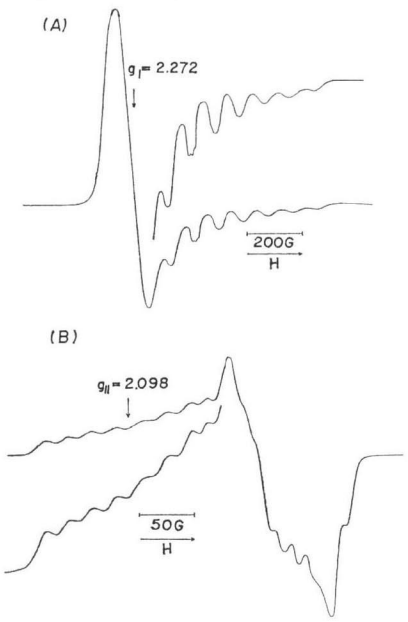
OXYGEN BINDING TO COBALT(II)-BLEOMYCIN

Sir:

Bleomycin (BLM), a glycopeptide antibiotic which has been used for the treatment of selected human neoplastic diseases,[1] was originally isolated as a Cu(II) complex from fermentation broths of *Streptomyces verticillus*.[2] The drug has both metal-chelating[3] and DNA-binding sites,[4] and its activity is presumably related to this bifunctionality. The BLM-induced degradation of DNA is enhanced by the presence of reducing agents such as 2-mercaptoethanol and ascorbate, Fe(II), and dissolved oxygen.[5] A mechanism involving a labile oxygen-iron complex, has been suggested for the strand scission of DNA by BLM.[5,6] Therefore, the structural and physico-chemical knowledge of BLM-metal complexes, in particular, oxygen binding to metal-BLM, appear to be a prerequisite to an understanding of its functions. Herein, it is shown by electron spin resonance (ESR) study that the 1:1 BLM-Co(II) complex has penta-coordinated square-pyramidal geometry and its oxygen adduct complex is mono-oxygenated low-spin Co(II) type.

Purified bleomycin-A_2 (BLM-A_2) was kindly supplied by Nippon Kayaku Co. Ltd. The 1:1 Co(II) complex of the antibiotic was obtained by mixing the metal-free BLM-A_2 and metal nitrate in aqueous solution (pH 6.8) under fully deaerated conditions. The X-band ESR spectra of magnetically dilute aqueous glasses containing the metal-antibiotic complex (10^{-3} M) 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).

Fig. 1. ESR spectra of BLM-A_2-Co (II) (A) and its oxygen adduct (B) complexes at 77 K



Under anaerobic conditions, the 1:1 BLM-A_2-Co(II) complex showed clearly an ESR spectrum which indicates a nearly axial symmetry about the Co(II) ion at 77 K (see Fig. 1A). The one axial ligand donor is a nitrogen atom, as demonstrated by the nitrogen (^{14}N, I=1) three-line superhyperfine pattern split, superimposed on the eight-line ^{59}Co (I=7/2) parallel hyperfine pattern with splittings, $A_\parallel^{Co}$. The X-band ESR feature of the 1:1 BLM-A_2-Co (II) complex is very similar to that of the 1:1 stoichiometric complexes of low-spin Co(II)-SCHIFF bases with nitrogenous bases,[7] deoxy Co(II)-myoglobin,[8] and deoxy Co(II)-peroxidase.[9] The observed relationship of $g_\perp > g_\parallel \cong 2.0$, the presence of superhyperfine splitting from one axial ^{14}N atom, and the apparent absence of superhyperfine splitting from the in-plane ^{14}N atoms indicate that the BLM-A_2-Co(II) complex is low-spin Co(II) ($3d^7$, S=1/2) complex in a pentacoordinated square-pyramidal configuration and that the unpaired electron is in the d_{z^2} orbital.[7] The $A_\perp^{Co}$ value is estimated to be less than 12.5 G, as judged from the peak-to-peak line width of the $g_\perp$ extremum. The A_{iso}^{Co} value of the BLM-A_2-Co(II) complex is estimated to be approximately 40 G from the equation of $A_{iso}^{Co} = (2A_\perp^{Co} + A_\parallel^{Co})/3$.

