ELECTROCHEMICAL AND ESR STUDIES ON Cu(II) COMPLEXES OF BLEOMYCIN AND ITS RELATED COMPOUNDS

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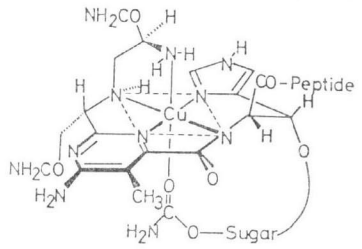
The 1:1 Cu(II) complexes of bleomycin (BLM) A2, BLM B2, *epi*-BLM B2, *iso*-BLM B2, depyruvamide-BLM A2, deglyco-BLM B2 and the structurally related peptides (P-5m, P-3A and P-3) have been comprehensively investigated by ESR and electrochemical methods. ESR spectra for Cu(II) complexes of BLM A2, *epi*-BLM B2, depyruvamide-BLM A2 and P-3 revealed the axially symmetric g-anisotropies. In contrast, ESR features of the *iso*-BLM B2, deglyco-BLM B2, P-5m and P-3A complexes, which lack the sixth ligation by the 3-*O*-carbamoyl group of mannose, exhibited rhombic g-anisotropies with decrease of the $A_{//}$ values. The cyclic voltamograms showed that all of the Cu(II) complexes underwent the well defined quasi-reversible one-electron Cu(II)/Cu(I) coupled redox reaction. The inverse of the redox potential, which measures the effective strength of the ligand field splitting, gave a linear relation with the observed $g_{//}$ value of each Cu(II) complex except for depyruvamide-BLM and P-3. The present results confirmed that the 3-*O*-carbamoyl group of the mannose moiety of the BLM molecule contributes to the stability of the metal site in BLM-Cu(II) complexes by ligation at the sixth coordination site.

On the basis of the square pyramidal structure shown by the X-ray crystallographic analysis of the P-3A-Cu(II) complex[1], a biosynthetic intermediate of bleomycin (BLM), the prolonged octahedral coordination geometry has been proposed for the chromophore of BLM-Cu(II) complex, where in the N^{π} and the deprotonated amide nitrogen of β-hydroxy-histidine, the N-1 of the 4-aminopyrimidine ring and the secondary amine occupy the square-basal positions with the primary amine at the square pyramidal position and the *O*-carbamoyl group at the sixth coordination site (Fig. 1)[2]. The ligation of the *O*-carbamoyl group has been suggested by the prohibition of the isomerization in BLM-Cu(II) complexes[2,3] which occurs in metal-free BLM under alkaline conditions by migration of the carbamoyl group from the 3-*O* to 2-*O* position of the mannose. The spectroscopic properties and potentiometric behaviors of BLM-Cu(II) complex have been consistently explained by the square pyramidal model[4]. The order of the stability of the Cu(II) complexes of BLM, *iso*-BLM[3] and *epi*-BLM[5] (which is formed in BLM-Cu(II) complex under an alkaline condition, see Fig. 2) has been shown to be *epi*-BLM > BLM > *iso*-BLM from the retention time of the CM-Sephadex chromatography[6]. This indicates that the ligation of the *O*-carbamoyl group contributes to the stability of the BLM-Cu(II) complex.

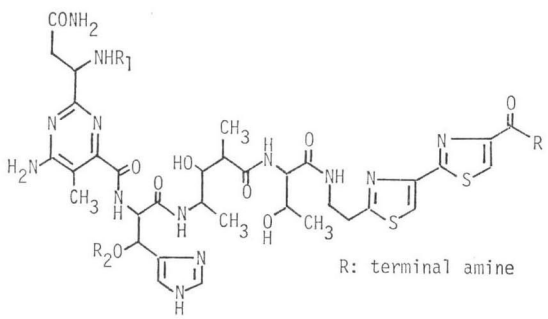
Fig. 1. Structure of BLM-Cu(II) complex.



The environment of the sixth coordination

Fig. 2. Structures of BLM and its related compounds.

