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Cu(II) Complexes of Glutathione. Coordination Mode, Spectroscopic Properties, and Lability

Akira HANAKI* and Hiroshi YOKOI+
National Institute of Radiological Sciences, Chiba 260
+Faculty of Engineering, Shizuoka University, Hamamatsu 432

Cu(II)-peptides were shown to coordinate to glutathione yielding transient ternary complexes as studied by both absorption and ESR spectrometries. Measurements were done by stopped-flow techniques. The ternary complexes in which glutathione functioned as a monodentate thiol ligand were stabilized significantly by ligation of an imidazole nitrogen to the metal ion.

The role of glutathione(GSH) in physiological processes depends upon its reducing and metal-coordinating abilities. However, attention of biochemists to GSH has been focused mainly on its intramolecular reducing ability.[1] Metal detoxification and probably transport may also be a possible physiological function of GSH. Several hypotheses have been proposed to explain the physiological role of copper. Free copper coordinates to cysteinyl thiol groups of proteins thereby inactivating essential proteins and enzymes. If molecular oxygen is present, the metal-thiolate bond may be oxidized, irreversibly inactivating enzymes.[2] In conjugation to this process, the oxygen may be reduced. Copper ion interacts with physiologically reduced oxygen species, such as superoxide anion and hydrogen peroxide, catalyzing the formation of highly toxic hydroxyl free radicals via Fenton-like reactions.[3] The production of hydroxyl radicals, associated with the cellular accumulation of copper, may occur soon after the metal enters the cell. Although metallothionein is an efficient copper chelator, its accumulation to a level sufficient to inhibit metal toxicity requires several hours after an initial exposure of the cell to the metal.[4] GSH may also function as an intramolecular detoxifying agent.[5] This proposal is based on observations that the depletion of GSH potentiates copper toxicity in rats[6] and mice.[7] The importance of GSH in the copper transport to metallothionein is also suggested.[8]

Since the Cu(II) complexes of GSH have been shown to undergo rapid oxido-reduction to yield oxidized glutathione(GSSG) under both aerobic and unaerobic conditions, the properties, which are considered important to explain and understand the metal toxifying and transporting abilities of GSH, have hitherto been unknown. In this paper we communicate coordination mode, spectroscopic properties and lability of Cu(II) complexes of GSH obtained by stopped-flow studies.

Stopped-flow techniques were used throughout for the detection and observation of transients.[9] The absorption spectrum at a desired time after the start of reaction was prepared by a point-by-point plot of absorbance which was measured with a UNION RA-401 stopped-flow spectrophotometer at pH 9.2 and 25 °C. The X band ESR spectrum was measured at pH 9.2 and 77 K with a JEOL JES-PE-2X spectrometer with a 100 kHz field modulation. Equilibrium constant of the Cu(II) and GSH reaction was determined spectrophotometrically by a stopped-flow molar-ratio method.

Transient complexes were produced immediately upon mixing a Cu(II) complex and GSH. The transients were expectedly unstable as studied by both absorption and ESR spectrometries. The absorption spectra at 1.5 ms after the start of reaction are shown in Fig. 1. The transient produced from the reaction of $Cu(H_{-1}GG)$ and GSH was a 1:1 complex.[10] On the other hand, $Cu(H_{-2}GGG)$ was stable so that the ligand exchange hardly occurred.

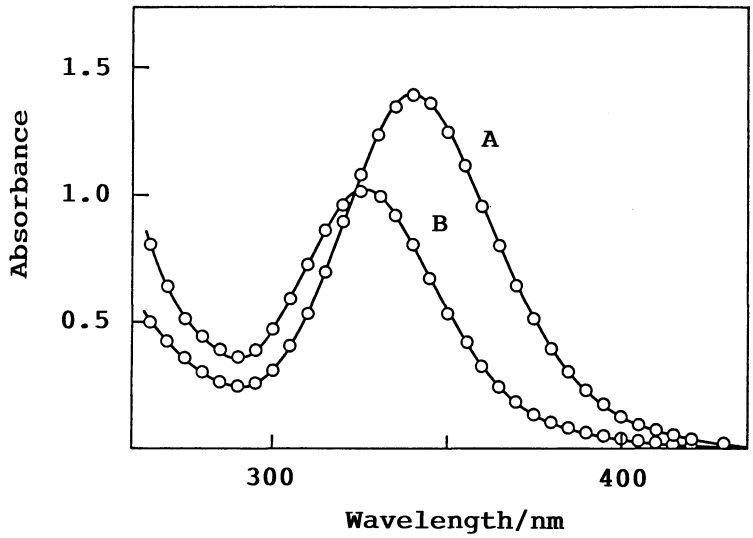


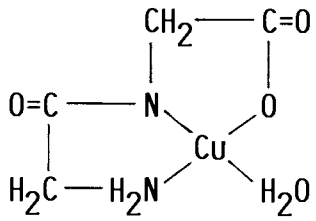
Fig. 1. Absorption spectra of the transients at 1.5 ms in the reaction of GSH with $Cu(H_{-1}GG)$ (A) and $Cu(H_{-2}GGG)$ (B).
(A); $[Cu(H_{-1}GG)] = 5.86 \times 10^{-4}$M, $[GSH] = 4.2[Cu(H_{-1}GG)]$,
(B); $[Cu(H_{-2}GGG)] = 5.89 \times 10^{-4}$M, $[GSH] = 12.5[Cu(H_{-2}GGG)]$.

