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METAL COORDINATION CORE OF BLEOMYCIN : COMPARISON OF METAL COMPLEXES BETWEEN BLEOMYCIN AND ITS BIOSYNTHETIC INTERMEDIATE

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Summary : On the basis of electron spin resonance results, the 1:1 Cu(II), Co(II), Co(II)-O_2, and Ni(III) complexes of bleomycin(BLM) have been compared with the corresponding metal complexes of its biosynthetic intermediate(P-3A). The present study suggests that (1) P-3A is an useful ligand for the clarification of metal-binding sites of BLM; (2) the secondary amine, pyrimidine ring nitrogen, deprotonated peptide nitrogen of histidine residue, and histidine imidazole groups as planar ligand donors, and the α-amino group as axial donor, are substantially important for metal-coordination of BLM; and (3) the sugar and bithiazole portions of BLM probably contribute to stabilization of Co(II)-O_2 adduct complex and axial sixth coordination of Cu(II) and Ni(III) complexes.

Introduction

BLM, a glycopeptide antibiotic which has been used for the treatment of selected human neoplastic disease,[1] was originally isolated as a Cu(II) complex from fermentation broths of Streptomyces verticillus.[2,3] The drug has both metal-chelating[4,5] and DNA-binding sites,[6,7] and its activity is presumably owing to this bifunctionality. However, the metal coordination core of BLM has never been established. The biosynthetic intermediate of BLM, P-3A, is a peptide isolated from a culture of Streptomyces verticillus and is structurally related to BLM. Herein, the coordination environment of BLM has been discussed through the comparison of metal complexes between BLM and P-3A.

Experimental

BLM-A_2 and P-3A purified were kindly supplied from Nippon Kayaku Co. Ltd. The 1:1 Cu(II) and Co(II) complexes of the antibiotics were obtained by the mixing of the ligand and metal nitrate in aqueous solution(pH 6.8), and a fully deaerated condition was kept for the preparation of the Co(II) complexes. The 1:1 Ni(III) complexes were also prepared

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Abbreviations used : BLM, bleomycin; P-3A, biosynthetic intermediate of bleomycin; ESR, electron spin resonance; Li-TCNQ, 7,7,8,8-tetracyanoquinodimethane lithium salt

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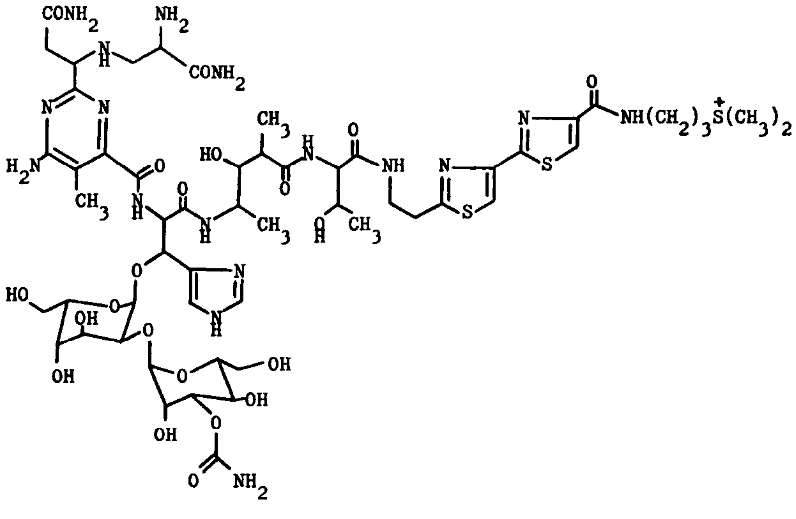
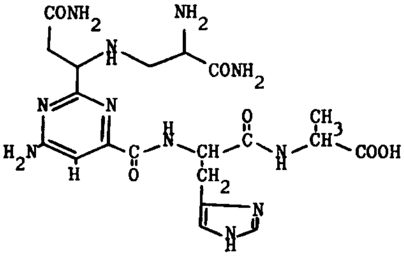


BLM-A_2



P-3A

by the oxidation of the corresponding Ni(II) complexes with $Ir^{IV}Cl_6^{2-}$.[8] X-Band ESR spectra of magnetically dilute aqueous glasses containing the metal-antibiotic complexes(1.0 mM) were measured at 77 K using a JES-FE-3X spectrometer operating with 100 KHz magnetic field modulation. The g values were determined taking Li-TCNQ(g=2.0026) as a standard, and the magnetic fields were calculated by the splitting of Mn(II) in MgO(ΔH_{3-4}=86.9 G).

