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INTERACTION OF BIOLOGICALLY RELEVANT Cu(II)-PEPTIDE AND CYSTEINE. TRANSIENT COMPLEXES WITH $Cu(II)N_3S$ AND $Cu(II)N_2S_2$ CHROMOPHORES

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Cu(II)-glycylglycine chelates with cysteine forming red-purple transient. A transient which is formed immediately upon mixing the reactants is a ternary Cu(II) complex. Another transient is a binary Cu(II) complex of cysteine produced successively from the ternary complex. Spectrophotometric properties of those transients are discussed.

The intensely purple copper complex of penicillamine or related thiols, which is produced in the presence of halide ions and has been structurally characterized, is a mixed-valence complex with a cluster structure,[1,2] and the intense purple color has been attributed to a S-Cu(II) charge transfer within the cluster unit.[2] The formation of the cluster is composed of several reactions including complexation and oxido-reduction.[3] When Cu(II) ion is added as the sulfate, nitrate or acetate, preparation of the cluster is not achieved, while a red-purple transient is observed.[4] The reaction of Cu(II) ion and cysteine also leads to the formation of the red-purple transient, which is probably a relevant electronic-structural model for a Cu(II)-S(cysteine) chromophore in the blue copper proteins. The author reports here the electronic spectra of the red-purple transients observed in the reaction of Cu(II)-glycylglycine and cysteine.

Solutions of Cu(II)-glycylglycine and cysteine were mixed with a rapid-mixing device and single-wavelength curves of optical density against time were recorded on a Union RA-401 stopped-flow spectrophotometer. The spectrum was obtained by a point-by-point scan in the range of 280-700 nm.

The spectra obtained at 5 ms, 50 ms and 2 s are shown in Fig. 1. Simultaneous curves of optical density against time at 330 and 400 nm are inserted in Fig. 1, from which the three stages of reactions are suggested. The first reaction, which is very rapid, rate constant $>10^6$ $M^{-1}s^{-1}$, and completed within the dead time of the instrument, gives a transient A with λ_{max} = 330 and 530 nm. The optical density observed immediately after the start of reaction, 2 ms, is linearly proportional to the concentration of Cu(II)-glycylglycine, where $[cysteine]_o/[Cu(II)]_o = 8$. The absorption coefficient based on the total concentration of copper is as follows; $\varepsilon = 4.3 \times 10^3$ $M^{-1}cm^{-1}$(330 nm) and $\varepsilon = 1.5 \times 10^2$ $M^{-1}cm^{-1}$(530 nm). The transient A is a paramagnetic copper complex giving an ESR spectrum with a ligand hyperfine splitting of seven lines.[5] In the second stage, another paramagnetic transient B with an additional λ_{max} at 390 nm, as well as λ_{max} = 330 and 530 nm, is formed. The reaction is first-order with respect to the transient A and cysteine, where $[cysteine]_o/[Cu(II)]_o > 2$. When the total concentration of cysteine is equimolar to Cu(II) or less, the transient A is decomposed to ESR silent

species with a broad absorption over 350-600 nm.[6] The transient B is decomposed in the third stage to ESR silent species with λ_{max} = 290 nm.

Those findings indicate that the transient A is a ternary complex of Cu(II), glycylglycine and cysteine with a Cu(II)N_3S chromophore and the transient undergoes subsequent ligand-substitution in the presence of excess cysteine forming the transient B, a binary complex of Cu(II) and cysteine with a Cu(II)N_2S_2 chromophore.[7] The reaction of Cu(II)-glycine and cysteine yields only the binary complex.[8]

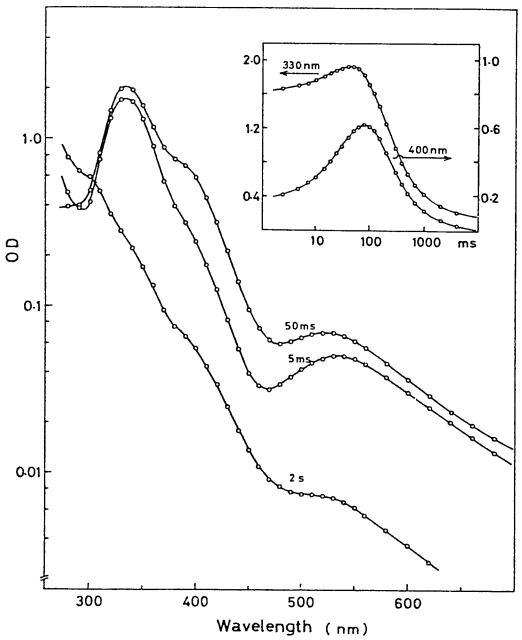

Fig. 1. Electronic spectra of the red-purple transients: [Cu(II)] = 4.0 x 10^{-4} M, [cysteine] = 3.2 x 10^{-3} M, pH 7.4(phosphate), T = 20°, I = 0.5(ClO_4^-).

References

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6) When the decomposition of the transient A is slow as shown in the reaction of Cu(II) and penicillamine, the purple-brown solution, containing both paramagnetic and diamagnetic complexes, displays a broad and intense absorption. Accordingly, though the ternary system, Cu(II)-glycylglycine-penicillamine, is examined by electronic spectroscopy (S.H.Laurie, T.Lund and J.B.Raynor, *J.C.S.Dalton*, 1389 (1975)), the spectrum is not well resolved.
7) The transients with the Cu(II)N_3S and Cu(II)N_2S_2 chromophores display distinctive ESR spectra at 77 K. A.Hanaki and H.Yokoi, to be submitted for publication.
8) Spectrophotometric parameters of the binary complex are as follows; ε = 5.2 x 10^3 $M^{-1}cm^{-1}$(330 nm), ε = 2.5 x 10^3 $M^{-1}cm^{-1}$(390 nm) and ε = 3.0 x 10^2 $M^{-1}cm^{-1}$(530 nm).

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