

Comparative Studies on the Stability of a Chelate-Ring Unit in Dynamic Aspects. Chelate-Rings of the Cu(II) Complexes Composed of [*N*-(Glycyl)], [*N*-(β-Alanyl)], and [*N*-(2-Aminoethyl)] Moieties

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Received July 11, 2003

The kinetic stability of a chelate-ring unit in Cu(H_{-1}GlyGly), Cu(H_{-1}β-AlaGly), and Cu(EDMA) complexes has been inspected by stopped-flow spectroscopy. Those three Cu(II) complexes reacted with cysteine (CysH) or penicillamine (PesH) to form primarily ternary complexes, formulated as Cu(H_{-1}L)(Rs^-); L = GlyGly and β-AlaGly, and Cu(EDMA)(Rs^-); where Rs^- represents the aminothiolates. The chelate-ring units composed of [*N*-(Gly)]-, [*N*-(β-Ala)]-, and [*N*-(2-aminoethyl)]-moieties in Cu(H_{-1}GlyGly), Cu(H_{-1}β-AlaGly), and Cu(EDMA) were conserved, respectively, in the corresponding ternary complexes. The rate of the ternary complex formation was very rapid, so much so that the rate constants, k_{1+}, could not be determined by the conventional stopped-flow techniques. The ternary complexes upon forming reacted with the RsH to produce the binary Cu(Rs^-)$_2$ species. Thus, the observables were limited to the rate constants, k_{2+}, for the transformation from Cu(H_{-1}L)(Rs^-) to Cu(Rs^-)$_2$. The kinetic stabilities of those chelate-ring units were compared based on the rate constant, k_{2+}, and equilibrium constant, K_2. The stability was arranged as follows: [*N*-(Gly)]- > [*N*-(2-aminoethyl)]- > [*N*-(β-Ala)]-unit. Though Cu(EDMA) was kinetically stable, the Cu(II) in Cu(EDMA)(Rs^-) was rapidly delivered by a nucleophilic attack of RsH to form Cu(Rs^-)$_2$. On the contrary, Cu(H_{-1}-GlyGly) was kinetically less stable than Cu(EDMA), but delivery of the Cu(II) from Cu(H_{-1}L)(Rs^-), by the proton-assisted mechanism, was comparatively slow.

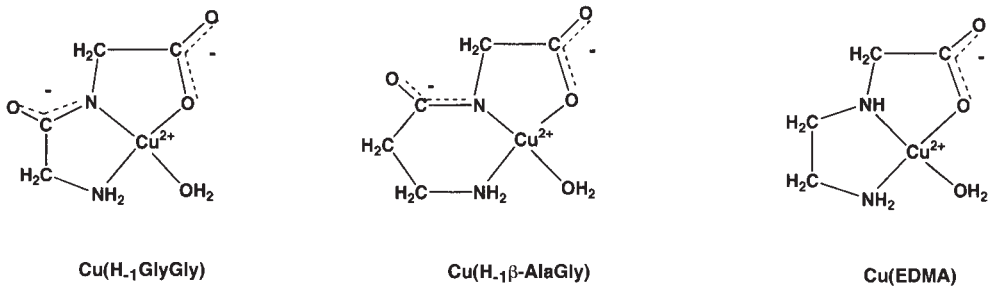
Vast numbers of chelating agents, which play a unique role in chemical and biochemical processes, have been studied in biomedical science for mobilizing toxic trace-metal ions.[1–3] However, chelating agents are no longer used only in medical practice to remove toxic metals from the body.[4,5] Some of their new uses are found in non-invasive diagnostic medicine, where they are used to carry γ-emitting or paramagnetic cations to specific parts of the body, and to manipulate of nucleic acids, a process which facilitates fragmentation into a designated sequence of nucleotides. Chelating agents might also be used as antimetabolites in the chemotherapy of viruses, microorganisms, and cancer.[6] Of basic interest is understanding the interaction of metal ions with drugs and proteins.[7] One of those purposes may be concerned with the uptake and delivery of the metal ion and metal-chelates from and to specific parts of the body. This is closely related to the kinetic stability, that is the stability in dynamic aspects, of the complexes. The kinetic stability is related to the exchangeability of the metal ion within the complexes and the metal-ion transport between the metal complexes and metal-receptors.

Synthetic chelating agents, which have been used worldwide for therapeutic purposes, are polyaminopolycarboxylic acids, such as ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA). The basic form of those chelating agents is *N*-(2-aminoethyl)glycine, generally named ethylenediaminemonoacetic acid (EDMA), a tridentate ligand with N,N,O^- donors. It can form a stable complex CuL, ($\log K_1$ 13.47), in which two parts of a five-membered chelate ring are fused.[8] Here, unless otherwise noted, the copper in the complex indicates Cu(II). The coordinated H_2O of CuL is easily hydrolyzed to yield the [CuL(OH)] species (pK_{OH} 4.77) without fundamentally changing the fused-chelate structure (Scheme 1).[9]

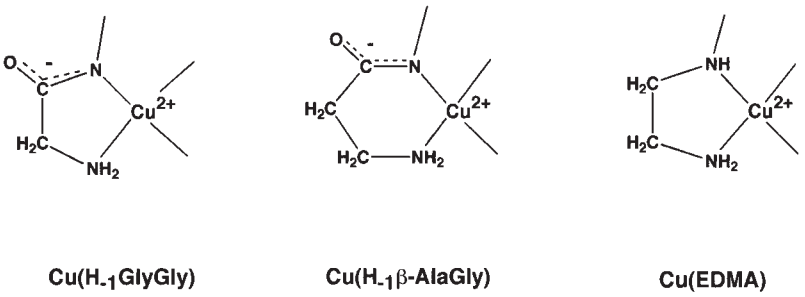
Glycylglycine (GlyGly), which is a naturally occurring peptide with a similar skeleton to that of EDMA, can form a Cu(II) complex (CuL) with a 5-membered fused chelate-ring through the terminal amino-nitrogen and the carbonyl oxygen of amide group ($\log K_1$ 5.26).[10,11] The amide group, –CO–NH–, coordinated to Cu(II) via oxygen, tends to undergo deprotonation to –CO–N^-–. Since the coordinated amide group is deprotonated at pH 4–5 (pK_c 4.31), the second donor changes from the carbonyl oxygen to the deprotonated amide nitrogen.[10,11] Thus newly formed complex is stable with the 5-5 membered fused chelate-ring, and is formulated as Cu(H_{-1}L) (Scheme 1). A similar observation was made for the β-AlaGly complex ($\log K_1$ 5.61, pK_c 4.29). The Cu(H_{-1}L) has a 6-5 membered fused chelate structure (Scheme 1).[12] In contrast, glycylsarcosine, which does not have an ionizable hydrogen in the amide group, can form only a CuL complex ($\log K_1$ 6.28), but not a Cu(H_{-1}L) species.[13]

The coordination modes of Cu(H_{-1}L) and Cu(EDMA) are different from each other. EDMA donates two amino nitrogens to the Cu(II), while GlyGly and β-AlaGly donate two nitrogens

Published on the web April 2, 2004; DOI 10.1246/bcsj.77.699



Scheme 1. Coordination structures of Cu(H_{-1}GlGly), Cu($H_{-1}\beta$-AlaGly), and Cu(EDMA).



