Monomeric Cobalt(II)-Oxygen Adducts of Bleomycin Antibiotics in Aqueous Solution. A New Ligand Type for Oxygen Binding and Effect of Axial Lewis Base

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Abstract: In aqueous solution, new Co(II) complexes and their dioxygen adducts of bleomycin (BLM) and its related ligands were characterized by electron spin resonance (ESR) spectroscopy. These ESR features are typical of a low-spin square-pyramidal Co(II) complex with the $(d_{xz})^2(d_{yz})^2(d_{xy})^2(d_{z^2})^1$ configuration and of a monooxygenated low-spin Co(II)-dioxygen adduct complex. The addition of DNA had a distinct effect on the ESR parameters of the BLM-Co(II)-O_2 complex, suggesting a change in the orientation of the bound O_2 molecule relative to the Co(II) plane. The $g_\perp$ and A^{Co} values in the depyruvamide- (dep) BLM-Co(II)-base (B) complexes decreased in the order pyridine > imidazole > *N*-methylimidazole > ammonia > methylamine and $H_2S > H_2O$, consistent with the order of pK_a in these external bases. In the dep-BLM-Co(II)-B-O_2 complexes, there was also a good correlation between the ^{59}Co hyperfine coupling constant and the pK_a of the bases. The pH-dependent axial donor change was observed in the Co(II) complex and its dioxygen adduct of deamido-BLM. BLM ligands showed a definite new and unique coordination environment for the monomeric Co(II)-dioxygen adduct complex.

Introduction

Monomeric Co(II)-dioxygen adduct complexes are of chemical and biological interest.[1] Typical examples include the Co(II)-porphyrins,[2] Co(II) complexes with synthetic tetraaza macrocyclic ligands,[3] and Co(II) complexes of salicylaldimine[4] and ketoimine.[5] All these Co(II) complexes form 1:1 adducts with dioxygen in nonaqueous solutions containing the added Lewis base. Four-coordinate Schiff base, porphyrin, and macrocyclic complexes of Co(II) normally are poor oxygen binders, whereas their corresponding five-coordinate base adducts readily bind dioxygen. On the other hand, most of the water-soluble dioxygen cobalt complexes are binuclear and can be correctly formulated as a μ-peroxo low-spin Co(III) complex. The use of protonic solvents often results in the irreversible dioxygen oxidation of the metal complex. The exceptions reported are the 1:1 complexes, $[Co(CN)_5(O_2)]^{3-}$,[6] vitamin $B_{12}(O_2)$,[7] and Co(hemoglobin)(O_2).[8] Thus, a delicately controlled ligand environment may produce a monomeric Co(II)-dioxygen complex, even in aqueous solution.

Bleomycins (BLM) are a family of glycopeptide antibiotics clinically prescribed for treatment of selected neoplastic diseases.[9] This drug, which both chelates metal ions and binds to deoxyribonucleic acid (DNA), induces a degradation of DNA in a reaction that depends on the presence of ferrous ion and molecular oxygen.[10] BLM appears to function by an iron-mediated redox mechanism. We have observed important effects of fifth axial nitrogen coordination to iron on oxygen activation of the BLM antibiotics, and of a site-specific oxygen radical on DNA cleavage by BLM.[11] From the comparison of several metal complexes, including BLM, its biosynthetic intermediate (P-3A), and pyruvamide- (dep) BLM, it was noted that the β-aminoalanine-pyrimidine-histidine portion of BLM is necessary for metal binding.[12] For the 1:1 P-3A-Cu(II) complex, in fact, recent X-ray crystallographic analysis discovered a unique distorted square-pyramidal structure with a N_5 donor set.[13] The ligand, P-3A, is an intermediate compound of BLM biosynthesis and lacks the sugar and bithiazole portions of the BLM molecule. In addition, a previous potentiometric study suggested that other divalent metal complexes of BLM have a coordination environment similar to that of the BLM-Cu(II) complex with a substantially square-pyramidal configuration.[14]

Using electron spin resonance (ESR) spectroscopy, the low-spin BLM-Co(II) complex and its dioxygen adduct were compared with those of P-3A, iso-BLM, dep-BLM, and deamido-BLM (see Figure 1). Iso-BLM[15] and deamido-BLM[16] are the products of carbamoyl migration in the sugar of the BLM molecule and of BLM inactivated by BLM hydrolase, respectively. In particular, the axial base effect on the dep-BLM complexes and the pH-dependent axial donor change in the deamido-BLM complexes are discussed in detail. The present results show a new ligand environment for monomeric Co(II)-dioxygen adduct complex in aqueous solution, and also provide useful information about an oxygenated BLM-Fe(II) complex which is significant on the action of BLM.

Experimental Section

Purified BLM-A_2, iso-BLM-A_2, dep-BLM-A_2, and deamido-BLM-A_2 which contain 3-aminopropyldimethylsulfonium ($R = -NH(CH_2)_3S^+(CH_3)_2$) at the terminal amine were kindly provided by Nippon Kayaku