After O_2-bubbling the 1:1 BLM-A_2-Co(II) complex, the ESR spectrum drastically changed to that as shown in Fig. 1B. This ESR characteristic resembles closely that of various mono-oxygenated low-spin Co(II) complexes (see Table 1). The A_{iso}^{Co} value is estimated to be 15.0 G for the BLM-A_2-Co (II)-O_2 adduct complex, from $A_\perp^{Co}$ and $A_\parallel^{Co}$ values. The effective g values, the relationship of $g_\parallel > g_\perp \cong 2.00$, and the relatively small A_{iso}^{Co} value of the BLM-A_2-Co (II)-O_2 complex suggest a considerable

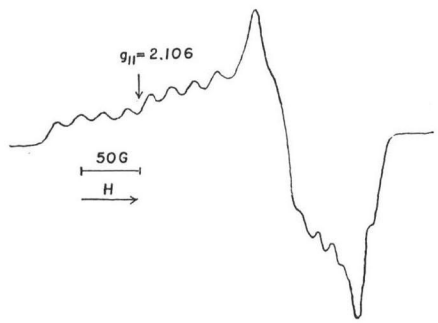
Table 1. ESR Parameters of some cobalt (II)–oxygen complexes

Compound	$g_\perp$	$g_\parallel$	$A_\perp^{Co}$, G	$A_\parallel^{Co}$, G	$A_\parallel^{N}$, G	Ref.
Co(bleomycin)	2.272	2.025		92.5	13	This work
Co(bleomycin)(O_2)	2.007	2.098	12.4	20.2		
Co(acacen)py	2.44	2.011		97.53	15.7	(7)
Co(acacen)py(O_2)	1.999	2.082	10.73	19.64		
Co(acacen)CN-py	2.39	2.013		101.17	16.24	(7)
Co(acacen)CN-py(O_2)	1.997	2.080	10.30	20.11		
Co(3-methoxysalen)py_2	2.33	2.038		78.0	12.5	(10)
Co(3-methoxysalen)py(O_2)	1.997	2.079	10.25	17.3		
Co(Ts-phthalocyanine)	2.27	2.068		107		(11)
Co(Ts-phthalocyanine)(O_2)	2.004	2.075	7.9	14.9		
Deoxy Co myoglobin	2.32	2.03	6.2	75	17	(8)
Oxy Co myoglobin	2.007	2.08	9	16		
Deoxy Co peroxidase	2.34	2.03	10.5	78	18.3	(9)
Oxy Co peroxidase	2.01	2.10	13.6	23.2		

delocalization of the unpaired electron from the Co (II) ion. HOFFMAN *et al.*[7] estimated that an A_{iso}^{Co} value of 10–14 G corresponds to about 90% transfer of the spin density from Co (II) to oxygen in their ESR study of mono-oxygenated Co (II)-SCHIFF bases complexes. Therefore, the paramagnetic center of the BLM–A_2–Co (II)–O_2 adduct complex may be also formally described as Co (III)–O_2^-.

Fig. 2 illustrates the ESR spectrum of the BLM–A_2–Co (II)–O_2 complex in the presence of calf thymus DNA (0.1 mM). The effective g values and A tensor obtained indicate that the Co (II)–O_2 coordination mode and the electron spin delocalization are substantially similar in the presence and absence of DNA. However, the addition of DNA gives a distinct effect on the ESR features of the BLM–A_2–Co (II)–O_2 complex. The $g_\parallel$ value (2.106) in the presence of DNA is larger by 0.008 unit than that in the absence of DNA, and the $g_\perp$ value (2.004) is smaller by 0.003. The line shape of hyperfine structure is also different from that in the absence of DNA. The narrowed hyperfine structure and small A^{Co} values ($A_\parallel^{Co}$=18.9 and $A_\perp^{Co}$=11.5 G) are clearly observed in the presence of DNA. These differences suggest that the orientation of the bound oxygen molecule relative to the Co (II) plane is somewhat different in the presence and absence of DNA. Presumably, the change of the orientation of the dioxygen molecule is due to the interaction of DNA with the bithiazole ring and terminal amine group of BLM–A_2 molecule.[4]

Fig. 2. ESR spectrum of BLM–A_2–Co(II)–O_2 complex in the presence of DNA at 77 K



In conclusion, the present ESR results strongly indicate that the 1:1 BLM–A_2–Co (II) complex has a square-pyramidal geometry with an axial nitrogen donor, and that oxygen is incorporated in the vacant sixth position of the BLM–A_2–Co (II) complex. The present direct evidence for oxygen binding to Co (II)–BLM provides useful information concerning an oxygen-labile BLM–Fe (II) complex which plays an important role in DNA degradation.

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