Scheme 2. Structures of the chelate-ring units in Cu(H_{-1}GlGly), Cu($H_{-1}\beta$-AlaGly), and Cu(EDMA).

from the amino and deprotonated amide groups. The Cu(II) complex of EDMA is thermodynamically more stable than Cu(H_{-1}GlyGly) and Cu($H_{-1}\beta$-AlaGly), but its stability in dynamic aspects is still unknown.

We attempted in the present work to evaluate and compare the exchangeability of the Cu(II) chelated to naturally occurring (peptides) and synthetic (polyaminocarboxylic acid) ligands with aminothiols (RsH), such as cysteine (CysH) and penicillamine (PesH). The Cu(II) complexes react with the RsH at unmeasureably rapid rates, forming ternary complexes, formulated as Cu(H_{-1}GlyGly)(Rs^-), Cu($H_{-1}\beta$-AlaGly)(Rs^-), and Cu(EDMA)(Rs^-), so much so that the rate constant, k_{1+}, could not be determined by the conventional stopped-flow kinetic techniques. Those ternary complexes subsequently react with another RsH molecule to form a binary complex, $Cu(Rs^-)_2$. A series of ligand-exchange reactions for Cu(H_{-1}L) can be briefly expressed as

$$\mathrm{Cu(H_{-1}L)} + \mathrm{RsH} \underset{k_{1-}}{\overset{k_{1+}}{\rightleftarrows}} \mathrm{Cu(H_{-1}L)(Rs^-)} + \mathrm{H^+}$$

$$\mathrm{Cu(H_{-1}L)(Rs^-)} + \mathrm{RsH} \underset{k_{2-}}{\overset{k_{2+}}{\rightleftarrows}} \mathrm{Cu(Rs^-)_2} + \mathrm{HL},$$

where L represents GlyGly or β-AlaGly. The observable rate constants in those reaction systems are limited to k_{2+} and k_{2-} for the reaction of Cu(H_{-1}L)(Rs^-) with RsH. In ternary complexes, one of the 5-5 or 6-5 membered fused-chelate rings is opened and the remained chelate-rings, constructed by [*N*-(Gly)]-, [*N*-(β-Ala)]-, or [*N*-(2-aminoethyl)]-moieties, remain unchanged (Scheme 2). A comparative study on the kinetic stability of those chelate-ring units is considered to offer an important problem in designing the chelating agent for biomedical uses. The kinetic stability of those three kinds of single chelate-ring units was evaluated based on the forward- and backward-rate constants, k_{2+} and k_{2-}, and the equilibrium constant, K_2.

Experimental

Materials. GlyGly and β-AlaGly were products from BACHEM Feinchemikalien AG. (Switzerland). They were pure, as checked by chromatography, and used without further purification. EDMA was obtained from Tokyo Kasei Co. (Tokyo). Copper(II) perchlorate, $Cu(ClO_4)_2 \cdot 6H_2O$, was obtained from G. Frederick Smith Chem. Co. (Columbus, Oh), and used after recrystallization from hot water. L-Cysteine (CysH) and D-penicillamine (PesH) were products from Sigma Chemical Co. (St. Louis, Mo). All other chemicals were of reagent grade and used without further purification.

Preparation of Stock Solutions. A stock solution of Cu(II) was prepared by dissolving $Cu(ClO_4)_2 \cdot 6H_2O$ in purified water, which was deionized once and doubly distilled from all glass apparatus; the first distillation was from alkaline permanganate. The solution was standardized by titration with standardized EDTA using merexide as an indicator.[14] Solutions of Cu(H_{-1}GlyGly), Cu($H_{-1}\beta$-AlaGly), and Cu(EDMA) were freshly prepared using aliquots of the Cu(II) solution with the corresponding ligand. A 3 mol% excess peptide or 10% excess EDMA was used to ensure complex formation. Solutions of the CysH and PesH were freshly prepared prior to spectroscopic measurements. The ionic strength, I, was maintained at 0.1 M (1 M = 1 mol dm^{-3}) with $NaClO_4$ for spectrophotometric measurements and at 0.5 M with $NaNO_3$ for ESR measurements.

Spectrophotometric Measurement. Solutions of 1.01×10^{-3} M Cu(II) complexes and of four equivalents CysH were equilibrated at 25 °C under nitrogen before measurements. After equilibration for 20 min, the reaction was initiated by mixing both solutions at 8 kg/cm^2 under nitrogen. Single-wavelength measurements of absorbance against time were carried out at intervals of either 5 nm or 10 nm from 255 nm to 750 nm, and analyzed on a computerized Union RA-401 stopped-flow spectrophotometer equipped with a 5 mm quartz cell. At least seven runs were repeated and averaged. The absorption spectrum at a certain time was obtained

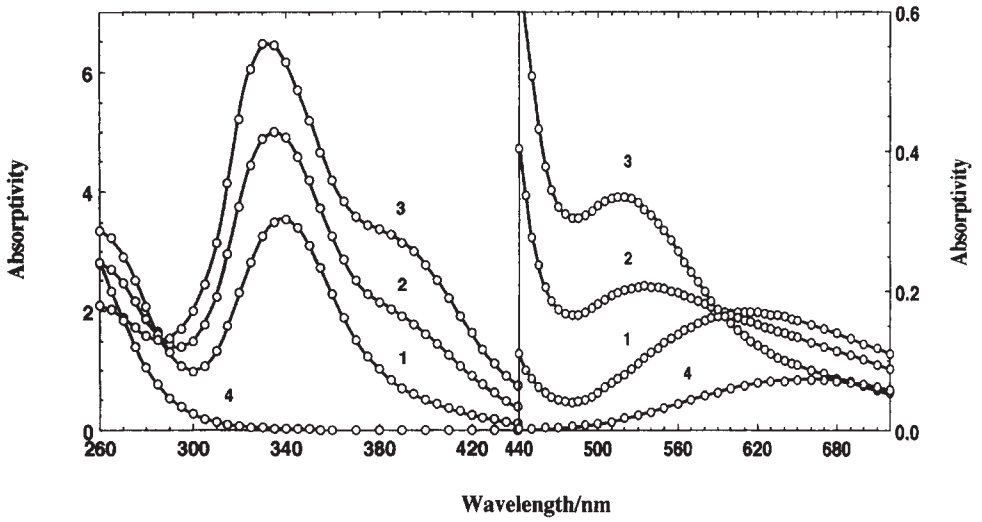


Fig. 1. Time-dependent spectral changes in the reaction of Cu(EDMA) with CysH at pH 9.3. [Cu(II)] = 5.12×10^{-4} M, [CysH] = 1.97×10^{-3} M, $I = 0.1$ M $NaClO_4$. (1), 0.3 ms, (2), 5 ms, (3), 50 ms, and (4), Cu(EDMA).

