Pre-equilibrium Determining the Rate and Selectivity in the Ligand-exchange of Cu(II) Complex, Cu(II)(H_{-2}Gly-Gly-X), with Cysteine to Yield a Ternary Complex, $(Cys)_{N,S}$Cu(II)(H_{-1}Gly-Gly-X)

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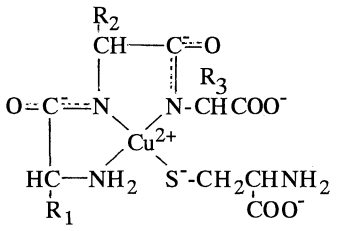
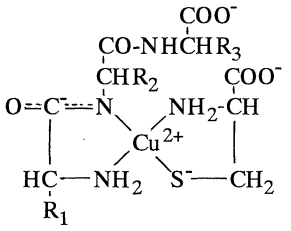
Equilibrium constant for the reaction of Cu(II)(H_{-2}Gly-Gly-X) with N-acetyl-L-cysteine to yield a ternary complex, $(AcCys)_S$Cu(II)(H_{-2}Gly-Gly-X), was determimed. A linear free energy relationship existed between this equilibrium constant and rate constant for the ligand-exchange with L-cysteine to form a ternary complex, $(Cys)_{N,S}$Cu(II)(H_{-1}Gly-Gly-X), indicating the presence of pre-equilibrium state in the ligand-exchange reaction.

The ligand-exchange reaction of peptide complexes with various chelating agents has been studied as a model reaction for the metal-ion transport in the body fluids.[1-3] The Cu(II) complex of doubly deprotonated tripeptide, generally formulated as Cu(H_{-2}L), reacts rapidly with cysteine forming a ternary complex, $(Cys)_{N,S}$Cu(II)(H_{-1}L), which displays a ligand-metal charge transfer(LMCT) band at 330-340 nm.[4] The reaction begins at the carboxylate end of the peptide in the Cu(II) complex;[5-7] the sulfur of cysteine probably attacks first on the Cu(II) and enters into the coordination site in place of the carboxylate-oxygen to form a ternary complex, $(Cys)_S$Cu(II)(H_{-2}L), in which the thiolate-sulfur functions as a monodentate ligand. In the next stage of reaction, intramolecular ligand-exchange would occur between nitrogens of the coordinated cysteine and the deprotonated peptide bond in the transient to yield a second ternary complex, $(Cys)_{N,S}$Cu(II)(H_{-2}L). Since the ligand-exchange reaction consisting of two steps appears to occur successively, the rate constants of individual steps



$(Cys)_S$Cu(II)(H_{-2}L)

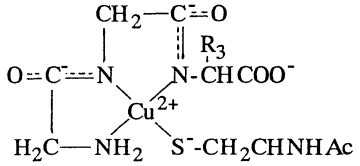


$(Cys)_{N,S}$Cu(II)(H_{-1}L)

could not have been determined. The first reaction is considered to be pre-equilibrium in the ligand-exchange reaction, and substituents of the amino acid residue at the carboxylate end would alter the equilibrium constant. We attempted to determine the equilibrium constant of the reaction between Cu(II)(H_{-2}L) and N-acetyl-L-cysteine (AcCys) to form $(AcCys)_S$Cu(II)(H_{-2}L) and the rate constant of the reaction between Cu(II)(H_{-2}L) and L-cysteine (Cys) to yield $(Cys)_{N,S}$Cu(II)(H_{-1}L), and found that the linear free energy relationship existed between both constants.

The parent complexes examined are Cu(II)(H_{-2}GlyGly-X), where X = Gly, β-Ala, D-Leu, L-Leu, L-nLeu, and L-Ile. Solutions of Cu(II)-tripeptide complexes and the thiols were prepared freshly prior to use. The reaction was monitored by a stopped-flow method at pH 9.2 and 25 °C under nitrogen. The equilibrium constant of the reaction between Cu(II)(H_{-2}Gly-Gly-X) and AcCys was determined at 330 nm for X=Gly and β-Ala and at 340 nm for X=D-Leu, L-Leu, L-nLeu, and L-Ile by a stopped-flow molar-ratio method using a 7.20×10^{-4} M Cu(II)(H_{-2}GlyGly-X) (1M = 1 mol dm^{-3}) at ionic strength 0.5 M ($NaClO_4$).[8] The rate constant k_{obsd} for the ligand-exchange between the Cu(II) complex and Cys to yield $(Cys)_{N,S}$Cu(II)(H_{-1}Gly-Gly-X) was obtained at 335 nm under pseudo first-order conditions using a 5.50×10^{-5} M Cu(II)(H_{-2}Gly-Gly-X) and a large excess of Cys at ionic strength 0.1 M ($NaClO_4$). Plot of k_{obsd} against [Cys] gave a straight line indicating the reaction to be first-order to both the Cu(II)-peptide complex and Cys. The forward rate constant (k_+) and the backward reaction constant (k_-) for the ligand-exchange were determined from the slope and the ordinate intercept, respectively.[9]

The rates of ligand-exchange reaction in many first row transition metal ions have been found to be dependent upon the metal ion, rather than the ligand.[10] Cu(II) exchanges exremely rapidly of all. Then, in the complex with AcCys formed within the dead-time(*ca* 1.2 ms) of the instrument, only the fourth site in Cu(II)(H_{-2}Gly-Gly-X), which had been occupied by a craboxylate-oxygen, was exchanged by a thiolate-sulfur, while other sites could not be altered.



