CHEMISTRY LETTERS, pp. 139-142, 1981.

Cu(II) ION TRANSFER FROM PEPTIDES TO CYSTEINE. FORMATION OF THE TERNARY COMPLEX, (CYSTEINE)-Cu(II)-(PEPTIDE), AS AN INTERMEDIATE

Akira HANAKI
National Institute of Radiological Sciences, Anagawa-4, Chiba 260

The transfer of Cu(II) ion from glycine peptides to cysteine was studied by a stopped-flow spectrophotometric method. The first reaction is the ligand substitution of Cu(II)-peptide, $Cu(H_{-2}peptide)^{-}$, by cysteine forming the ternary complex, $(cysteine)Cu(H_{-1}peptide)^{2-}$. The subsequent reaction is the nucleophilic attack of cysteine upon the ternary complex producing Cu(II)-biscysteinate. The rate of those reactions depends on the steric factors around the metal site.

The transfer of Cu(II) between peptide and amino acid may be of biological significance in understanding the mechanism by which copper is transported between blood and tissues in the body.[1,2] The substitution reactions of metal-peptides are those in which another ligand, namely amino acid, coordinates to the metal replacing one or more of the metal-N(peptide) bonds. Those nucleophilic reactions result initially in the formation of mixed-ligand, ternary, complexes and finally in the complete displacement of the peptide from the metal ion.[3] However, little is known about properties of the ternary complex of peptide-metal-amino acid. An essential way to detect the ternary complex may be to lead the metal ion in a completely new environment of different characters with a strong complexing agent so that the reaction is easily followed. In order to characterize and understand fully the ternary complex, we have been investigating the Cu(II) transfer reaction from peptide to cysteine.[4,5]

In a previous paper, the author demonstrated that the ternary complex with a S→Cu(II) charge transfer band at 330 nm was produced immediately upon mixing solutions of Cu(II)-glycylglycine and cysteine at pH 7.4.[5] The ternary complex possesses a $Cu(II)N_3S$ chromophore; ligands forming the Cu(II)-N(amino terminal) and -N(deprotonated peptide) bonds are derived from glycylglycine and the Cu(II)-N(amino) and -S(thiolate) bonds from cysteine. The reactions of Cu(II)-triglycine or -glycine peptides and cysteine also afford a violet transient which is expected to be the ternary complex with the same chromophore as (cysteine)Cu(glycylglycine). In the present communication are reported the mechanism for the formation and subsequent reactions and the spectral properties of the ternary complex.

Glycine peptides employed were glycylglycine(GG), triglycine(GGG), tetraglycine (GGGG), pentaglycine(GGGGG) and hexaglycine(GGGGGG). Solutions of Cu(II)-peptides were prepared freshly prior to each kinetic run using aliquots of the standardized Cu(II) solution with a 2-5 mole % excess peptide ligands to ensure complex formation. The reactions were monitored spectrophotometrically by following the absorbance

changes at 260, 300, 330 and 390 nm using a Union RA-401 stopped-flow spectrophotometer with a 10 mm observation cell thermostatted at 25°C. The spectrum was obtained by a point-by-point plot at intervals of 5-10 nm in the range of 250-700 nm, and the appropriate cysteine solution was used as the reference. The optical density at each wavelength is the average of at least six runs.