by a point-by-point method.[15]

The spectrometer was checked before measurements by a previously reported procedure.[15] The dead time of the instrument, determined by the reaction of ascorbate with 2,4-dichlorophenolindophenol, was 1.2 ms.[16]

Species Distribution. Time course of the reaction was monitored at three different wavelengths (265 nm, 330 nm, and 390 nm) for the Cu(H_{-1}GlyGly)/CysH reaction, and at 265 nm, 335 nm, and 390 nm for the Cu(EDMA)/CysH reaction. The concentrations of the ternary and binary complex during the reaction were calculated by a previously reported method.[15]

ESR Measurement. ESR measurements were conducted at room temperature on a JEOL JES-RE 1X spectrometer with 100 kHz field modulation. The spectrum of the transient was measured by a continuous-flow method using a JES-SM-1 mixing apparatus equipped with a M07-K04 sample mixer and a JES-LC-01 flow cell. The concentration of Cu(II) was 3.0×10^{-3} M in 0.05 M phosphate buffer ($I = 0.5$ M $NaNO_3$) at pH 7.8. The g value and the hyperfine constant of the spectrum were calculated by comparisons with a standard Mn(II) doped in MgO_2. The minimum dead time was 4.5 ms at a flow rate of 5 mL/s.

Stopped-Flow Kinetic Measurements. The reaction was conducted at 25 °C under pseudo first-ordered conditions using a large excess of thiolate. A Cu(II) complex solution at 1.11×10^{-4} M was mixed rapidly with CysH or PesH solutions, and subsequent absorbance changes at 390 nm were recorded. The pseudo first-ordered rate constant was obtained over the range from pH 6.5 to pH 11.0 at $I = 0.1$ M $NaClO_4$. A plot of the observed rate constant against the concentration of RsH, [RsH], gave a straight line, indicating that the reaction was first-order to both the Cu(II) complexes and the thiolate. The forward rate constant (k_+) and backward rate constant (k_-) were determined from the slope and the intercept on the ordinate, respectively.[17,18]

Results

Absorption Spectrum of the Transient; Cu(EDMA)-(Cys^-). Upon mixing with CysH, Cu(EDMA) instantly afforded red-brown transients, which disappeared thereafter. The absorption spectra of the transients observed at 0.3 ms, 5 ms, and 50 ms at pH 9.3 are shown in Fig. 1. The Cu(EDMA) had been rapidly and completely transformed to a transient, which exhibited two characteristic absorption bands. The absorption at 340 nm was assignable to the S → Cu(II) charge transfer band. The weak absorption around 610 nm is assignable to the d–d transition band of Cu(II). A stopped-flow molar-ratio plot at 0.3 ms revealed the formation of a ternary complex, Cu(EDMA)(Cys^-), as shown in Fig. 2. In the subsequent stage, the Cu(EDMA)(Cys^-) reacted with another CysH, forming Cu(Cys^-)$_2$, which exhibited a new absorption band at around 390 nm, in addition to the band at 333 nm.

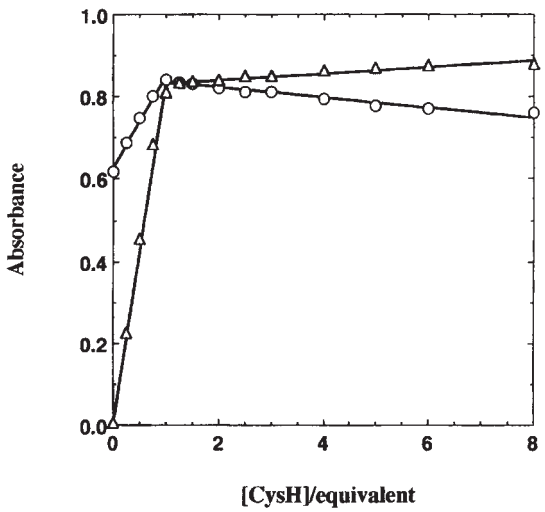


Fig. 2. Stopped-flow molar ratio plot for the first transient produced in the reaction of Cu(EDMA) with CysH at pH 9.3 ($t = 0.3$ ms); △, 265 nm, ○, 335 nm. [Cu(II)] = 2.60×10^{-4} M.

The same was observed in the reaction of Cu(H_{-1}GlyGly) with CysH.[15] The reaction of Cu($H_{-1}\beta$-AlaGly) with CysH afforded Cu(Cys^-)$_2$, but not the Cu($H_{-1}\beta$-AlaGly)(Cys^-) species, at the beginning of the observation. The Cu($H_{-1}\beta$-AlaGly)(Cys^-) upon forming would change to Cu(Cys^-)$_2$ at an unmeasurably rapid rate.

Time-Dependent Distribution of Copper Species in the

Reactions of CysH with Cu(EDMA) and Cu(H_{-1}GlyGly). The reactions of CysH with Cu(EDMA) and with Cu(H_{-1}GlyGly) were very rapid. More than 90% of the total Cu(II) had been transported to the ternary complexes at the beginning of the observation, i.e., 1.2 ms. The time-dependent distribution of $Cu(Cys^-)_2$ in the CysH reactions with Cu(EDMA) and Cu(H_{-1}GlyGly) are shown in Fig. 3, which suggest that the Cu(EDMA)(Cys^-) are labile to be almost quantitatively converted to $Cu(Cys^-)_2$ within 50 ms. The Cu(II) complexed via the amino and deprotonated-amide groups, as observed in Cu(H_{-1}GlyGly), was kinetically stable. The Cu(II) in Cu(EDMA)(Cys^-) was sensitive toward the oxido-reduction and rapidly reduced after 50 ms.

ESR Spectrum of the Transients. As noted above, Cu(EDMA)(Cys^-) was labile, changing rapidly to $Cu(Cys^-)_2$. The ESR spectrum at room temperature may be assignable to mixtures of Cu(EDMA)(Cys^-) and $Cu(Cys^-)_2$ under the experimental conditions: $[Cu(II)] = 3.0 \times 10^{-3}$ M and $[CysH] = 6.0 \times 10^{-3}$ M at pH 8.0. The spectra, recorded at 4.5 ms and 5.5 ms, along with $Cu(Cys^-)_2$, are shown in Fig. 4. It is shown that most of the Cu(EDMA)(Cys^-) changed to $Cu(Cys^-)_2$, as indicated by the species distribution curve. The signals indicated by arrows are assignable to the ternary complex. The ESR parameters, g_{av} and A_{av}, are given in Table 1.