$(AcCys)_S$Cu(II)(H_{-2}Gly-Gly-X)

Ternary complexes with X = Gly and β-Ala showed LMCT bands at λ_{max} = 327 and 332 nm respectively, while those with other residues showed them at 340 nm. Thus, the LMCT band appears to undergo bathochromic shift by hydrophobic groups at the carboxylate end. The equilibrium constant (K') for the $(AcCys)_S$Cu(II)(H_{-2}Gly-Gly-X) formation determined by using a stopped-flow molar-ratio method was very small. Cu(II)(H_{-2}Gly-Gly-X) is

thermodynamically very stable and would resist partial dissociation of the fused-chelate ring. It is indicated that the fused 5-5-6 membered chelated ring in the β-Ala containing complex is more stable than the 5-5-5 membered ring in the Gly containing complex and that the side chain on X remarkably stabilized the fused-chelate ring.

Table 1. Equilibrium constant K' for the formation of $(AcCys)_S Cu(II)(H_{-2}Gly\text{-}Gly\text{-}X)$

X	log K'
Gly	2.45
β-Ala	1.18
L-Leu	0.45
D-Leu	0.40
L-nLeu	0.27
L-Ile	0.02

The ligand-exchange reaction with L-cysteine to yield $(Cys)_{N,S}Cu(II)(H_{-1}L)$ was first-order to both the parent complex and the thiol. Then, the rate expression can be shown in Eq. (1);

$$v = k'_{+}[Cu(II)(H_{-2}L)][Cys] \quad (1)$$

where k'_{+} represents the forward rate constant. The rate in $Cu(II)(H_{-2}Gly\text{-}Gly\text{-}Gly)$ was extremely rapid so that the reaction had completed within the dead-time of the instrument. Probably, k'_{+} is bigger than 10^7 $M^{-1}s^{-1}$. The constants in other parent complexes were distributed in the range of 10^5 - 10^6 $M^{-1}s^{-1}$. The rate of ligand-exchange appeared to depend on the bulkiness of the substituent on the X. The backward rate constant k'_{-} was several orders of magnitude less than k'_{+}, and the ligand-exchange behaved irreversible.

Initial reaction for the Cu(II) transport from the peptide to cysteine is assumed to be a nucleophilic attack of the thiolate-sulfur on the metal ion. Thus, a transient, $(Cys)_S$-$Cu(II)(H_{-2}L)$, would be formd. Since the rate of reaction

$$Cu(II)(H_{-2}L) + Cys \rightleftarrows (Cys)_S Cu(II)(H_{-2}L) \quad (2)$$

is extremely rapid, the reaction (2) can be characterized by equilibrium constant, $K_{11} = k_{11+}/k_{11-}$, where k_{11+} and k_{11-} represent the rate constants of forward and backward reactions, respectively. The ternary complex thus formed undergoes spontaneous transformation to yield the second ternary complex as shown in reaction (3);

$$(Cys)_S Cu(II)(H_{-2}L) \rightarrow (CyS)_{N,S}Cu(II)(H_{-1}L) \quad (3)$$

where k_{12+} is the rate constant of the forward reaction. Since the backward reaction in Eq.(3) was several orders of magnitue slower than the forward reaction, the reaction could be treated kinetically irreversible. Similarly, successive ligand-exchange was observed in the reaction of $Cu(II)(H_{-2}Gyl\text{-}Gly\text{-}Gly)$ with homocysteine. The rate of $(CyS)_{N,S}Cu(II)$-$(H_{-1}L)$ formation from the peptide complex and Cys is given by Eq. (4):

$$v = k_{12+}[(Cys)_S Cu(II)(H_{-2}L)]$$
$$= (k_{11+}/k_{11-} + k_{12+})k_{12+}[Cu(II)(H_{-2}L)][Cys] \quad (4)$$

The ligand-exchange in Cu(II) is extremely rapid; k_{11+}, and probably k_{11-}, is bigger than 10^8 M^{-1} s^{-1}. When $k_{11-} \gg k_{12+}$, Eq. (4) can be rewritten to give Eq. (5):

$$v = k_{12+}K_{11}[Cu(II)(H_{-2}L)][Cys] \quad (5)$$

The constant K_{11}, which could not be determined in the cysteine reaction, is estimated from the constant K' in the AcCys reaction and related to the rate constant k'_{+} obtained experimentally, as shown in Eq. (6) :

$$k'_{+} = K' k_{12+} \quad (6)$$

In Fig. 1 is shown the plot of k'_{+} against K'. A fairly good linear-relationship (LFER) with slope = 1 existed between both constants. The rate of ligand-exchange is first-ordered dependent on the equilibrium constant, indicating the rate and probably stereo-selectivity are controlled and determined by the pre-equilibrium.

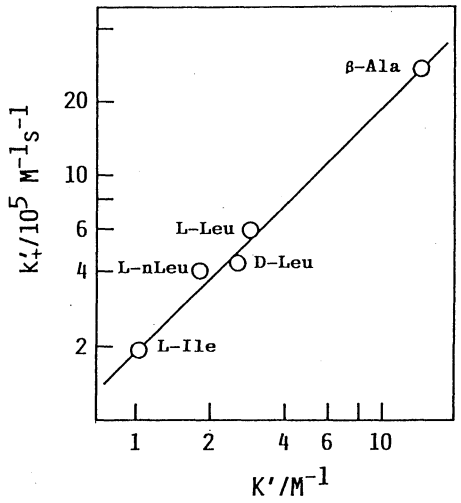


Figure 1. Plot of equilibrium constant K' for the AcCys reaction against rate constant k'_{+} for the Cys reaction.

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