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DIMER FORMATION OF COPPER(II) PEPTIDE COMPLEXES IN AQUEOUS SOLUTION AS STUDIED BY ESR

Hiroshi YOKOI* and Akira HANAKI†

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

†National Institute of Radiological Sciences, Anagawa-4, Chiba 260

It has been first revealed by ESR spectroscopy that copper(II) peptide complexes form dimers in aqueous solutions, if they have two or more deprotonated peptide nitrogens, and that this dimer formation is quite similar in many respects to the dimerization or self-association of porphyrins, metal porphyrins, nucleotides, dyes, and some aromatic heterocycles in aqueous solutions. Computer simulation of the dimer ESR spectra has shown that the dimers of the above peptide complexes are of a parallel planar type; two coordination planes in most of them lie almost right above each other with the separation of ≈4 Å.

Recently, the deprotonated peptide nitrogen has been shown to be a potent copper(II)-specific binding site under physiological conditions of pH in small amide ligands, oligopeptides, and copper transport proteins such as serum albumin.[1] In recent years, actually, there have been many reports on copper or some other metal complexes of biological significance, which all contain deprotonated peptide nitrogens.[2] Much attention is being denoted to the chemistry of metal complexes with those nitrogens. We report here a new fact that most planar copper(II) complexes of glycine peptides or related ligands have tha ability to form dimers in aqueous solutions, if they contain two or more deprotonated peptide nitrogens. The ESR technique has been used in the present work.

The copper(II) complexes of amines such as ethylenediamine and of amino acids have no ability to dimerize in aqueous solutions. Similarly, the 1:1 complex of copper(II) with glycylglycine does not dimerize at any pH value (glycine peptide are here abbreviated as G_n, as exemplified by G_2 for glycylglycine). We have found, however, that the 1:1 complexes of copper(II) with G_n ($n \geq 3$) can form dimers at pH > 9. Figure 1 shows an example of ESR evidences for the dimer formation; obviously, this type of ESR line shapes with half-field ($\Delta M = 2$) signals are not due to any kind of monomeric copper(II) complexes, but to a certain kind of dimeric or binuclear ones ($1\ T = 10^4\ G$). These dimer ESR spectra were analyzed by the computer simulation method developed for the system of parallel planar copper(II) dimers,[3] using the coordinate system shown in Fig. 2; two structural parameters, r and ξ, are introduced in this simulation, where r is the Cu-Cu distance and ξ, the angle between the Cu-Cu direction and the normal to the molecular plane.[4] In Fig. 1, simulation spectra are shown by dotted lines, and they fit in with the observed dimer ones, although the

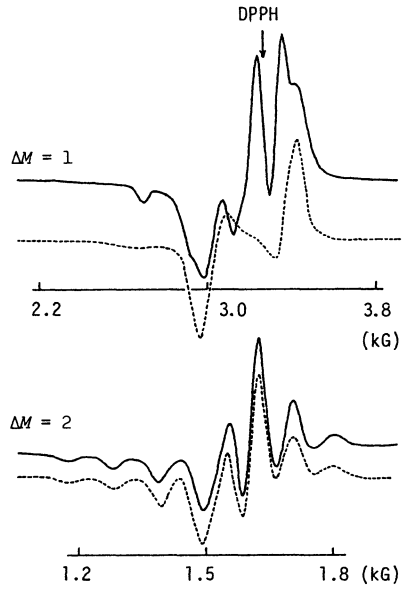

Fig. 1. ESR spectra of $Cu(H_{-3}G_5)$ in frozen aqueous solutions at 77 K: solid lines, observed spectra; dotted lines, simulation spectra obtained with the parameters of $g_{\parallel} = 2.21$, $g_{\perp} = 2.04$, $A_{\parallel} = 0.0112\ cm^{-1}$, $A_{\perp} = 0.001\ cm^{-1}$, $r = 4.25$ Å, $\xi = 5°$, $\Delta H_1 = 45$ G, and $\Delta H_2 = 25$ G (ΔH_1 and ΔH_2 express the line widths of $\Delta M = 1$ and 2 spectra, respectively). The sample solution was 0.010 M in $Cu(H_{-3}G_5)$, 0.30 M in $NaNO_3$, and buffered at pH 10.0 (at room temperature) with 0.05 M borate.

observed $\Delta M = 1$ spectrum due to dimers is superimposed by a monomer one. The 1:1 complexes of copper(II) with G_3 and G_n ($n \geq 4$) at pH ≈10 have planar coordination and are generally expressed as $Cu(H_{-2}G_3)$ and $Cu(H_{-3}G_n)$ respectively, where the m of $Cu(H_{-m}G_n)$ corresponds to the number of deprotonated peptide nitrogens. All the values of r and ξ thus determined are summarized in Table 1.[5)]

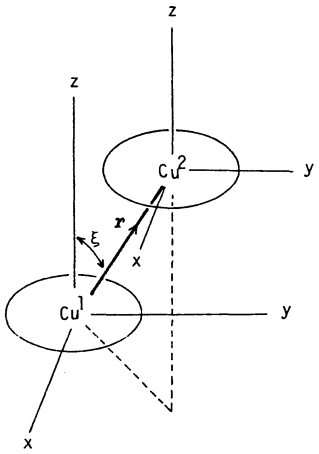

Fig. 2. Coordinate system for the dimers.

