. [Cu(II)] = 1.03×10^{-4} M, [CysH] = 2.93×10^{-3} M (28.4 equiv.).

Table 2. Rate and Equilibrium Constants for the Reaction of Cu(H$_{-i}$L) with Cys at pH 9.3

Cu(H$_{-i}$L)	k_{1+}/M^{-1} s^{-1}	log K_1/M^{-1}	k_{2+}/M^{-1} s^{-1}	log K_2/M^{-1}
Cu(H$_{-2}$GlyGlyGly)	$>5.0 \times 10^6$		3.78×10^3	3.04
Cu(H$_{-2}$GlyGly-β-Ala)	5.0×10^6		2.54×10^3	3.09
Cu(H$_{-2}$GlyGly-γ-Aba)	$>5.0 \times 10^6$		3.10×10^3	3.05
Cu(H$_{-2}$Gly-β-AlaGly)	3.03×10^4		3.72×10^3	3.07
Cu(H$_{-2}\beta$-AlaGlyGly)	1.66×10^4		$>5.0 \times 10^6$	
Cu(H$_{-2}$Gly-β-Ala-β-Ala)	8.04×10^5		3.04×10^3	3.01
Cu(H$_{-2}\beta$-AlaGly-β-Ala)	6.78×10^4		$>5.0 \times 10^6$	
Cu(H$_{-2}$Gly-β-Ala-γ-Aba)	$>5.0 \times 10^6$		3.05×10^3	3.01
Cu(H$_{-2}\beta$-AlaGly-γ-Aba)	$>5.0 \times 10^6$		$>5.0 \times 10^6$	
Cu(H$_{-2}$GlyGly-L-Ile)	1.20×10^5		2.16×10^3	2.86
Cu(H$_{-3}$penta-Gly)	1.25×10^4	3.21	2.45×10^3	3.22

In 0.01 M borate buffer ($I = 0.1$ M $NaClO_4$) at pH 9.3.

The rates of the Cu(H$_{-2}$L) and Cys reaction were primarily pH dependent. As the pH decreased, both the ligand-exchange and the reductions of the Cu(II) species, including Cu(H$_{-1}$L)(Cys$^-$) and Cu(Cys$^-$)$_2$, were remarkably enhanced. At a physiological pH, since the Cu(H$_{-1}$L)(Cys$^-$) was rapidly reduced instantly upon forming, reliable rate constants could not be determined.[6] Reliable k_{1+} and k_{2+} values could be obtained above pH 9, where the pH variations of the rate constants were small. The k_{1+} value was obtained from a rate plot at 335 nm, and the k_{2+} value was determined from a plot at 390 nm. The backward rate constants, k_{1-} and k_{2-}, could be determined from the intercept on the vertical axis at [CysH] $= 0$. Reliable k_{1-} values could hardly be obtained, because they were far smaller than k_{1+}. The equilibrium constant (K) could be obtained by the ratio of the forward-to-backward rate constants (k_+/k_-). The rate and equilibrium constants for the reaction are summarized in Table 2, where the constants for the reactions with Cu(H$_{-2}$GlyGly-L-Ile) and Cu(H$_{-3}$penta-Gly) are shown as references.

The formation of the Cu(H$_{-1}$L)(Cys$^-$) species from Cu(H$_{-2}$GlyGlyGly), Cu(H$_{-2}$GlyGly-β-Ala), and Cu(H$_{-2}$-GlyGly-γ-Aba) was very fast, so much so that k_{1+} was either hardly determined or not obtained by the conventional stopped-flow technique. In those cases, the k_{1+} was expressed as $k_{1+} > 5.0 \times 10^6$ M^{-1} s^{-1} in Table 2. Both Cu(H$_{-2}$Gly-β-AlaGly) and Cu(H$_{-2}\beta$-AlaGlyGly) were sluggish toward the ligand-exchange, and the k_{1+} values were two orders of magnitude smaller than Cu(H$_{-2}$GlyGlyGly). The Cu(H$_{-2}$L), involving two β-Ala residues, were also rather sluggish, and the k_{1+} for Cu(H$_{-2}\beta$-Ala-Gly-β-Ala) was a little smaller than that of Cu(H$_{-2}$Gly-β-Ala-β-Ala). Both Cu(H$_{-2}$Gly-β-Ala-γ-Aba) and Cu(H$_{-2}\beta$-AlaGly-γ-Aba) could rapidly form ternary complexes, so much so that k_{1+} could not be obtained, because the fourth-donors in those complexes were either loosely associated to Cu(II), as in Cu(H$_{-2}\beta$-AlaGly-γ-Aba), or free from Cu(II) in Cu(H$_{-2}$Gly-β-Ala-γ-Aba).[14] Those results suggests that the peptides composing of one β-Ala and two Gly residues can form the kinetically stable Cu(H$_{-2}$L) species, and that the Cu(H$_{-2}$L) species involving the β-α-amino-acid sequence at the amino terminus were kinetically more stable than those with the α–β sequence.