Bleomycin and its derivatives



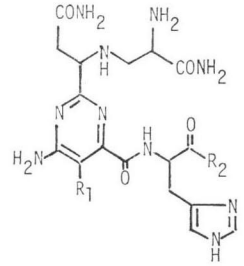
	R_1	R_2
BLM	: $-CH_2-CH(NH_2)-CONH_2$	disaccharide*
depyruvamide-BLM	: -H	disaccharide*
deglyco-BLM	: $-CH_2-CH(NH_2)-CONH_2$	-H

*2-O-(3-O-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl

iso-BLM : carbamoyl group at 2-O position of the mannose

epi-BLM : the stereochemistry of α-methine at the pyrimidine-2 is R (BLM: S)

Biosynthetic intermediates



	R_1	R_2
P-3 :	-H	-OH
P-3A:	-H	-Ala
P-5m:	$-CH_3$	$-NH-CH(CH_3)-CH(OH)-CH(CH_3)-CO-Thr$

site should be emphasized in BLM-Co(II) and BLM-Fe(II) complexes, because the electronic configuration, magnetic properties and the chemical reactivities are strongly dependent on the interaction between the d-orbital and the sixth axial ligand in the octahedral coordination geometry[7,8].

In the present work, the effects of the axial ligation of the α-amino and the *O*-carbamoyl group were investigated by electronic, CD, ESR and electrochemical measurements carried out for copper complexes of BLM and related compounds as shown in Fig. 2. In order to compile more detailed data on the coordination chemistry of the BLM-Cu(II) complex, in particular, the effect of the 3-*O*-carbamoyl ligation, ^{65}Cu-enriched complexes of BLM and related compounds were prepared. Their ESR parameters and redox potentials were studied in terms of ligand field splitting as a measure of the distortion of the metal chromophores in the Cu(II) complexes.

Experimental

All of the BLM and its related compounds used in this experiment were provided by A. FUJII, Y. MURAOKA and their associates at the research laboratory in Nippon Kayaku Co. Ltd. The aqueous solutions of Cu(II) complexes (5×10^{-3} M, pH 9) were prepared by dissolving the isolated Cu(II) complexes and/or by mixing the metal-free ligands with $CuSO_4$ in aqueous solution. In order to avoid the overlap of ESR hyperfine structures due to the natural-abundant copper isotopes (^{63}Cu and ^{65}Cu, both I=3/2), the ^{65}Cu-enriched isotope was used for the experiment. ESR spectra were recorded at 77 K, and g-values were calibrated with Li-TCNQ (g=2.0026) and Mn(II) in MgO (ΔH_{3-4}=86.9 G). Cyclic voltamograms were obtained by a Yanagimoto P-1000H cyclic voltammeter (1×10^{-4} M aqueous

solution at 25°C). Reagent grade $NaClO_4$ was used as the supporting electrolyte (0.1 M) and the pH-value was adjusted to 9.2 with sodium borate (0.05 M) or to 6.8 with phosphate buffer (0.025 M Na_2HPO_4 -0.025 M KH_2PO_4). Cyclic voltammetries of the freshly prepared Cu(II) complex solutions were carried out in 0.1 M ion-strength with a three-electrode system including a dropping mercury electrode.

Results and Discussion

Electronic and Circular Dichromism Spectra

Table 1 presents electronic and CD spectral data for the Cu(II) complexes of BLM, P-3A[9] and depyruvamide-BLM. Both the visible absorption maximum and the CD extrema for P-3A-Cu(II) complex were shifted to longer wave lengths compared with those for the BLM-Cu(II) complex. In contrast, those of the depyruvamide-BLM-Cu(II) complex were shifted to the shorter wave length. This complex appears to have a square-planar Cu(II) configuration because of the lack of the apical α-amino group.

According to the MO assumption of FABBRIZZI[10], i) the observed visible absorption band (ν_{d-d}) corresponds to the d_{xz}, $d_{yz} \rightarrow d_{x^2-y^2}$ transition for CuN_4 series and ii) the in-plane σ antibonding will rise in energy as the ligand coplanarity strengthens, while the π antibonding d_{xz} and d_{yz} orbitals will decrease in energy. Thus the blue shift in the ν_{d-d} as observed for the depyruvamide-BLM complex means a weakened axial interaction and an increase in the in-plane ligand field strength by reducing the number of N donors from five to four. The red shift of ν_{d-d} for the P-3A complex was recognized as an indication of the distortion from square-planar to square-pyramidal stereochemistry, apparently caused by the steric constraint.

