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Stereo-Selectivity and Hindrance in the Ligand-Exchange of the Complex, $[(CyS)Cu(II)(H_{-1}X\text{-}Gly)]^{2-}$, with Cysteine Diastereomers. X ; L-Amino Acid Residues

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Stopped-flow kinetic studies were carried out on the stereo-selectivity and hindrance in the ligand-exchange of the ternary complex, $[(CyS)Cu(II)(H_{-1}X\text{-}Gly)]^{2-}$ with cysteine diastereomers, where X represented L-amino acid resudues. The rate of ligand-exchange with the D-diastereomer was approximately 1.7 fold faster than that of the L-diastereomer. The complexes with aspartyl, asparaginyl and threonyl residues were labile for the ligand-exchange.

The transfer of Cu(II) between peptides and amino acids may be of biological significance in understanding the pathway in which copper is transported in the body fluids.[1,2] The copper complex of dipeptide, $Cu(II)(H_{-1}dipeptide)$, in which the peptide coordinates with Cu(II) by a terminal amino nitrogen, a deprotonated peptide nitrogen and a carboxylate oxygen,[3] reacts with thiol-containing amino acids to yield instantly a transient, which has been determined as the ternary complex shown by $[(CyS)Cu(II)(H_{-1}dipeptide)]^{2-}$ and can be characterized by the S-Cu(II) charge transfer band in ultraviolet region.[4] The transient subsequently undegoes the second ligand-exchange to yield the binary complex, $Cu(II)(CyS)_2^{2-}$.[5,6] Thus, the copper ion is transported from peptides to amino acids. If the peptide contains a chiral amino acid residue and a diastereomer of thiol-containing amino acids is used as an attacking ligand, both the stereoselectivity and steric efftcts will be expected in the ligand-exchange reaction. In this paper is communicated the ligand-exchange of diastereomers of cysteine with $[(CyS)Cu(II)(H_{-1}X\text{-}Gly)]^{2-}$; where X represents L-amino acid residues including glycine, alanine, valine, phenylalanine, tryptophan, threonine, asparatic acid, asparagine, glutamic acid, and lysine.

The peptides used were products from BACHEM Feinchemikalien AG (Switzerland) and D- and L-cysteine, abbreviated as D- and L-CySH respectively, from SIGMA Chem. Co.(Mo. USA). Those reagents were used as received. Solutions of the Cu(II)-peptide complex were prepared freshly using aliquots of the standardized Cu(II) solution with a 3 mole% excess peptide to ensure the complex formation. The reaction was examined by the stopped-flow technique at 25 °C. A 1.11 x 10^{-4} M Cu(II)-dipeptide solution(1 M =1 mol dm^{-3}) was mixed rapidly with CySH solutions and subsequent absorbance changes at 390 nm were recorded on a UNION RA-401 stopped-flow spectrophotometer. An apparent rate for the ligand-exchange was obtained under pseudo first-order conditions using a large excess of CySH at pH 9.5 and ionic strength 0.1($NaClO_4$). Plot of the observed rate constant k_{obsd} against [CySH] gave a straight line indicating the reaction to be first-order to both the complex and CySH. The forward rate constant (k_+) and backward rate constant (k_-) for the ligand-exchange were determined from the slope and the intercept on ordinate, respectively.[7)]

The reaction of Cu(II)(H_{-1}L-X-Gly)$^-$ and CySH to yield the ternary complex, [(L-CyS)Cu(II)-(H_{-1}L-X-Gly)]$^{2-}$ was extremely rapid so that the reaction had been completed within the dead-time, *ca.* 1 ms, of the insturment. The rate constant was estimated to be at least 1 x 10^7 $M^{-1}s^{-1}$.[8)] Then, the reaction discussed here is the ligand-exchange of [(CyS)Cu(II)(H_{-1}X-Gly)]$^{2-}$ with CySH diastereomers to Cu(II)$(CyS)_2^{2-}$. Both the ternary and binary complexes have been shown to possess the ligand-metal charge transfer (LMCT) bands. Since the former is at λ_{max} = 332 nm and the latter at λ_{max} = 332 nm and 385 nm, the rate constants of forward and backward reactions were determined at 390 nm. Plots of k_+s for the reaction of [(L-CyS)Cu(II)(H_{-1}L-X-Gly)]$^{2-}$ with L-CySH against those of [(D-CyS)Cu(II)(H_{-1}L-X-Gly)]$^{2-}$ with D-CySH are shown in Fig. 1. There existed a fairly good relationship between the k_+ values.

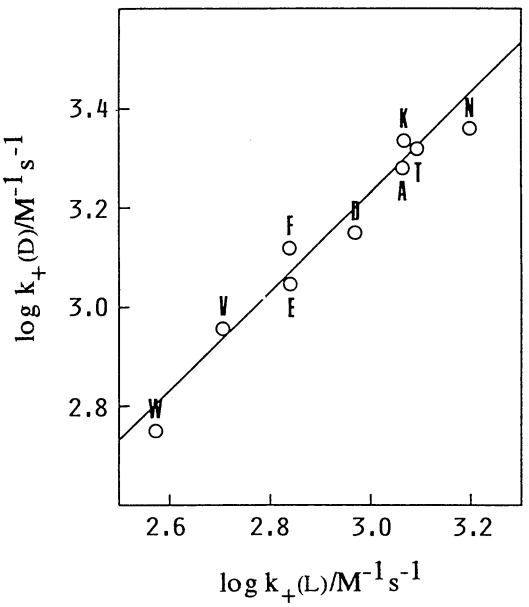
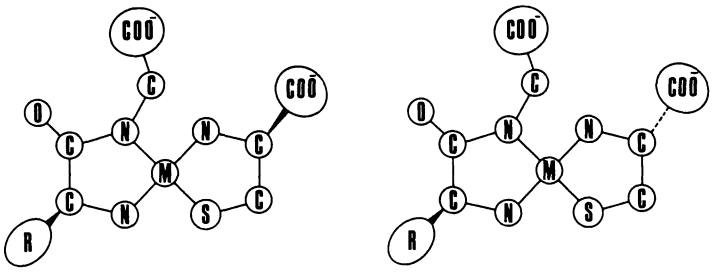


Fig. 1. Plot of log k_+(L) and log k_+(D) : G; Gly-Gly, A; Ala-Gly, V; Val-Gly, F; Phe-Gly, W; Try-Gly, T; Thr-Gly, D; Asp-Gly, E; Glu-Gly, N; Asn-Gly, K; Lys-Gly.