Results and Discussion

Figure 1 and Table I summarize the ESR spectral data for the Cu(II), Co(II), Co(II)-O_2, and Ni(III) complexes of the two antibiotics at pH 6.8. The average g values and A tensor of the P-3A-Cu(II) complex are close to those of the BLM-A_2-Cu(II) complex, though the spectral anisotropy(g_{zz}=2.214, g_{yy}=2.133, g_{xx}=2.078, $A_{\parallel}$=167 G, $A_{\perp}$=72 G) of the former

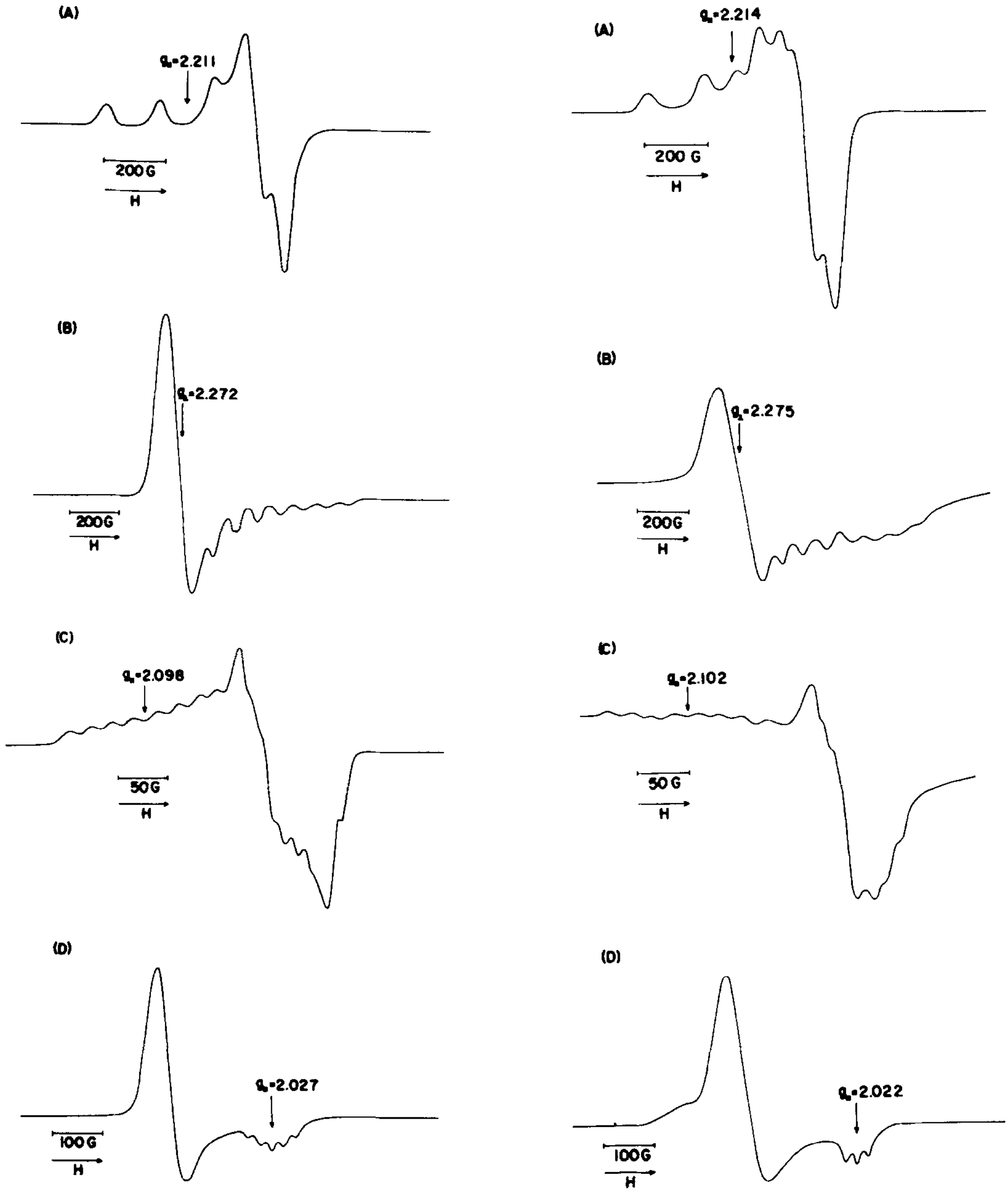


Figure 1 ESR spectra for Cu(II)(A), Co(II)(B), Co(II)-O_2(C), and Ni(III)(D) complexes of BLM-A_2(left) and P-3A(right) at 77 K

reveals lower symmetry of the Cu(II) site(see Figure 1A). Recent X-ray crystallographic result for the 1:1 P-3A-Cu(II) complex indicated a distorted square-pyramidal structure