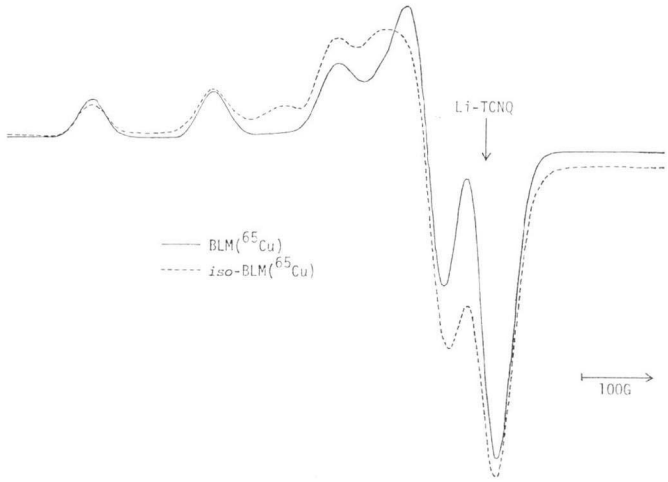
Table 1. Electronic and circular dichroism spectral data for Cu(II) complexes of BLM, P-3A and depyruvamide-BLM.

Complex	λ_{max}, nm(ε)	CD Extrema, nm ($\Delta\varepsilon$)	ΔH, nm
BLM-Cu(II)	595 (120)	555 (+1.21) 665 (−0.60)	110
P-3A-Cu(II)	625 (125)	580 (+0.56) 700 (+0.21)	120
Depyruvamide-BLM-Cu(II)	560 (120)	505 (+0.60) 590 (−0.35)	85

ESR Spectra

ESR spectra of BLM-^{65}Cu(II) and *iso*-BLM-^{65}Cu(II) complexes at pH 9.2 are shown in Fig. 3. These ESR spectra are typical of tetragonal-Cu(II) complex with $d_{x^2-y^2}$ ground state doublets. The ESR hyperfine component determined are consistent with the assignment of the anisotropic g- and A-tensor components as previously determined[4,11]. The spin Hamiltonian of the paramagnetic BLM-Cu(II) chromophore can be described by assuming axially symmetric g- and A-tensor components; $g_z=g_{//}=2.211$, $g_x=g_y=g_\perp=2.055$, $A_z=A_{//}=178\times10^{-4}$ cm^{-1} and $A_x=A_y=A_\perp$, respectively. Similar ESR spectra were obtained for depyruvamide-BLM-Cu(II) and P-3-Cu(II) complexes. We previously advanced the assumption that the considerable reduction in $A_{//}$ value noted for the BLM-Cu(II) complex is attributed to the apical coordination of the α-amino nitrogen. The effect of the apical coordination is now evident in comparison with the parameters of depyruvamide-BLM-Cu(II) complex ($g_{//}=2.174$, $A_{//}=193\times10^{-4}$ cm^{-1} and $g_\perp=2.055$), in which the $A_{//}$ value becomes larger than that of BLM-Cu(II) and conversely the $g_{//}$ value is smaller. A similarity is recognized between the ESR parameters of the depyruvamide-BLM-Cu(II) complex and the glycylglycyl-L-histidine (GGH)-Cu(II)

Fig. 3. ESR spectra of ^{65}Cu-enriched BLM and *iso*-BLM complexes at pH 9.2.



complex [$g_{//}=2.172$, $g_{\perp}=2.065$ and $A_{//}=206\times10^{-4}$ cm^{-1}][12]. In addition, the CD spectral parameters of the GGH-Cu(II) complex [490 nm (+0.91) and 580 nm (−0.40)] are also close to those of the depyruvamide-BLM-Cu(II) complex. The ESR parameters for depyruvamide-BLM-Cu(II) also imply that the Cu-N_4 chromophore involving the secondary amine nitrogen, pyrimidine (N-1) ring nitrogen, deprotonated peptide and N^{π} nitrogen of β-hydroxy-histidine is likely to take a square-planar geometry in the same manner as is already confirmed for GGH-Cu(II) complex[13].

ESR spectra of the *iso*-BLM, deglyco-BLM, P-5m and P-3A complexes showed the copperhyperfine structures with lower symmetric g-anisotropies. The typical ESR spectrum [*iso*-BLM-Cu(II) complex] is shown in Fig. 3. The line-width of $g_{\perp}$ branch is broadened by the non-equivalent spin-orbital interactions in the equatorial plane, that is $g_x \neq g_y$. Furthermore, the overlapped extra copper hyperfine structure gives rise to the $g_{//}$-hyperfine components probably due to a rhombic distortion in the octahedral ligand geometry of the copper chromophore. P-3A, P-5m and deglyco-BLM also exhibited rhombic g-anisotropies with a slight increase of the g_z-component and an decrease in the A_z values. A lower field shift of the g_z component may be caused by the displacement of the Cu(II) ion from the square basal N_4 plane as demonstrated by the X-ray crystallographic result of the P-3A-Cu(II) complex[1].