The ligand-exchange with the D-diastereomer was approximately 1.7 fold faster than that with the L-diastereomer. Since CySH was prompt to form a five-membered chelate ring with the Cu(II), it was hard to distinguish which of the two donors, thiolate-sulfur and amino-nitrogen, was the first ligating group. On other hand, Cu(II) was chelated stepwise by homocysteine to form a six-membered ring and the thiolate-sulfur lagated at first.[9] Those findings suggest that the side chains occupy the *trans* position with respect to the square-plane in $[(\text{L-CyS})\text{Cu(II)}(\text{H}_{-1}\text{L-X-Gly})]^{2-}$ and the *cis* position in $[(\text{D-CyS})\text{Cu(II)}(\text{H}_{-1}\text{L-X-Gly})^{2-}$. It is indicative of more steric hindrance in the reaction of $[(\text{L-CyS})\text{Cu(II)}(\text{H}_{-1}\text{L-X-Gly})]^{2-}$ and L-cysteine.



Schematic representation for $[(\text{D-CyS})\text{Cu(II)}(\text{H}_{-1}\text{L-X-Gly})]^{2-}$(left) and $[(\text{L-CyS})\text{Cu(II)}(\text{H}_{-1}\text{L-X-Gly})]^{2-}$(right)

Both hydrophilic and hydrophobic side chains of X appeared to retard the forward reaction in the ligand exchange. Remarkable steric and/or electronic effects were observed in the backward reaction. In Fig. 2 is shown the plot of k_+ against k_- in the reaction of $[(\text{L-CyS})\text{Cu(II)}(\text{H}_{-1}\text{X-Gly})]^{2-}$ and L-CySH. The ratio of k_+/k_- corresponds to the equilibrium constant (K) in dynamic aspects. An average of log K for the complexes containing alkyl and aromatic side chains was approximately 3.15. In Fig. 2 is shown a straight line at log K = 3.15. The dots for the complexes with hydrophobic side chain

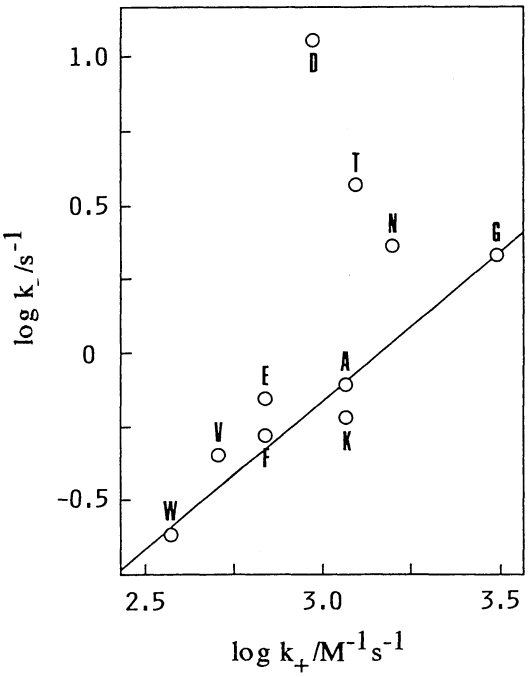


Fig. 2. Plot of $\log k_+$ against $\log k_-$: abbreviations same as under Fig. 1.

were distributed around the straight line, while those of the complexes with hydrophilic side chains were spreaded over the line. Valyl and threonyl residues, having similar frame and bulkiness but different electronegativity and hydrophilicity, gave different stabilities in dynamic aspects to the complexes; especially, the backward reaction for the Thr-containing complex was extraordinarily rapid as compared with the Val-complex. Aspartyl residue stimulated dramatically the backward reaction. Though both aspartyl and glutamyl residues have a negatively charged carboxylate, the k_- value is approximately 15 fold bigger in the Asp-containing complex than in the Glu-complex. The carboxylate group in aspartyl residue is attached at β-carbon, while in the glutamyl is at γ-carbon. Both threonyl and asparaginyl residues, having hydrophilic group, i.e. hydroxyl and carbonyl groups respectively, at the β-carbon, enhanced the backward reaction, while the lysyl residue with the positive charged $-NH^{3+}$ group at ε-carbon did no affect. The hydrophilicity in the vicinity of the metal-site appears to afford lability for the ligand-exchange to the complex. It may be concluded that hydrophilicity and hydrophobicity, electric charge, and conformation of the side chains contibute variously to the dynamics of the metal-ion transport. Investigation of the mechanism in detail is now in progress.

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8) The value of k_+ for the ligand-exchange of Cu(II)(H_{-2}GlyGly-β-Ala) with L-CySH to form the ternary complex, $[(CyS)Cu(II)(H_{-1}GlyGly-\beta-Ala)]^{2-}$, was determined as 2.6×10^6 $M^{-1}s^{-1}$. The rate constant for the Cu(II)(H_{-1}GlyGly) and CySH reaction was appeared to be at least one order of magnitude bigger than that of the above reaction.

9) A. Hanaki, to be published elsewhere.

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