Kinetics of the Ligand-Exchange. The rate constants, which could be determined, were limited to k_{2+} and k_{2-} for the reaction between the ternary complexes and CysH. The forward rate constant (k_{2+}) and backward rate constant (k_{2-}) were determined from the slope of the straight line and the intercept on the ordinate, respectively.

The rate constants k_{2+} and k_{2-} of ligand-exchange with CysH and PesH were obtained over the pH range from 6.5 to 11.0 by the stopped-flow spectrophotometric methods. In the reactions with CysH, the ternary complexes, $Cu(Cys^-)_2$, or both were likely to be rapidly reduced at lower pH, so that the rate constants k_{2+} could not be determined below pH 8. The rate constant k_{2+} and equilibrium constant K_2, i.e., (k_{2+}/k_{2-}), in the reactions with CysH and PesH are shown in Table 2. The k_{2+} values for the PesH reaction were one order of magnitude smaller than those in the CysH reaction. Probably, the bulky $-C(CH_3)_2$ group in ternary complexes hinders the attack of thiolate sulfur on the Cu(II) (Scheme 3). The k_{2+} values in the Cu(EDMA) reaction were one order of magnitude bigger than those for the Cu(H_{-1}GlyGly) reaction. The Cu($H_{-1}\beta$-AlaGly)(Pes^-) rapidly changed, within the dead time, to $Cu(Pes^-)_2$, so that the k_{2+} value could be determined

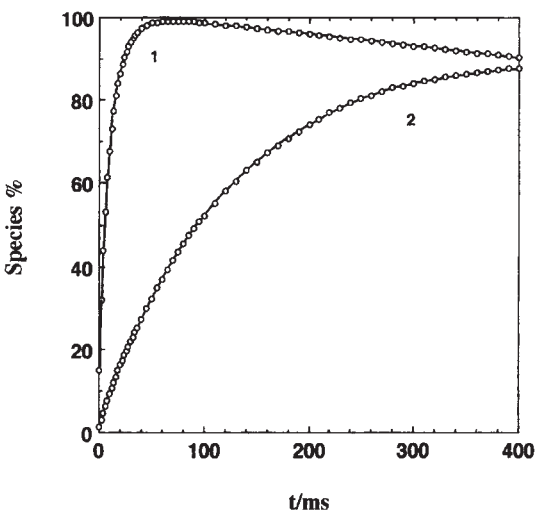


Fig. 3. Time-dependent distribution of $Cu(Cys^-)_2$ in the reaction of CysH with (1), Cu(EDMA) and (2), Cu(H_{-1}GlyGly) at pH 9.3. $[Cu(II)] = 5.12 \times 10^{-4}$ M, $[CysH] = 1.97 \times 10^{-3}$ M.

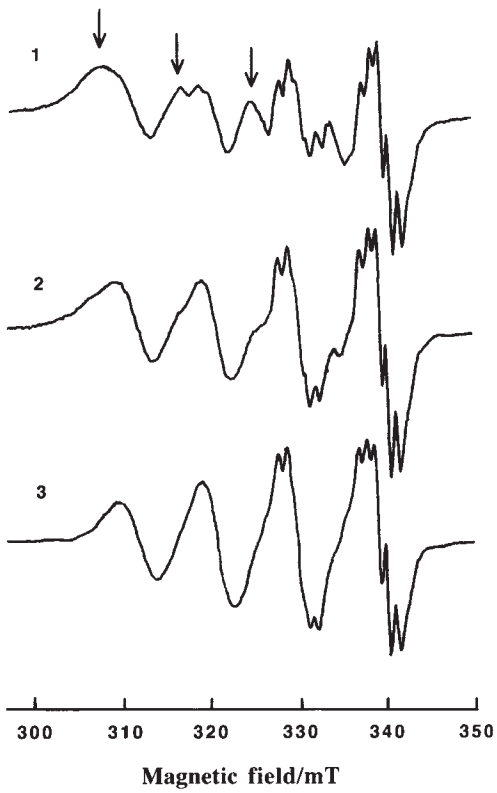


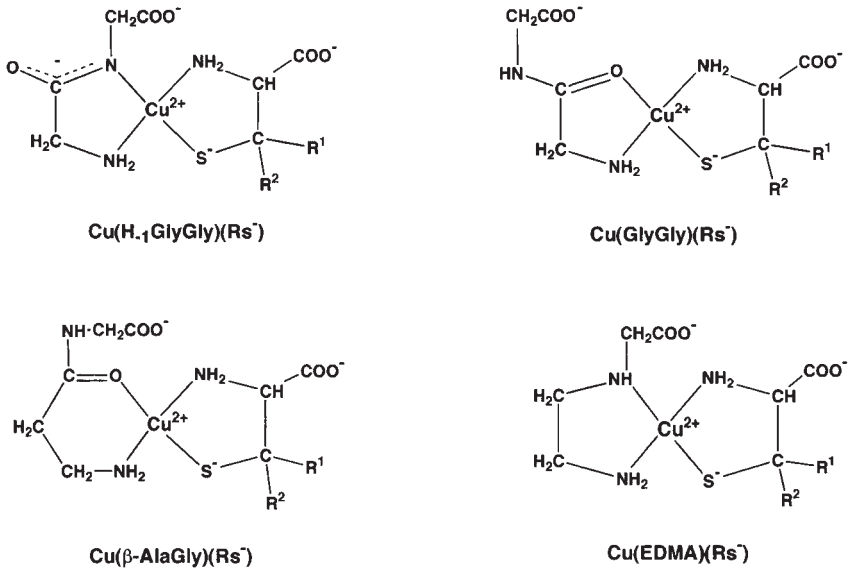
Fig. 4. ESR spectra at room temperature of transients in the reaction of Cu(EDMA) with CysH at pH 8.0. $[Cu(II)] = 3.0 \times 10^{-3}$ M, $[CysH] = 6.0 \times 10^{-3}$ M, in 0.05 M borate buffer ($I = 0.5$ M $NaNO_3$), (1), $t = 4.5$ ms, (2), $t = 5.5$ ms, and (3), $Cu(II)(Cys^-)_2$.