If $CuL^{(2-n)+}$ and GSH forms a 1:1 complex, the equilibrium constant K_f would be given by Eq. 1.

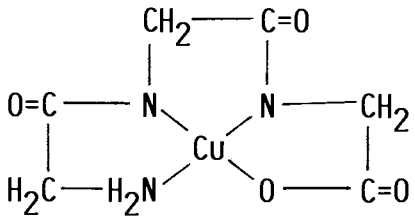
$$K_f = ß/[CuL](1 - ß)(n - ß) \quad (1)$$

where ß and n represent the fraction of CuL bound to GSH and equivalent GSH to the total copper, respectedly. When (n - ß) is plotted against ß/(1 - ß), a straight line should be obtained. The plot in the reaction of $Cu(H_{-2}GGG)$ and GSH gave a straight line, indicating the formation of a 1:1 complex with a small K_f value.

The transients showed a S-Cu(II) charge transfer band which varied depending on the coordination mode of the peptide moieties. Either $Cu(H_{-1}GG)$ or $Cu(H_{-2}GGG)$ forms a transient with cysteine(CySH), which is a N,S-bidentate ligand.[11] Both transients were determined to be ternary complexes with the coordination structure formulated as $(CyS)Cu(H_{-1}peptide)$. On the other hand, $Cu(H_{-1}GG)$ and $Cu(H_{-2}GGG)$ produced spectroscopically different 1:1 complexes with a S-monodentate N-acetylcysteine(AcCySH). In addition, the ternary complex of a certain Cu(II)-peptide with either GSH or AcCySH showed identical spectroscopic parameters.



$Cu(H_{-1}peptide)$



$Cu(H_{-2}peptide)$

The spectroscopic parameters along with K_f values of the transients are summarized in Table 1. It was confirmed that GSH functioned as a monodentate ligand forming a ternary complex in which the coordination structure of the peptide moiety is probably conserved with a little perturbation.

Table 1. Thermodynamic and spectroscopic parameters of glutathione-containing ternary complexes

Complex	log K_f/M^{-1}	$\lambda_{max}/nm(\varepsilon/M^{-1}cm^{-1})$	$g_{\parallel}$	$A_{\parallel}/cm^{-1}$
$(CyS)Cu(H_{-1}GG)$	> 5	332(4.35×10^3)	2.173	0.0200
$(CyS)Cu(H_{-1}GGG)$	> 5	333(4.24×10^3)	2.173	0.0200
$(GS)Cu(H_{-1}GG)$	4.59	340(4.78×10^3)	2.196	0.0164
$(AcCyS)Cu(H_{-1}GG)$	4.58	343(4.72×10^3)	2.195	0.0162
$(GS)Cu(H_{-2}GGG)$	2.44	326(5.26×10^3)	2.170	0.0200
$(AcCyS)Cu(H_{-2}GGG)$	2.46	327(5.29×10^3)	2.170	0.0199
$(GS)Cu(H_{-1}GHG)$	4.05	344(5.57×10^3)	2.175	0.0203

Spectroscopic properties and probably kinetic stability, lability, of the GSH-containing ternary complex appeared to depend on the coordination mode of the peptide moiety. The ligation of an imidazole nitrogen to the metal enhanced the stability of Cu(II)-S bond in the ternary complex. It has been shown that Cu(II) complexes with GHG to form $Cu(H_{-1}GHG)$, in which the metal ion coordinates with a terminal amino-nitrogen, a neighboring deprotonated peptide-nitrogen and an imidazole-nitrogen of the histidyl residue, and that a residual site of the metal is occupied by either a labile H_2O or OH^-. $Cu(H_{-1}GHG)$ was shown to associate with GSH, forming a relatively stable ternary complex, $(GS)Cu(H_{-1}GHG)$. The half life at pH 9.2 and 25 °C estimated from the decay curve of absorbance at λ_{max} was approximately 1 min, while the ternary complex without histidyl ligands were labile with a half life of a few hundred sec. There exist physiologically relevant histidine-containing peptides with a H-X-(histidyl)--Y-OH backbone such as GHK, a growth factor in liver cell.[12] The Cu(II) complexes of those peptides are capable of yielding a rather stable ternary complex with GSH, which is proposed to function transport of Cu(II) to the biological pool such as metallothionein or ceruloplasmin. It is considerably of interest that a labile Cu(II)-S bond in the complex is stabilized by ligation of the imidazole nitrogen to the metal ion.

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