Table I ESR Parameters for Cu(II), Co(II), Co(II)-O_2, and Ni(III) Complexes of BLM-A_2 and P-3A

	Cu(II)			Co(II)			Co(II)-O_2			Ni(III)		
	$g_\parallel$	$g_\perp$	$A_\parallel^{Cu}$,G	$g_\parallel$	$g_\perp$	$A_\parallel^{Co}$,G	$g_\parallel$	$g_\perp$	$A_\parallel^{Co}$,G	$g_\parallel$	$g_\perp$	$A_\parallel^{N}$,G
BLM-A_2	2.211	2.055	183	2.025	2.272	92.5	2.098	2.007	20.2	2.027	2.169	22.4
P-3A	2.214	2.133 2.078	167	2.027	2.275	93.8	2.102	2.007	22.4	2.022	2.235 2.163	23.5

which involves the secondary amine, pyrimidine, deprotonated peptide, histidine imidazole, and α-amino nitrogen donors.[9] The four Cu-N distances of the basal plane range from 1.86 to 2.12 Å, and the fifth axial Cu-N distance is 2.28 Å. Whereas, the sugar and bithiazole portions in BLM-A_2 probably make a contribution to approximately axial symmetry of the 1:1 BLM-A_2-Cu(II) complex.

Of special interest is the fact that P-3A forms low-spin Co(II) and its oxygen adduct complexes similar to BLM-A_2.[5] Under the anaerobic condition, the 1:1 P-3A-Co(II) complex also showed the ESR spectrum which is a nearly axial symmetry with the relationship of $g_\perp > g_\parallel \simeq 2.0$ and is characteristic of a penta-coordinated square-pyramidal configuration with the unpaired electron in the d_{z^2} orbital(see Figure 1B).[10] By oxygen-bubbling of the P-3A-Co(II) complex, the ESR spectrum drastically changed to that of mono-oxygenated low-spin Co(II) complex(see Figure 1C). The A_{iso}^{Co} values were estimated to be 15.0 and 16.7 G for the Co(II)-O_2 complexes of BLM-A_2 and P-3A, respectively. A_{iso}^{Co} values were calculated from the equation of $A_{iso}^{Co}=(A_\parallel^{Co}+2A_\perp^{Co})/3$. The $A_\parallel^{Co}$ and $A_\perp^{Co}$ values were as follows; $A_\parallel^{Co}=20.2$ and $A_\perp^{Co}=12.4$ G(BLM-A_2 complex) and $A_\parallel^{Co}=22.4$ and $A_\perp^{Co}=13.8$ G(P-3A complex). The effective g values, the relationship of $g_\parallel > g_\perp \simeq 2.00$, and the relative small A_{iso}^{Co} value of these oxygen adduct complexes suggest a considerable delocalization of the unpaired electron from the Co(II) ion. However, it is noted that the oxygenated P-3A-Co(II) complex is more unstable than the BLM-A_2-Co(II)-O_2 complex and undergoes further oxidation to a marked degree. The detailed kinetic and thermodynamic studies of these Co(II)-O_2 complex systems are now under way.

Oxidation of the Ni(II) complexes of BLM-A_2 and P-3A in aqueous solution(pH 6.8) yielded paramagnetic products characterized as Ni(III) complexes(see Figure 1D). The ESR results are best described by a tetragonally distorted octahedral geometry with the electron in an orbital which has a large amount of d_{z^2} character.[11] The 1:1 BLM-A_2-Ni(III) complex gave the ESR spectrum with $g_{\perp} > g_{\parallel} (g_{zz})$ and five-line hyperfine splittings in the $g_{\parallel}$ region. The g_{xx} and g_{yy} values are approximately equal and are not resolved. On the other hand, the ESR spectrum of the 1:1 P-3A-Ni(III) complex showed $g_{xx}, g_{yy} > g_{zz}$ and three-line hyperfine patterns in the g_{zz} region. These results indicate species which have two and one nitrogen nuclei(^{14}N, I=1) bound in the axial position, respectively, for the Ni(III) complexes of BLM-A_2 and P-3A. Complexes of Ni(III) which show strong axial nitrogen coordination have been known to be both kinetically and thermodynamically more stable than those in which the axial donor is water.[11]

In conclusion, P-3A is an useful ligand to elucidate the metal-binding sites of BLM in which the secondary amine nitrogen, pyrimidine(N-1) ring nitrogen, deprotonated peptide nitrogen of histidine residue, and histidine imidazole(N-1) nitrogen as planar ligand donors, and the α-amino nitrogen as axial donor, are substantially important. The sugar and bithiazole portions of BLM, however, would contribute to stabilization of the Co(II)-O_2 adduct complex and axial sixth coordination of the Cu(II) and Ni(III) complexes.

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