Table 1. Spectral Parameters of the Ternary and Binary Complexes

Complex	Absorpion spectrum λ_{max}/nm (ε/M^{-1} cm^{-1})			ESR	
	$\sigma(S) \rightarrow Cu(II)$	$\pi(S) \rightarrow Cu(II)$	d–d	g_{av}	$A_{av}/10^{-4}$ cm^{-1}
Cu(H_{-1}GlyGly)(Cys^-)	332 (4250)	406 (193)	542 (162)	2.084	79.6
Cu(EDMA)(Cys^-)	339 (4340)	395 (397)	614 (213)	2.083	78.9
$Cu(Cys^-)_2$	333 (6170)	390 (2760)	525 (350)	2.073	87.6

Table 2. Forward Rate Constant k_{2+} and Equilibrium Constant K_2 in the Ligand-Exchange Reaction at 25 °C

Complex	Cysteine		Penicillamine		pH
	$k_{2+}/M^{-1}\,s^{-1}$	$\log K_2/M^{-1}$	$k_{2+}/M^{-1}\,s^{-1}$	$\log K_2/M^{-1}$	
Cu(EDMA)			6.54×10^3	2.93	7.0
	3.59×10^4	3.71	5.16×10^3	3.64	8.0
	6.56×10^4	>4.5	5.54×10^3	3.98	9.0
	2.75×10^5	>5.0	2.09×10^4	3.39	10.0
Cu(H_{-1}GlyGly)			4.84×10^2	1.74	7.0
	3.55×10^3	2.86	3.70×10^2	1.99	8.0
	3.43×10^3	3.14	2.75×10^2	1.92	9.0
	2.95×10^3	3.15	2.29×10^2	1.86	10.0
Cu($H_{-1}\beta$-AlaGly)			1.30×10^5	>5.0	6.5
			4.22×10^5	>5.0	7.0



Scheme 3. Coordination structure of the ternary complexes, Cu(H_{-1}GlyGly)(Rs^-), Cu(GlyGly)(Rs^-), Cu(β-AlaGly)(Rs^-), and Cu(EDMA)(Rs^-). RsH; CysH: R^1, R^2 = H, PesH: $R^1 = CH_3$, $R^2 = CH_3$.

above pH 7.5.

The rate constants, k_{2+} and k_{2-}, and equilibrium constant, K_2, depended on the pH. The pH dependences of the k_{2+} and k_{2-} values in the Cu(H_{-1}GlyGly)/PesH and Cu(EDMA)/PesH reactions are shown in Figs. 5, 6, and 7. The k_{2+} value in the Cu(H_{-1}GlyGly) reaction tended to decrease as the pH was raised. On the contrary, the k_{2+} in the Cu(EDMA) reaction increased slowly with decreasing the pH values below 8.5, while it increased steeply as the pH was raised over pH 8.5, probably because of increasing concentrations of the nucleophile Pes^-. Significant variations in k_{2-} were not observed in both reaction systems in the region above pH 8.5: $(2.52 \pm 0.26)\ s^{-1}$ for the Cu(H_{-1}GlyGly)/CysH reaction, $(3.23 \pm 0.13)\ s^{-1}$ for the Cu(H_{-1}GlyGly)/PesH reaction, and $(0.71 \pm 0.09)\ s^{-1}$ for the Cu(EDMA)/PesH reaction, while it considerably increased with decreasing pH values below pH 8.0, as shown in Fig. 7.

The pH dependence of the equilibrium constant, K_2, which is defined as the ratio of k_{2+}/k_{2-}, is shown in Fig. 8. The K_2 values for each reaction system were arranged as follow: Cu(EDMA)(Pes^-)/(Pes^-) > Cu(H_{-1}GlyGly)(Cys^-)/(Cys^-) > Cu(H_{-1}GlyGly)(Pes^-)/(Pes^-). As the pH decreased below pH 8.0, the K_2 values tended to decrease in all of the systems. The ternary complexes were likely to become thermodynamically unstable as the pH decreased, so that they were easily dissociated back to the parent complexes; i.e., Cu(H_{-1}GlyGly) or Cu(EDMA). The K_2 values for the Cu(H_{-1}GlyGly)(Pes^-)/PesH reaction system did not change above pH 8.0, while the Cu(EDMA)(Pes^-)/PesH system increased steeply over pH 9.0, probably because of a steep increase in the k_{2+} values.

Discussion

Absorption Spectrum of the Ternary Complexes. Cu(EDMA)(Rs^-), upon forming, rapidly reacted with RsH to be transformed to Cu(Rs^-)$_2$. Accordingly, in the Cu(EDMA)/RsH reaction system, Cu(EDMA)(Rs^-) and Cu(Rs^-)$_2$ coexisted at the beginning of the observation. Then, the absorption spectrum of Cu(EDMA)(Rs^-) was calculated by subtracting the contribution of the spectrum due to Cu(Rs^-)$_2$ from the observed value. A detailed procedure has been described in a previous paper.[15] The parameters of the absorption and ESR spec-

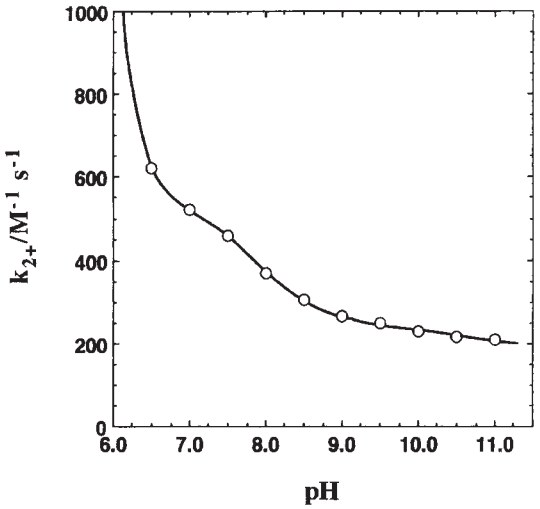


Fig. 5. pH dependence of k_{2+} in the reaction of Cu(H_{-1}GlyGly) with PesH; ○, Observed, full line, Simulated.

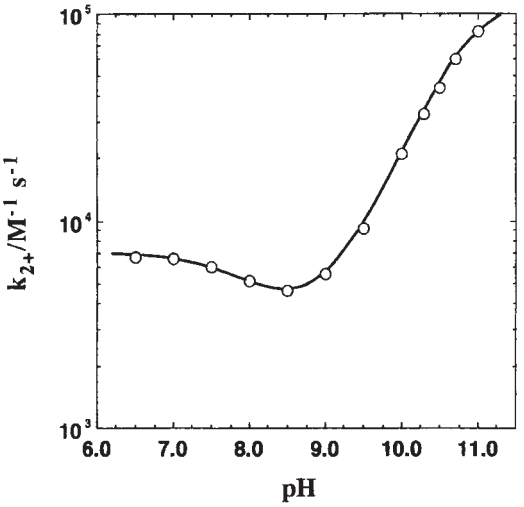


Fig. 6. pH dependence of k_{2+} in the reaction of Cu(EDMA) with PesH; ○, Observed, full line, Simulated.