The major species of the copper-peptide under the experimental conditions is $Cu(H_{-2}peptide)^-$ which involves a Cu-N(amino terminal) and two Cu-N(deprotonated peptide) bonds in addition of a Cu-O(carboxylate), Cu-O(peptide) or Cu-O(H_2O) bond.[2] In glycylglycine, $Cu(H_{-1}GG)$ is major. When solutions of $Cu(H_{-2}peptide)^-$ are mixed with cysteine, a rapid increase of absorbance which is followed by sequential absorbance change is observed. The large absorbance change occurs in the region of 260-420 nm, especially at 260, 330 and 390 nm. Simultaneous curves of absorbance against time at 330 and 390 nm are analyzed for three stages of reaction which is the same as that observed in the reaction of $Cu(H_{-1}GG)$ and cysteine.[5] Absorption spectra obtained from the stopped-flow experiment for the reactions of $Cu(H_{-2}GGGGGG)^-$ and $Cu(H_{-1}GG)$ with cysteine are shown in Fig. 1. The initial spectra obtained immediately after mixing, at 10 ms for $Cu(H_{-2}GGGGGG)^-$ and at 5 ms for $Cu(H_{-1}GG)$, show three characteristic absorption bands at 260, 330 and 535 nm. Ultraviolet-visible absorption and ESR spectral similarities reveal substantial structural homology between those violet transients.[6,7] The first reaction in $Cu(H_{-2}GGG)^-$, as well as in $Cu(H_{-1}GG)$, is very rapid enough to be complete within the dead-time of the instrument, 2 ms, but is moderately rapid in $Cu(H_{-2}GGGGGG)^-$. The intensity of the 330 nm band immediately after mixing, in the $Cu(H_{-2}GGG)^-$-cysteine reaction, is proportional to the concentration of the peptide complex where [cysteine]/[Cu(II)] = 8. The formation of the 1:1(cysteine:Cu(II)-peptide) species was verified by a stopped-flow molar-ratio method. Those results indicate that the violet transient produced in the first stage is the monomeric ternary complex and a Cu-N(deprotonated peptide) bond is broken first.

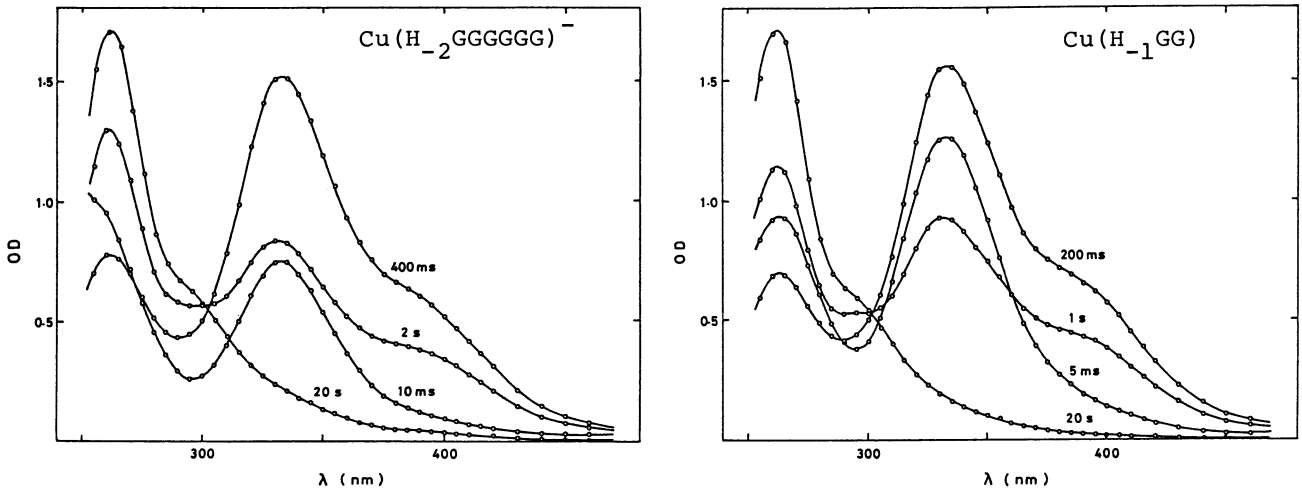

Fig. 1. Successive absorption spectra during the cysteine reactions with $Cu(H_{-2}GGGGGG)^-$ and $Cu(H_{-1}GG)$ at pH 8.3 and 25°C.
[Cu(II)-peptide] = 2.85×10^{-4} M, [cysteine] = 1.70×10^{-3} M.