An inspection of Table 1 shows that the dimeric structure of $Cu(H_{-2}G_3)$ is quite different from the others'. Interestingly, its parameter values substantially agree with those for a dimer in crystals ($r = 3.03$ Å and $\xi = 40°$),[6)] where each of the two copper atom in a dimer is bonded to the terminal amino nitrogen and the two deprotonated peptide nitrogens of one peptide, and to the carboxylic oxygen of another peptide. This fact clearly demonstrates that $Cu(H_{-2}G_3)$ is dissolved in aqueous solutions as a dimer of the same structure as in crystals. On the other hand, the other dimers of $Cu(H_{-3}G_n)$ ($n \geq 4$) in frozen aqueous solutions have almost a common structure, where the monomeric halves of the parallel planar dimer lie almost right above each other with the separation of ≈4 Å (roughly, $\xi \approx 0°$). $Cu(H_{-3}G_5)$ in crystals form a quite different parallel planar dimer with much larger r and ξ values,[7)] indicating that there are at least two modes of intermolecular interaction for this complex.

The dimer formation constants (K for $2M \rightleftharpoons M_2$) of the present complexes at ≈0°C can be roughly estimated by comparison between the real integrated $\Delta M = 1$ spectral intensities of monomer and dimer species; the K values thus estimated are also listed in Table 1. The K value of $Cu(H_{-2}G_3)$ is the largest in the table, and this is probably associated with its unique dimeric structure, where the monomeric halves of

Table 1. Structural Parameters (r and ξ)[a] and Dimer Formation Constants (K)[b] for the Dimers of $Cu(H_{-3}G_n)$

n	r (Å)	ξ (degree)	K (M^{-1})
3[c]	3.3	40	4100
4	4.3	15	580
5	4.25	5	850
6	4.25	5	1200
8	4.25	5	580

a) Errors (limits of simulation): r, ±0.10 Å; ξ, ±5°.
b) K at ≈0°C. Errors: ±15%.
c) The dimer of $Cu(H_{-2}G_3)$.

the dimer are linked together more tightly and stably with two extra bridging carboxylic groups.[6] Interestingly, there is a "n" dependence of K for $Cu(H_{-3}G_n)$ ($n \geq 4$); the K value of $Cu(H_{-3}G_n)$ is subtly influenced by the non-coordinating part of the peptide. In fluid aqueous solutions of the present complexes, some degree of ESR line broadening caused by the dimerization was detected at low temperatures near 0°C, indicating that the K value becomes remarkably larger with a decreasing in temperature. In addition, this dimer formation was markedly depressed by the addition of ethanol. The K values of $Cu(H_{-3}G_n)$ ($n \geq 4$) in Table 1 are smaller than those for water-soluble porphyrins or metal porphyrins,[7a,b] but are comparable to those for nucleotides,[7c,d] dyes,[7e] phenanthroline and dipyridyl.[7f] These compounds are planar molecules with large π-electron systems, and their dimerization or self-association is the same in the temperature dependence and in the effect of alcohol as that of $Cu(H_{-3}G_n)$. Obviously, $Cu(H_{-3}G_n)$ also has a π-electron system composed of deprotonated amide groups and the metal ion. All these facts suggest that the dimerization of $Cu(H_{-3}G_n)$ in aqueous solutions proceeds by a mechanism similar to those for porphyrins, nucleotides, etc. $Cu(H_{-1}G_2)$ does not form dimers in aqueous solutions, as mentioned above, but do $Cu(H_{-2}G_2$-amide) and $Cu(H_{-1}G$-amide$)_2$, where G_2-amide and G-amide are glycylglycinamide and glycinamide, respectively. $Cu(H_{-2}$biuret$)_2$ also exhibits a remakable dimer formation. From these facts, it is clear that at least two deprotonated peptide nitrogens coordinating to a metal ion or the corresponding π-electron system is necessary for planar metal complexes to dimerize in aqueous solutions.

In conclusion, it has been first revealed in this study that there exists an attractive force between metal peptide complexes which possess deprotonated peptide nitrogens as ligand atoms; the force is sometimes strong enough for the formation of parallel planar dimers of the complexes. This fact is suggestive of the extreme importance of π-interaction between peptide bond units in biological systems.

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