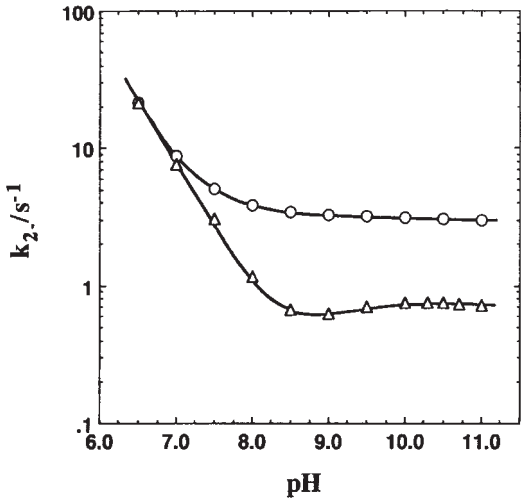


Fig. 7. pH dependence of k_{2-} in the reactions of PesH with Cu(H_{-1}GlyGly) and Cu(EDMA); ○, Cu(H_{-1}GlyGly), △, Cu(EDMA).

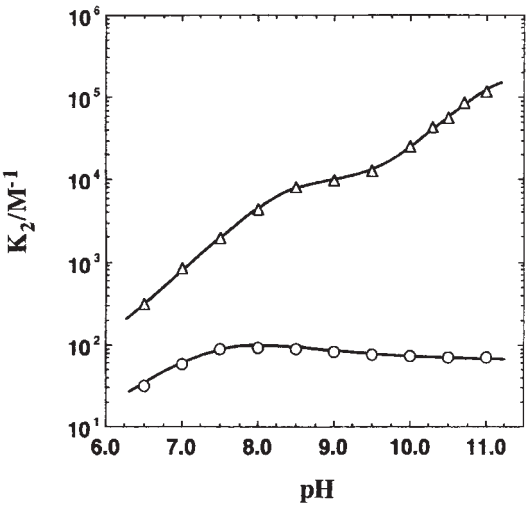


Fig. 8. pH dependence of K_2 in the reactions of PesH with Cu(H_{-1}GlyGly) and Cu(EDMA); ○, Cu(H_{-1}GlyGly), △, Cu(EDMA).

tra of Cu(H_{-1}GlyGly)(Cys^-) and Cu(EDMA)(Cys^-) are given in Table 1. The absorption spectrum was resolved by a previously reported method.[20] All of the spectrum exhibited two LMCT absorptions: $\sigma(S) \rightarrow Cu(II)$ and $\pi(S) \rightarrow Cu(II)$, and a d–d band of Cu(II). Both of the $\sigma(S) \rightarrow Cu(II)$ CT and d–d transition bands in Cu(EDMA)(Cys^-) shifted to a lower energy as Cu(H_{-1}GlyGly)(Cys^-): 600 cm^{-1} in the CT and 2200 cm^{-1} in the d–d transitions.

Kinetic Stability of a Single Chelate-Ring Unit; Kinetics and Mechanism of the Ring Opening. The results from kinetic experiments reveal that the reaction was first order with respect to both the ternary complex and RsH. Thus, the rate of the forward-reaction, v, can be expressed by

$$v = k_{2+}[Cu(X)(Rs^-)][RsH], \quad (1)$$

where [Cu(X)(Rs^-)] and [RsH] represent the concentrations of the ternary complex and RsH, respectively. Over the pH range examined, the [Cu(X)(Rs^-)]; X = peptide, is the sum of the concentrations of two species and given by Eq. 2-1 (Scheme 3),

$$[Cu(X)(Rs^-)] = [Cu(GlyGly)(Rs^-)] + [Cu(H_{-1}GlyGly)(Rs^-)], \quad (2\text{-}1)$$

where Cu(GlyGly)(Rs^-) and Cu(H_{-1}GlyGly)(Rs^-) are equilibrated as follows,

$$Cu(GlyGly)(Rs^-) \overset{K_c}{\rightleftarrows} Cu(H_{-1}GlyGly)(Rs^-).$$

Eq. 2-1 in the Cu(EDMA)/PesH system can be written as

$$[Cu(X)(Rs^-)] = [Cu(EDMA)(Rs^-)], \quad (2\text{-}2)$$

RsH involves three species (RsH, Rs, and Rs^-), which are in equilibrium, under the conditions examined. The equilibrium in PesH is shown as follows[21–24]

$$\overset{\mathbf{RsH}}{\mathrm{HSC(CH_3)_2CH(NH_3^+)COO^-}}$$

$$\overset{K_{a2}}{\rightleftarrows} \overset{\mathbf{Rs}}{\mathrm{^-SC(CH_3)_2CH(NH_3^+)COO^-}}$$

$$\overset{K_{a3}}{\rightleftarrows} \overset{\mathbf{Rs^-}}{\mathrm{^-SC(CH_3)_2CH(NH_2)COO^-}}, \quad (3)$$

where K_{a2} and K_{a3} are the proton-ionization constants, $10^{-7.9}$ and $10^{-10.7}$, respectively. Then, Eq. 1 is rearranged as

$$\begin{aligned} v &= k_{2+}([\mathrm{Cu(L)(Rs^-)}] + [\mathrm{Cu(H_{-1}L)(Rs^-)}]) \\ &\quad \times ([\mathrm{RsH}] + [\mathrm{Rs}] + [\mathrm{Rs^-}]) \\ &= (\mathbf{A}\cdot\mathbf{B})[\mathrm{Cu(X)(Rs^-)}]_o[\mathrm{RsH}]_o, \end{aligned} \quad (4)$$

where $[\mathrm{RsH}]_o$ and $[\mathrm{Cu(X)(Rs^-)}]_o$ represent the total concentrations of the RsH and Cu(X)(Rs^-), respectively. **A** and **B** are given as

$$\mathbf{A} = \frac{(k_{ML}[H^+] + k_{M(H_{-1}L)}K_c)}{([H^+] + K_c)}, \quad (5)$$

where k_{ML} and $k_{M(H_{-1}L)}$ are the rate constants of the $Cu(Rs^-)_2$ formation from Cu(GlyGly)(Rs^-) and Cu(H_{-1}GlyGly)(Rs^-), respectively, and

$$\mathbf{B} = \frac{(k_{RsH}[H^+]^2 + k_{Rs}K_{a2}[H^+] + k_{Rs^-}K_{a2}K_{a3})}{([H^+]^2 + K_{a2}[H^+] + K_{a2}K_{a3})}. \quad (6)$$

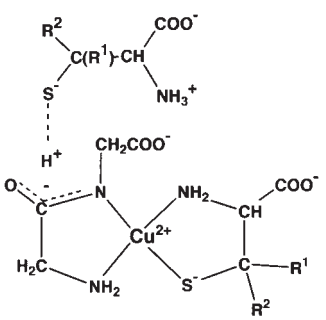
where k_{RsH}, k_{Rs}, and k_{Rs^-} represent the rate constants of the $Cu(Rs^-)_2$ formation concerning the corresponding RsH species.