The rate of the cysteine reactions with Cu(II)-glycine peptides is influenced greatly by the numbers of the glycine residues. The reaction of $Cu(H_{-1}GG)$ with cysteine is extremely rapid to be complete within the dead-time of the instrument. The presence of a second Cu-N(deprotonated peptide) bond in $Cu(H_{-2}GGG)^-$ has relatively little effect on the kinetic reactivity at pH 8.6. However, the addition of a third peptide group by glycine which is not coordinated or, if any, weakly associated to the metal ion causes a substantial change in the rate of nucleophilic attack by cysteine species. Of much interest is the reactivity of the bis-peptide complexes of copper. Upon the addition of excess triglycine to a solution of $Cu(H_{-2}GGG)^-$, a drastic effect on the cysteine reaction is observed, but excess glycylglycine which also causes the bis-peptide complex formation is less effective. Though both bis-peptide complexes possess the same metal center, namely Cu-$(H_{-1}\text{peptide})(\text{peptide})^-$,[8] the reactivities are greatly different. Probably, the steric factors due to the axial-ligation by peptide ligand control the rate of nucleophilic attack on the metal site by cysteine. The reactivity of Cu(II)-glycine peptides at pH 8.6 in decreasing order is as follows;

$$Cu(H_{-1}GG) \sim Cu(H_{-1}GG)(GG)^- \gtrsim Cu(H_{-2}GGG)^- > Cu(H_{-2}GGGG)^-$$
$$> Cu(H_{-1}GGG)(GGG)^- > Cu(H_{-2}GGGGG)^- \sim Cu(H_{-2}GGGGGG)^-.$$

In the second stage of reaction, increases of the 330 and 390 nm bands and a concurrent decrease of the 260 nm band are observed. The transient produced in this stage displays three characteristic bands at 330, 390 and 535 nm, and the spectrum corresponds to that obtained at 400 ms for hexaglycine or that at 200 ms for glycylglycine in Fig. 1. The rate for the increase of the 390 nm band is first order in the ternary complex and cysteine, respectively. According to the spectral similarities and kinetic behaviors, the transient is identified as the binary complex, namely Cu(II)-biscysteinate, which is produced in the reaction of Cu(II)-bisglycinate and cysteine.[6] The rate of the cysteine reactions with the ternary complexes is also greatly influenced by the numbers of the glycine residues. The formation and subsequent or consecutive transformation of the ternary complexes was overlapped, but separable in the cysteine reactions with $Cu(H_{-1}GG)$ and Cu-$(H_{-2}GGG)^-$. Then, the second-order rate constant for the cysteine reaction with $(\text{cysteine})Cu(H_{-1}GGG)^{2-}$ can be estimated as 5×10^{-3} $M^{-1}s^{-1}$. The reactivity of the ternary complexes to form Cu(II)-biscysteinate decreases within the following series;

$$(\text{cysteine})Cu(H_{-1}GG)^{2-} \gtrsim (\text{cysteine})Cu(H_{-1}GGG)^{2-} > (\text{cysteine})Cu(H_{-1}GGGG)^{2-}$$
$$\gtrsim (\text{cysteine})Cu(H_{-1}GGGGG)^{2-} \sim (\text{cysteine})Cu(H_{-1}GGGGGG)^{2-}.$$

In the pH region below 7, the ternary complex is very unstable and directly decomposed to Cu(I) species.

The third stage of reaction is the oxido-reductive decomposition of the Cu(II)-biscysteinate. Successive spectra during the reaction show an isosbestic point at 300 nm which provides evidence that a single species is concerned in the reaction. The rate depends on the concentration of free cysteine.

A complete analysis of the Cu(II) ion transfer from peptide to cysteine seems difficult due to the complexity of the spectra. The consecutive stages of

nucleophilic attack on the metal site by cysteine may be occurring. Figure 2 shows the possible pathway of the formation and subsequent transformation of the ternary Cu(II) complex in which a Cu(II)-N(deprotonated peptide) bond is broken first by the attack of cysteine species.

