Complexation Reaction of a Copper(II)–Glycinepeptide Complex with Cysteine: Electron Spin Resonance Evidence for the Formation of a Ternary Complex from Copper(II), Glycinepeptide and Cysteine

AKIRA HANAKI*

National Institute of Radiological Sciences, Anagawa, Chiba 260, Japan

and HIROSHI YOKOI

Chemical Research Institute of Non-aqueous Solutions, Tohoku University, Katahira, Sendai 980, Japan

Received September 17, 1985

Amino acid complexes containing the copper(II)–sulfur(thiol) bond are of biological interest and are probably implicated in the exchange, transport and excretion of the trace metals in body fluids [1]. For example, D-penicillamine, a thiol compound, appears to be effective in removing excess copper from patients with Wilson's disease and its use in the treatment of this disease may be related to its affinity for copper ion [2]. In the metal transport reaction, the intermediary ternary complex involving a macromolecule, such as serum albumin, and a thiol as the ligands is suggested as being a key compound [3, 4]. However, since the Cu(II)–S bond is generally so chemically labile as to undergo rapid redox reaction [5, 6], a primary study has been recently carried out concerning the spectroscopic properties of the Cu(II)–S bond in the complex [7–9]. We previously demonstrated the formation of a transient ternary complex produced by mixing a Cu(II)–peptide complex and cysteine, detected by a stopped-flow spectrophotometric technique, and which characterized the Cu(II)–S bond in the complex [10, 11]. The present work reveals additional ESR evidence and a probable mechanism for the formation of the ternary complex as mentioned above.

Solutions of the Cu(II)–peptide complex and cysteine at pH 9.5 (0.03 M borate buffer in 0.3 M $NaClO_4$) were rapidly mixed and transients produced were trapped by a rapid freezing technique (quenching time less than 5 ms). A detailed procedure for such experiments by the rapid-freezing apparatus has been already described [12].

The ESR spectra of transients produced on mixing solutions of Cu(II)–triglycine, $Cu(II)(H_{-2}trigly)^{-1}$, and cysteine at pH 9.5 are shown in Fig. 1. These spectra are well-resolved at the low field of $g_{\parallel}$ components and are, obviously, due to two complex species. The intensity ratio of the spectra due to these two complex species depends on the concentration of cysteine relative to the Cu(II)–peptide complex. At the molar ratio of 1:1.5(Cu(II):cysteine), the main species gives the spectrum of $g_{\parallel} = 2.17$, and its intensity decreases in proportion to an increasing concentration of cysteine, accompanying the appearance of a new spectrum of $g_{\parallel} = 2.14$. The intensity of the new spectrum increases with increasing cysteine. The transient formed in the presence of excess cysteine gives the ESR spectrum identical with that of Cu(II)–biscysteinate, $Cu(II)(cys)_2^{2-}$ (Fig. 1, D). These findings indicate that the consecutive reactions (1) and (2) occur after the solutions are mixed.

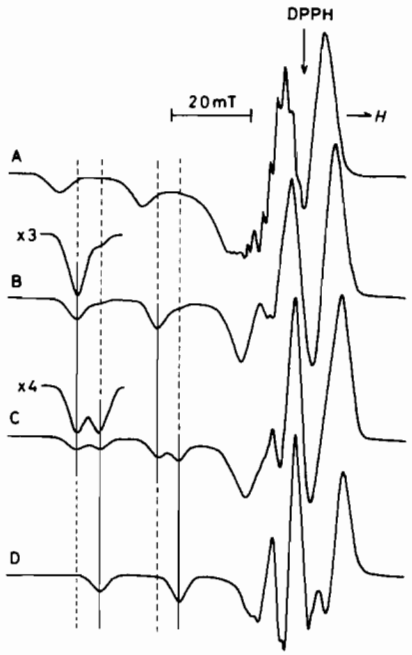


Fig. 1. ESR spectra at 77 K of $Cu(II)(H_{-2}trigly)^{1-}$ and transients resulting from the reaction of the peptide complex with cysteine at pH 9.5. (A) $Cu(II)(H_{-2}trigly)^{1-}$ ($[Cu(II)] = 1.25 \times 10^{-3}$ M); (B) transient at [cys]/[Cu(II)] = 1.5; (C) transient at [cys]/[Cu(II)] = 4.0; (D) $Cu(II)(cys)_2^{2-}$.

$$Cu(II)(H_{-2}trigly)^{1-} + cys \longrightarrow \text{transient X} \quad (1)$$

$$\text{transient X} + cys \longrightarrow Cu(II)(cys)_2^{2-} + trigly \quad (2)$$

Since the transient X is an intermediate from the parent peptide complex to $Cu(II)(cys)_2^{2-}$ and shows a Cu(II)–S charge transfer band 330 nm, as observed in $Cu(II)(cys)_2^{2-}$, it is assignable as a ternary complex, $(cys)Cu(II)(H_{-1}trigly)^{2-}$. This assignment is also supported by the ESR fact that the

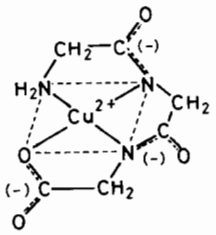
*Author to whom correspondence should be addressed.