The rate constants are expected to be functions of the pH, and concentrations of RsH and Cu(X)(Rs^-). In the reaction with Cu(H_{-1}GlyGly)(Rs^-), the rate constant k_{2+} increased as the pH decreased. These results are suggestive of the formation of an adduct, as follows:

$$\begin{aligned} &\mathrm{Cu(H_{-1}GlyGly)(Rs^-)} + \mathrm{RsH} \\ &\quad \rightleftarrows [\mathrm{Cu(H_{-1}GlyGly)(Rs^-)}]\mathrm{H^+\cdots s^-R}. \end{aligned} \quad (7)$$

The proton in the $[\mathrm{Cu(H_{-1}GlyGly)(Rs^-)}]\mathrm{H^+\cdots s^-R}$ species (Scheme 4) would easily associate with the deprotonated amide nitrogen to form Cu(L)(Rs^-),

$$\begin{aligned} &[\mathrm{Cu(H_{-1}GlyGly)(Rs^-)}]\mathrm{H^+\cdots s^-R} \\ &\quad \rightleftarrows \mathrm{Cu(GlyGly)(Rs^-)\cdots s^-R}. \end{aligned} \quad (8)$$



Scheme 4. Proposed coordination structure for the protonated ternary complex, $H^+[Cu(H_{-1}GlyGly)(Rs^-)]$. RsH; cysteine: R^1, R^2 = H, penicillamine: $R^1 = CH_3$, $R^2 = CH_3$.

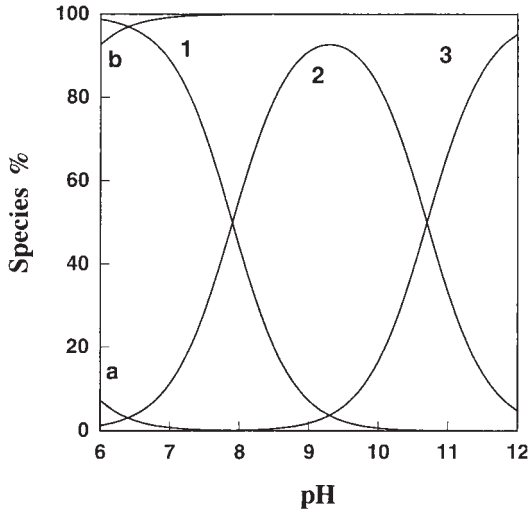


Fig. 9. Species distribution for Cu(II)–GlyGly complex and PesH as a function of pH. (a), Cu(GlyGly)(Pes^-), (b), Cu(H_{-1}GlyGly)(Pes^-), (1), PesH, (2), Pes, and (3), Pes^-.

The chelate ring unit in Cu(GlyGly)(Rs^-), having a labile coordination structure as Cu(H_{-1}HisGlyGly)(Cys^-),[25] is spontaneously replaced by Rs^- to form $Cu(Rs^-)_2$,

$$\mathrm{Cu(GlyGly)(Rs^-)\cdots s^-R} \rightleftarrows \mathrm{Cu(Rs^-)_2} + \mathrm{GlyGly}. \quad (9)$$

The pK_c of Cu(GlyGly) is shown to remarkably increase upon forming a ternary complex with 2,2′-bipyridyl (Bpy).[26] This was also confirmed in a ternary complex involving aliphatic sulfates as another ligand. The ΔpK_c is bigger than 1.0, where ΔpK_c denotes the difference of pK_c between the ternary and parent complexes.[27] In the pH–k_{2+} plot in Fig. 5, the full line is the simulated by using Eqs. 4 and $\Delta pK_c = 1$. The individual rate constants in Eq. 4 were calculated by referring to the distribution curve, shown in Fig. 9, of each molecular species: Cu(GlyGly)(Pes^-), Cu(H_{-1}GlyGly)(Pes^-), PesH, Pes, and Pes^-. The proper rate constants ($k_{2+(RsH)}$, $k_{2+(Rs)}$, and $k_{2+(Rs^-)}$) for Cu(H_{-1}GlyGly)(Pes^-), and $k_{2+(RsH)}$ for Cu(GlyGly)(Pes^-), obtained from the best fits, are summarized in Table 3. The rate constants, $k_{2+(Rs)}$ and $k_{2+(Rs^-)}$, for the Cu(GlyGly)(Pes^-) reaction were not given, because of the following reasons. At pH 7.0 or less, the Cu(H_{-1}GlyGly)(Pes^-) complex could occupy less than 0.8% of the total copper, and the RsH and Rs approximately 90% and 10%, respectively, of the total penicillamine, as shown in Fig. 9. Then, RsH is considered to be only a probable active species on Cu(H_{-1}GlyGly)(Pes^-).

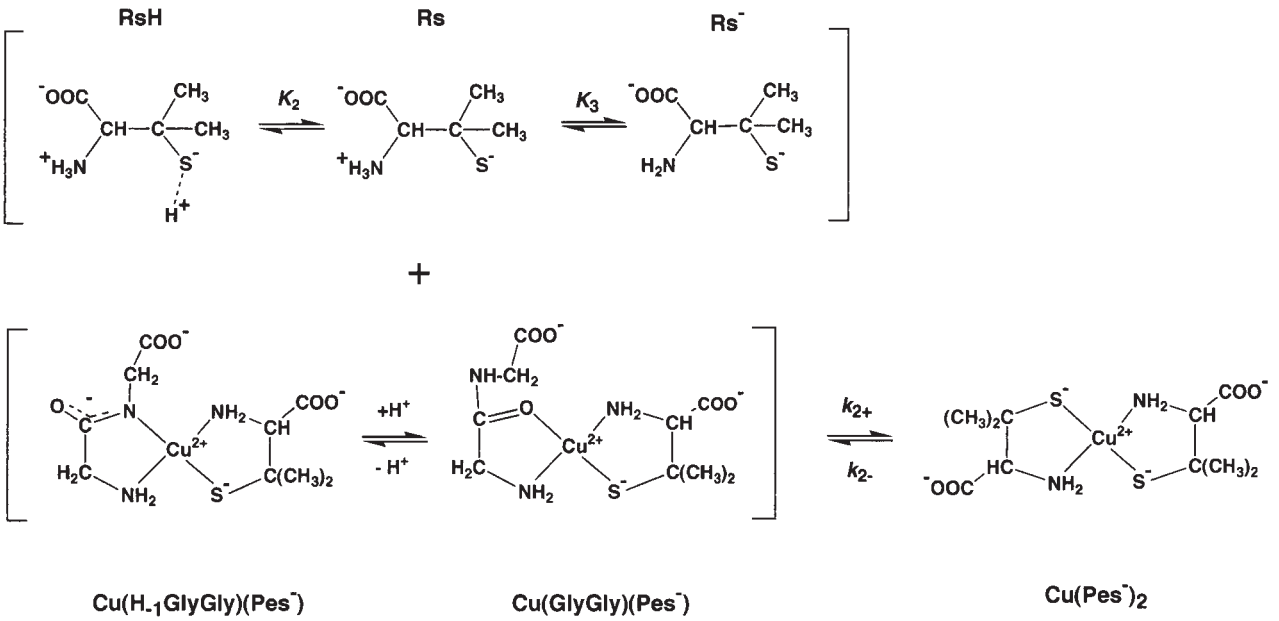
The ternary complex Cu(GlyGly)(Pes^-) was shown to react approximately 300-fold faster with PesH than did Cu(H_{-1}GlyGly)(Pes^-). Though the reaction of Cu(H_{-1}GlyGly)(Rs^-) with RsH occurs primarily by the nucleophilic mechanism, in the weakly acidic region below pH 8, the proton from RsH may assist the reaction (Scheme 5).