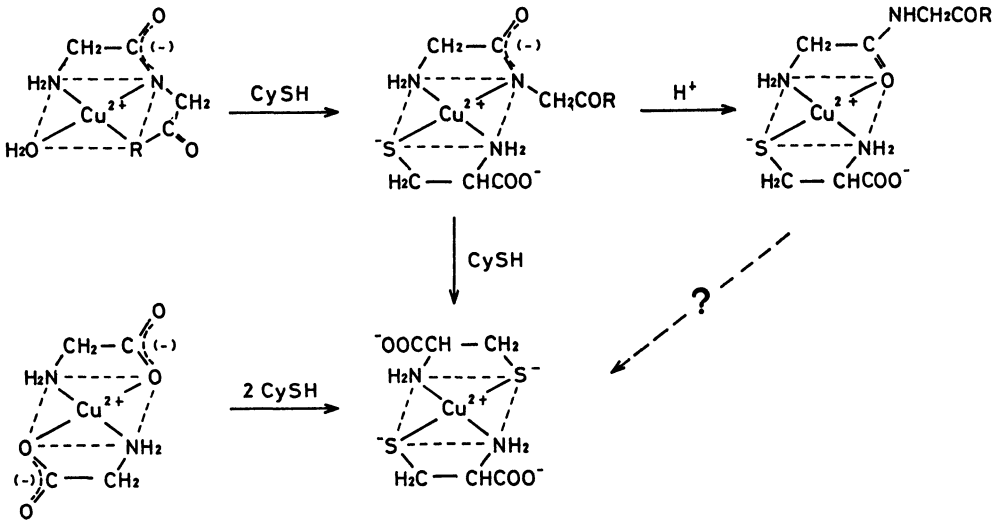

Fig. 2. Mechanistic scheme for the Cu(II) ion transfer from glycine peptide to cysteine.

References

1) B. Sarkar and T. P. A. Kruck, "The Biochemistry of Copper", ed. by J. Peisach, P. Aisen and W. E. Blumberg, Academic Press, New York (1966) p. 183.
2) D. W. Margerum et al., "Bioinorganic Chemistry", Adv. in Chem. Series 162, ed. by K. N. Raymond, Am. Chem. Soc., Washington, D. C. (1977) p. 281.
3) H. Hauer, E. J. Billo and D. W. Margerum, J. Am. Chem. Soc., 93, 4173 (1971). T. P. A. Kruck and B. Sarkar, Inorg. Chem., 14, 2383 (1975). S. H. Laurie, T. Lund and J. B. Raynor, J. Chem. Soc. Dalton, 1389 (1975). T. Sakurai and A. Nakahara, Inorg. Chem., 19, 847 (1980).
4) A. Hanaki, Chem. Lett., 1225 (1976).
5) A. Hanaki, Chem. Lett., 629 (1980).
6) Spectrophotometric data at pH 8.6:

Complex	λ_{max}, nm (ε, $M^{-1}cm^{-1}$)		
$(CyS)Cu(H_{-1}GG)^{2-}$	330(4400)		535(160)
$(CyS)Cu(H_{-1}GGG)^{2-}$	330(4300)		535(150)
$(CyS)Cu(CyS)^{2-}$	330(6000)	390(3000)	535(310)

7) ESR data: A. Hanaki and H. Yokoi, unpublished.
8) M. S. Scheinblatt and E. D. Becker, J. Biol. Chem., 242, 3159 (1967). R-P. Martin, L. Mosoni and B. Sarkar, J. Biol. Chem., 246, 5944 (1971). A. Kaneda and A. E. Martell, J. Coord. Chem., 4, 140 (1975). A. Gergely and I. Magypál, J. Chem. Soc. Dalton, 1104 (1977).

(Received November 14, 1980)