In a previous paper, we reported that the affinity of the Cu(II) for the fourth-donor atom in Cu(H$_{-i}$L) determined the rate of the Cu(H$_{-1}$L)(Cys$^-$) formation, based on the evidence that Cu(H$_{-2}$penta-Gly), in which the exchangeable carbonyl oxygen occupied the fourth site, suffered the rapid ligand-exchange ($k_+ > \sim 5 \times 10^6$ M^{-1} s^{-1}), while the Cu(H$_{-3}$penta-Gly), in which the less exchangeable deprotonized-amide nitrogen occupied the fourth-site, was sluggish toward ligand-exchange ($k_+ = 1.25 \times 10^4$ M^{-1} s^{-1}).[2,5] Cu(H$_{-2}$Gly-β-Ala-Gly) and Cu(H$_{-2}\beta$-Ala-GlyGly), though the carboxylate oxygen occupied at the fourth site, were sluggish toward ligand-exchange as Cu(H$_{-3}$penta-Gly). The stability of the whole structure of a fused-chelate may be important in deciding the k_{1+} values.

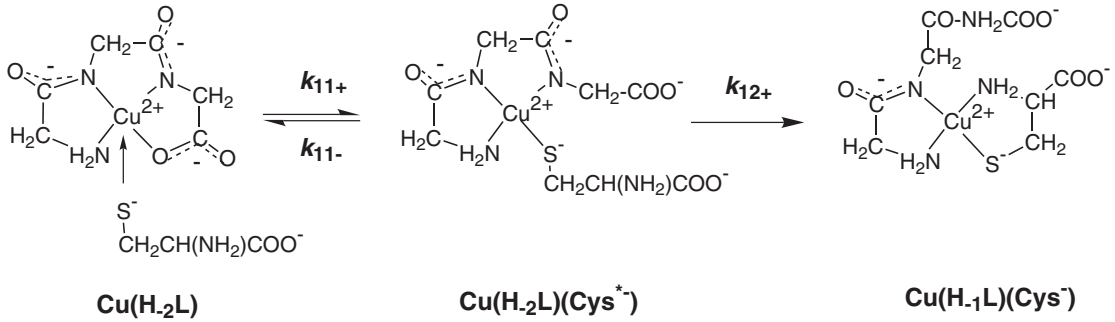
The formation of Cu(H$_{-1}$L)(Cys$^-$) is postulated to be composed of a series of two ligand-replacement reactions, (1-1) and (1-2) (Scheme 2).[15] Kinetically stable complexes, including Cu(H$_{-2}$Gly-β-Ala-Gly), Cu(H$_{-2}\beta$-Ala-GlyGly), and Cu(H$_{-3}$penta-Gly), showed very small formation constants (K_{11}) for Cu(H$_{-2}$L)(Cys^{*-}),[15]

$$\mathrm{Cu(H_{-2}L) + Cys^- \underset{k_{11-}}{\overset{k_{11+}}{\rightleftarrows}} Cu(H_{-2}L)(Cys^{*-}),} \quad (1\text{-}1)$$

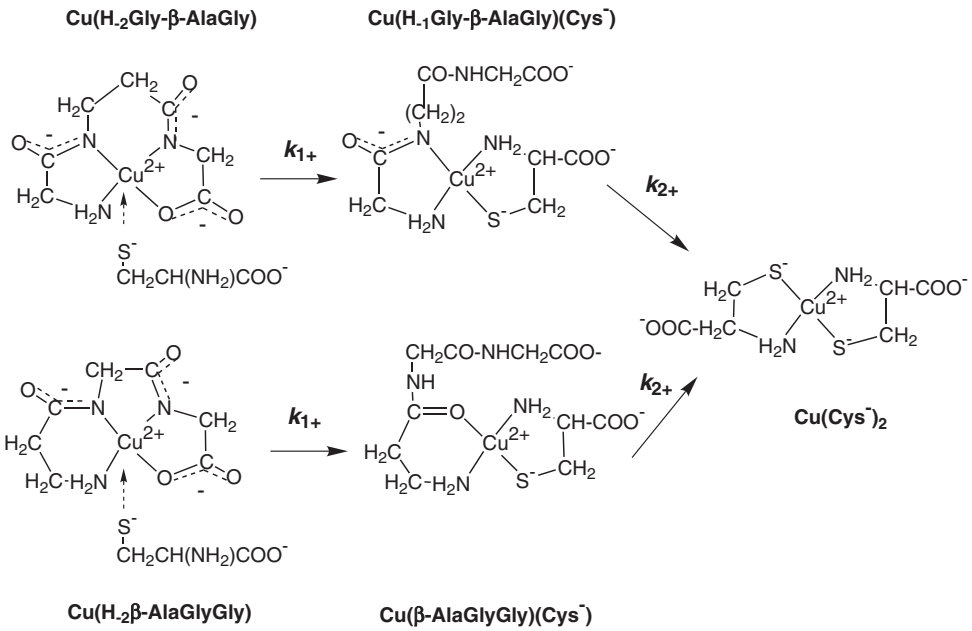
$$\mathrm{Cu(H_{-2}L)(Cys^{*-}) + H^+ \overset{k_{12+}}{\rightarrow} Cu(H_{-1}L)(Cys^-).} \quad (1\text{-}2)$$