Cyclic Voltammetry (CV)

Although detailed structures of the coordination environment of the Cu(II) site have been often discussed based on the ESR parameters, perturbations on the g- and A-tensors resulted by axial ligations are more or less insensitive for $d_{x^2-y^2}$ ground state. In order to characterize the coordination environment of each Cu(II) complex more rigorously, the redox potentials ($E_{1/2}$) were measured, and relationships between the redox potentials and ESR parameters were studied.

The cyclic voltamograms measured for the Cu(II)/Cu(I) coupled reaction in the BLM and deglyco-BLM complexes are shown in Fig. 4. In both cases the cyclic voltamograms displayed the figures typical of quasi-reversible redox-reaction at $E_{1/2}$ (*vs.* SCE); −0.57 V (BLM complex) and −0.42 V (deglyco-BLM complex) respectively. DABROWIAK *et al.* observed one-electron reduction wave at −0.53 V (*vs.* SCE) and totally irreversible CV behavior for the Cu(II)/Cu(I) couple in BLM-Cu(II) com-

plex[14]. We assumed that the observed electrode potential can be attributed to the one electron reduction of divalent copper ion in the metal-chromophore, because the Cu(II) complexes of P-3A and pentaazacyclopentadecane (15-ane-N_5) gave nearly reversible cyclic voltamograms which are well defined to the one-electron metal centered reduction at $E_{1/2}=-0.38$ V (P-3A) and -0.57 V (15-ane-N_5) respectively[15].

In terms of the MO binding scheme as defined to the Cu(II)-complex with $d_{x^2-y^2}$ ground state[16], a positive hole occupying the $d_{x^2-y^2}$ orbital with lowest energy level should be effectively stabilized by the in-plane ligand field splitting. Therefore, larger ligand field splitting results in a higher degree of negative shift in the observed $E_{1/2}$ value. The positive shift of $E_{1/2}$ for deglyco-BLM-Cu(II) complex indicates that the ligand field splitting is weakened in comparison with that in the BLM-Cu(II) complex. Thus the role of the sugar moiety in contributing to the stability of the BLM-Cu(II) chromophore can be clearly demonstrated based on the present CV experiments. We studied the relationship between the $E_{1/2}$ values and observed ESR parameters of these Cu(II) complexes. With reference to MARGERUM's bonding scheme proposed for Cu(III)/Cu(II) couple of peptide complex[17], the redox potential measuring the difference in the ground state energy of Cu(II) and Cu(I) in the crystal field can be written as

$$E_{1/2}=(1-k)\Delta+E' \quad (1)$$

where E' is the energy difference between the Cu(I) and the Cu(II) ions without ligand field, which is independent of ligand effect over the limited energy range of the Cu(II) complexes.

Fig. 4. Cyclic voltamograms for Cu(II) complexes of BLM and deglyco-BLM at pH 9.2.

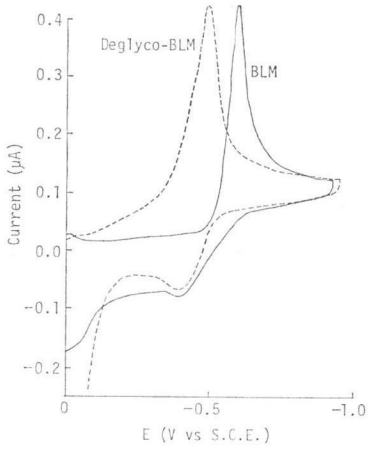
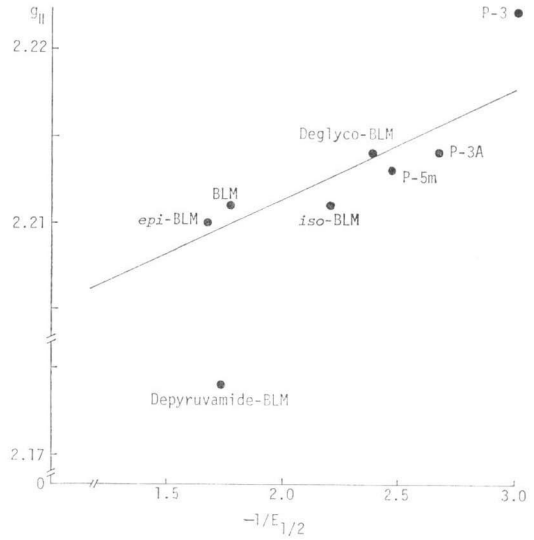


Fig. 5. Relationship between $g_{//}$ and $-1/E_{1/2}$ values of BLM and its related compounds.