0020-1693/86/$3.50

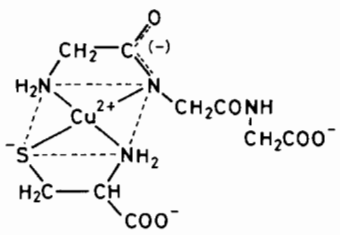
TABLE I. ESR Parameters and Characteristic Absorption Bands for the Cu(II)–Peptide Complexes and their Ternary Complexes with Cysteine

Complex	$g_{\parallel}$	$A_{\parallel}$ (cm^{-1})	S–Cu(II) CT band λ_{max} (nm)
Cu(II)(H_{-1}glygly)	2.242	0.0179	
(cys)Cu(II)(H_{-1}glygly)$^{2-}$	2.173	0.0200	333
Cu(II)(H_{-2}trigly)$^{1-}$	2.197	0.0208	
(cys)Cu(II)(H_{-1}trigly)$^{2-}$	2.173	0.0200	333
Cu(II)(H_{-3}tetragly)$^{2-}$	2.193	0.0200	
(cys)Cu(II)(H_{-1}tetragly)$^{2-}$	2.173	0.0205	333
Cu(II)(H_{-3}hexagly)$^{2-}$	2.176	0.0215	
(cys)Cu(H_{-1}hexagly)$^{2-}$	2.171	0.0204	333
Cu(II)$(cys)_2^{2-}$	2.139	0.0192	333, 385

transient is intermediate in the $g_{\parallel}$ value between the Cu(II)–peptide having a Cu(II)(N_3O) center and Cu(II)–biscysteinate having a Cu(II)$(NS)_2$ center [12], as will be demonstrated later.



Cu(II)(H_{-2}trigly)$^{1-}$



(cys)Cu(II)(H_{-1}trigly)$^{2-}$

The ESR parameters determined for the Cu(II)–peptide complexes and their ternary complexes with cysteine are shown in Table I, together with the optical absorption spectral data. From Table I, it can be seen that the ternary complexes are similar in ESR parameters, as well as in spectrophotometric properties, irrespective of the difference in the peptide segments. Accordingly, the ternary complexes are shown to have a common coordination center, Cu(II)(N_2)(NS), where the Cu(II) ion coordinates to the peptide segment via an amino nitrogen and an adjacent deprotonated peptide nitrogen and to cysteine via an amino nitrogen and a thiol sulfur.

In the reaction of a bidentate cysteine with the Cu(II)–peptide complexes, ESR studies provide evidence that the path of the ligand exchange begins at the coordinated carboxylate or peptide group. Either of the sulfur or nitrogen atoms of the bidentate cysteine attacks first at the Cu(II)–O(carboxylate) bond in Cu(II)(H_{-2}trigly)$^{1-}$ and at the Cu(II)–N(peptide) bond in Cu(II)(H_{-3}hexagly)$^{2-}$. Consecutively, the cysteine replaces the coordinated groups from the carboxylate end. When the cysteine has replaced two coordinated groups and occupied the coordination sites of the Cu(II), the ternary complex with the common Cu(II)(N_2)(NS) center is formed.

References

1 R. I. Henkin, in M. Friedman (ed.), 'Protein–Metal Interactions', Plenum, New York, 1974, p. 299.
2 J. M. Walshe, in J. Peisach, A. Aisen and W. E. Blumberg (eds.), 'The Biochemistry of Copper', New York, 1966, p. 475.
3 S. Lau and B. Sarkar, *J. Biol. Chem., 246,* 5938 (1971).
4 S. Lau and B. Sarkar, *Can. J. Chem., 53,* 710 (1975).
5 H. K. Back, R. L. Cooper and R. A. Holwerda, *Inorg. Chem., 24,* 1077 (1985).
6 C. H. Anderson and R. A. Holwerda, *J. Inorg. Biochem., 23,* 29 (1985).
7 D. Cavallini, C. de Marco, S. Duprè and G. Rotilio, *Arch. Biochem. Biophys., 130,* 354 (1969).
8 S. H. Laurie, T. Lund and J. B. Raynor, *J. Chem. Soc., Dalton Trans.,* 1389 (1975).
9 F. J. Davis, B. C. Gilbert, R. O. C. Norman and M. C. R. Symons, *J. Chem. Soc., Perkin Trans, 2,* 1763 (1983).
10 A. Hanaki, *Chem. Lett.,* 629 (1980).
11 A. Hanaki, *Chem. Lett.,* 139 (1981).
12 H. Yokoi and A. Hanaki, *Chem. Lett.,* 481 (1984).