Though the thermodynamic stability constant for Cu($H_{-1}\beta$-AlaGly) was similar to that of Cu(H_{-1}GlyGly),[10,11,13] the former was kinetically labile. Cu($H_{-1}\beta$-AlaGly) reacted with PesH, changing rapidly to $Cu(Pes^-)_2$, so much so that the ternary complex could not be identified. Probably, the ΔpK_c of the amide group in Cu($H_{-1}\beta$-AlaGly)(Pes^-) is bigger than that

Table 3. Proper Rate Constants to Cu(EDMA)(Pes$^-$), Cu(H$_{-1}$GlyGly)(Pes$^-$), and Cu(GlyGly)(Pes$^-$) Species

Rate constant/M^{-1} s^{-1}	Cu(EDMA)(Pes$^-$)	Cu(H$_{-1}$GlyGly)(Pes$^-$)	Cu(GlyGly)(Pes$^-$)
$k_{2+(RsH)}$	7.00×10^3	5.50×10^2	1.80×10^5
$k_{2+(Rs)}$	3.20×10^3	2.40×10^2	not determined[a)]
$k_{2+(Rs^-)}$	1.18×10^5	1.90×10^2	not determined[a)]

a) The rate constants were not determined, because Cu(GlyGly)(Pes$^-$) did not encounter Rs and Rs$^-$ as shown in Fig. 9.



Scheme 5. Pathway of the reaction between Cu(H$_{-1}$GlyGly)(Pes$^-$) and PesH.

of Cu(H$_{-1}$GlyGly)(Pes$^-$), so that Cu(H$_{-1}$$\beta$-AlaGly)(Pes$^-$) upon forming is spontaneously rearranged to the labile Cu(β-AlaGly)(Pes$^-$) species. It is shown that, in the ternary complex Cu(β-AlaGly)(bpy), the β-AlaGly coordinates to the Cu(II) via a N-terminal amino nitrogen and an amide oxygen.[26] The k_{2+} value increased with increasing pH and became unmeasurably large above pH 7.5; $k_{2+}/M^{-1}\,cm^{-1}$; 1.30×10^5 (pH 6.5), 1.94×10^5 (pH 6.7), and 4.22×10^5 (pH 7.0). Those rate constants are close to $k_{2+(RsH)}$ for the Cu(GlyGly)(Pes$^-$) reaction. The reaction probably progresses by the nucleophilic mechanism.

The chelate-ring unit from [*N*-(2-aminoethyl)] moiety was maintained in the Cu(EDMA)(Pes$^-$) complex over the examined pH range, and the rate equation can be simplified as

$$v = (\mathbf{B})[\mathrm{Cu(X)(Rs^-)}]_o[\mathrm{RsH}]_o. \quad (4')$$

The reaction occurs by the nucleophilic mechanism, and the rate constant (k_{2+}) depends mainly on [Rs$^-$], and partly on [Rs]. The full line in the pH–k_{2+} plot in Fig. 6 was simulated using Eq. 4′. A fairly good correlation exists between the observed and calculated rate constants. The proper rate constants ($k_{2+(RsH)}$, $k_{2+(Rs)}$, and $k_{2+(Rs^-)}$) obtained from the best fits are summarized in Table 3. The $k_{2+(Rs)}$ value for Cu(EDMA)(Pes$^-$) was similar to the $k_{2+(RsH)}$ for Cu(GlyGly)(Pes$^-$).

The equilibrium constant for the ligand-exchange, as well as the rate constant, might be related to the kinetic stability of the chelate ring. Since at a lower pH value below 8, both the k_{2+} and k_{2-} increased, as shown in Figs. 5, 6, and 7, the equilibrium constant K_2 correspondingly decreased. Above pH 8.5, however, the reaction with Cu(EDMA)(Pes$^-$) accelerated proportionally with an increase of the pH, but k_{2-} did not change significantly. Then, the K_2 value tended to steeply increase as shown in Fig. 8. The K_2 value for the Cu(H$_{-1}$GlyGly)(Pes$^-$)/PesH reaction, by the proton-assisted nucleophilic mechanism, did not change significantly over the pH range from pH 8 to pH 11, and was the 10^2 orders of magnitude smaller than those of the Cu(EDMA)(Pes$^-$) reaction.

Conclusion

The five-membered chelate-ring unit in Cu(H$_{-1}$GlyGly), which is composed of the naturally occurring α-amino acid residue at the amino terminus, has been found to be kinetically stable. The ternary complex, Cu(H$_{-1}$GlyGly)(Rs$^-$), upon forming, reacts with RsH to produce Cu(Rs$^-$)$_2$. The RsH would first attacks the Cu(II) in Cu(H$_{-1}$GlyGly)(Rs$^-$) and protonate the deprotonated amide nitrogen to form the [Cu(H$_{-1}$GlyGly)(Rs$^-$)]H$^+$···s$^-$R species, which subsequently undergoes an intramolecular rearrangement to form a labile species, Cu(L)(Rs$^-$). This transient would spontaneously react with a nucleophile Rs$^-$, forming Cu(Rs$^-$)$_2$. Accordingly, the reaction occurs through a proton-assisted nucleophilic mechanism. The six-membered chelate-ring unit in Cu(H$_{-1}$$\beta$-AlyGly)(Rs$^-$),

which is easily protonated, is labile, undergoing a rapid ligand-exchnage with RsH through the nucleophilic mechanism. The five-membered chelate-ring unit in Cu(EDMA) is labile, though thermodynamically stable.

The kinetic stabilities of the chelate-ring units constructed from three different kinds of moieties were arranged in decreasing order as follows: [*N*-(NH_2CH_2CO)]- > [*N*-(NH_2–(CH_2)$_2$)]- > [*N*-(NH_2–(CH_2)$_2$CO)]- moiety.

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