On the other hand, the $d_{x^2-y^2}$ ground state electronic configuration can be mixed with the electronic exited state due to the spin-orbital interaction, whose magnitude is inversely proportional to the ligand field splitting (Δ). The observed ESR parameters are thus expressed as a function of Δ, the spin densities on the copper 3d orbital, α^2, and the spin-orbital coupling constant for free Cu(II) ion, $\lambda=-828$ cm^{-1};

$$g_{//}=2.0023(1-4\alpha^2\lambda/\Delta) \quad (2), \qquad g_{\perp}=2.0023(1-\alpha^2\lambda/\Delta) \quad (3)$$

$$A_{//}=p[-K-(4/7)\alpha^2+(g_{//}-2.0023)+3/7(g_{\perp}-2.0023)] \quad (4)$$

$$A_{\perp}=p[-K+(2/7)\alpha^2+11/14(g_{\perp}-2.0023)] \quad (5)$$

where P is the free ion dipole term (0.036 cm^{-1}) and K denotes the Fermi's isotropic term, which is usually taken to be K=(3/7)[18]. The α^2-value calculated was actually the same value (~0.73) for the BLM, BLM isomers and the biosynthetic intermediates. The result means that the α^2-value can be fixed to be the constant value everywhere. With replacing the relation (2) into (1), a linear relationship holds between the $g_{//}$-components and the $1/E_{1/2}$ values. In fact, an excellent linear relation has been already confirmed between the $g_{//}$ and $1/E_{1/2}$ values for the Cu(II) complexes of several tetra and pentadentate cyclic polyamines[15]. A plot of $g_{//}$ value *versus* $1/E_{1/2}$ also shows the linear relation for the BLM, BLM isomers and the intermediates complexes, except for the cases of depyruvamide-BLM and P-3 as seen in Fig. 5. The figure shows that the stability for each Cu(II) chromophore of BLM and its related compounds decreases in the following order; *epi*-BLM>BLM>*iso*-BLM>deglyco-BLM>P-5m>P-3A>P-3. Of particular interest is the fact that the order of stability determined for the *epi*-BLM, BLM and *iso*-BLM-Cu(II) complexes is consistent with that assumed from the ion-exchange chromatography, where the retention time of the elution delayed in the reversed order; *epi*-BLM>BLM>*iso*-BLM.

Based on the present experiments, some important features of the ESR and electrochemical properties of the BLM-Cu(II) complex can be extracted.

i) There is an apparent difference in the $A_{//}$ and $E_{2/1}$ values between the Cu(II) complexes with and without the sugar group in BLM ligands. Removal of the sugar group from the BLM molecule destabilizes the Cu(II) chromophore as seen from the parameters of deglyco-BLM, where the $A_{//}$ value decreases and the $E_{1/2}$ shows a positive shift in comparison with those of the BLM-Cu(II) complex.

ii) The symmetry of the anisotropic g-tensor is reduced by removal of the sugar moiety, but not much by elimination of the terminal amine and the bithiazole group. $E_{1/2}$ values also were not affected by removal of the terminal peptide part.

iii) Of interest is the fact that the P-3-Cu(II) complex, the Cu(II) complex with the smallest molecular size examined, again showed the axially symmetric g- and A-anisotropies. This may be due to the involvement of the C-terminal carboxylate of P-3 in the ligation.

iv) The quasi-reversible redox reaction of the BLM-Cu(II) complex probably can be attributed to the slow diffusion rate as caused by the large molecular size of the metallo-BLM. In fact, the P-3A-Cu(II) complex with much smaller molecular dimension revealed the nearly reversible CV pattern well defined to the metal-centered one-electron redox reaction.

v) The effect of the *O*-carbamoyl ligation in BLM is now evident; the $E_{1/2}$ of *iso*-BLM positively shifted from that of BLM-Cu(II) complex can be ascribed to a distortion, which may be caused by lacking the ligation of the 3-*O*-carbamoyl at the sixth coordination site.

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