Then, the Cu(H$_{-2}$L)(Cys^{*-}) easily dissociates back to Cu(H$_{-2}$L). The k_{12+} would not vary widely, because Cu(H$_{-2}$L)(Cys^{*-}) species have similar coordination modes in each reaction system. Provided that k_{12+} in reaction (1-2) is on the magnitude of 10^6 M^{-1} s^{-1}, the rate constants k_{1+} for Cu(H$_{-2}$Gly-β-Ala-Gly), Cu(H$_{-2}\beta$-Ala-GlyGly), and Cu(H$_{-3}$penta-Gly) are assessed to be 10^4–10^5 M^{-1} s^{-1}. Since the k_{1+} values for other Cu(H$_{-2}$L) species were on the magnitude of 10^6 M^{-1} s^{-1}or bigger, K_{11} would be bigger than 10^2 M-1. A detailed account of the mechanism will be presented in the next paper.

A bulky side chain in the chelate ring **C** hindered the formation of the Cu(H$_{-1}$L)(Cys$^-$) species. The k_{1+} value (1.2×10^5 M^{-1} s^{-1}) for Cu(H$_{-2}$GlyGlyIle) was at least two orders of magnitude smaller than that of Cu(H$_{-2}$GlyGlyGly), and approximately ten folds bigger than those for Cu(H$_{-2}$Gly-β-Ala-Gly) and Cu(H$_{-2}\beta$-Ala-GlyGly). Those results reveal that the whole structure of a fused-chelate ring, rather than the side chain, does contribute to the stabilization of the Cu(H$_{-2}$L) species.



Scheme 2. Proposed pathway for the formation of Cu(H$_{-1}$L)(Cys$^-$) from Cu(H$_{-2}$L) and Cys.



Scheme 3. Pathways from Cu(H$_{-2}$Gly-β-AlaGly) and Cu(H$_{-2}\beta$-AlaGlyGly) to Cu(Cys$^-$)$_2$.

Since the ternary Cu(H$_{-1}$L)(Cys$^-$) complex, having a 5-membered ring **A**, was rather stable ($k_{2+} = 2.5$–3.5×10^3 M^{-1} s^{-1}) so that it could be detected by stopped-flow spectrometries. On the contrary, the Cu(H$_{-1}$L)(Cys$^-$) complex, with a 6-membered ring **A**, was labile ($k_{2+} > 5.0 \times 10^6$ M^{-1} s^{-1}), so that it could not be detected by stopped-flow spectrometries.

Conclusion

The kinetic stability of the whole structure of a fused-chelate ring in Cu(H$_{-2}$L) was evaluated based on the k_{1+} value for the formation of Cu(H$_{-1}$L)(Cys$^-$). The Cu(H$_{-2}$L) species, having 5–6–5- and 6–5–5-membered fused-chelate rings, were rather stable. The k_{1+} values for those complexes were 2–3 $\times$ 10^4 M^{-1} s^{-1}. The k_{1+} values for other Cu(H$_{-2}$L) species were bigger than 10^5 M^{-1} s^{-1}. Because the Cu(H$_{-1}$L)(Cys$^-$) species, involving a 6-membered chelate ring **A**, were labile, Cu(H$_{-1}$L)(Cys$^-$) could not be detected.

Though Cu(H$_{-2}$L) having 5–6–5- and 6–5–5-membered chelate rings are kinetically stable, the features of metal delivery are quite different from each other (Scheme 3). The carboxylate O$^-$ in both complexes resisted being replaced by the thiolate S$^-$, and thereby the formation of the Cu(H$_{-1}$L)(Cys$^-$) species was sluggish. The ternary complex from Cu(H$_{-2}$Gly-β-AlaGly) was fairly stable, while Cu(H$_{-2}\beta$-AlaGlyGly)(Cys$^-$) was promptly transformed to Cu(Cys$^-$)$_2$.

The fates of Cu(H$_{-2}$Gly-β-AlaGly) and Cu(H$_{-2}\beta$-AlaGlyGly) in biological fluids are considered to depend on the whole structure of the fused-chelate ring in the Cu(H$_{-2}$L) species. The Cu(H$_{-2}$L) complexes with the 5-membered ring **A** could form ternary complexes with some chelators of biological origin, while the Cu(II) in the Cu(H$_{-2}$L) complexes involving the 6-membered ring **A** would be rapidly delivered without forming the ternary complexes.

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