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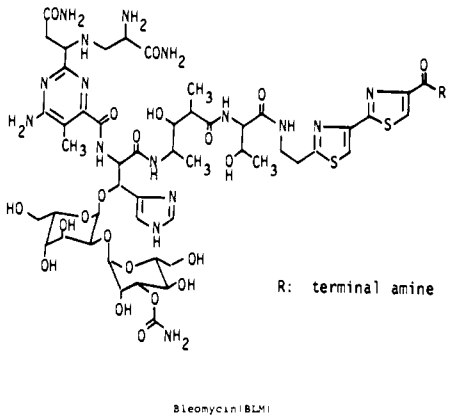
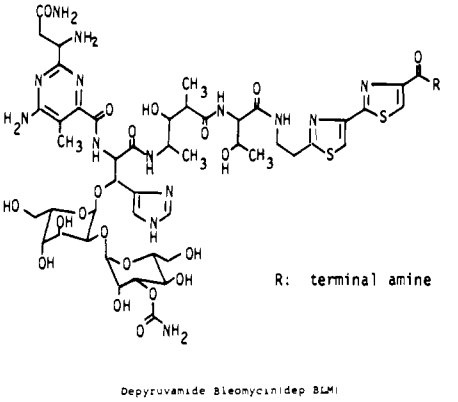
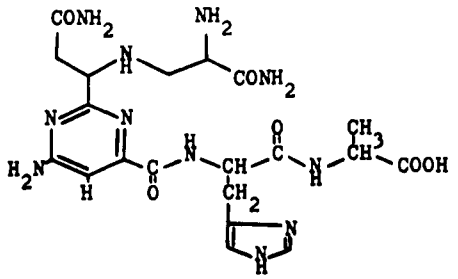


Bleomycin(BLM)



Depyruvamide Bleomycin(dep BLM)



P-3A

Figure 1. Bleomycin and its related compounds.

Co. Ltd. P-3A, a biosynthetic intermediate of BLM, was a generous gift of Dr. T. Takita, Institute of Microbial Chemistry. A standard Co(II) solution was prepared from reagent-grade material ($Co(NO_3)_2 \cdot 6H_2O$) and standardized by EDTA complexation. Calf thymus DNA was purchased from P-L Biochemicals, Inc. All other reagents were the highest quality available, and deionized water was used throughout the experiments.

The 1:1 Co(II) complexes of BLM and its related compounds were prepared by mixing the antibiotics and Co(II) ion in an aqueous solution (pH 6.8) under fully deaerated conditions. In the case of dep-BLM complexes, samples were made up with a large molar excess of bases. Anaerobiosis was achieved by using a vacuum line or by addition of a few milligrams of sodium borohydride. Deoxygenated samples were anaerobically transferred to an ESR quartz tube and immediately frozen in liquid nitrogen (77 K). These samples could be oxygenated by exposure to air or by 100 mm of oxygen pressure at 20 °C for a few seconds, and again frozen rapidly in liquid N_2. Samples of oxygenated species could also be prepared directly by the reaction of the antibiotics and Co(II) ion in an aqueous solution (pH 6.8) in the open air.

The X-band ESR spectra of magnetically dilute aqueous glasses containing the antibiotic-Co(II) complex (1.0 mM) were measured at 77 K using a JES-FE-3X spectrometer operating with a 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($\Delta H_{3-4} = 86.9$ G).

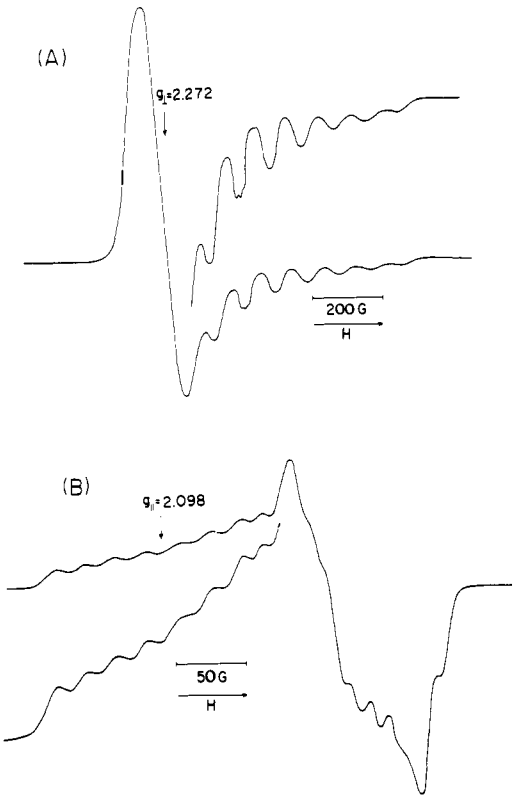


Figure 2. ESR spectra of BLM-Co(II) (A) and its oxygen adduct (B) complexes at 77 K.

Results and Discussion

ESR Characteristics of BLM-Co(II) and Its Dioxygen Adduct Complexes. Under anaerobic conditions, the 1:1 BLM-Co(II) complex clearly exhibits an ESR spectrum with a nearly axial symmetry about the Co(II) ion and an eight-line parallel hyperfine pattern due to the interaction with ^{59}Co ($I = {}^7/_2$) nucleus (see Figure 2A). This ESR feature is characteristic of the low-spin d^7 ($S = {}^1/_2$) and five-coordinated square-pyramidal Co(II) type, with the unparied electron in the d_{z^2} orbital. This is inferred from the presence of three-line superhyperfine splittings from the one axial nitrogen (^{14}N, $I = 1$), the observed relationship of $g_\perp > g_\parallel \simeq 2.0$, and the apparent absence of superhyperfine splitting from the in-plane ^{14}N donor atoms. The ESR parameters for the BLM-Co(II) complex were as follows: $g_\perp = 2.272$, $g_\parallel = 2.025$, $A_\parallel^{Co} = 92.5$ G, and $A_\parallel^{N} = 13$ G. The $A_\perp^{Co}$ value was estimated to be less than 12.5 G, as judged from the peak-to-peak line width of the $g_\perp$ extremum.[17] The ESR parameters of the BLM-Co(II) complex are remarkably similar to those of five-coordinate Co(II) complexes of the Schiff base (SB) and prophyrin (Por) (see Table 1). The (SB)Co(B) complexes typically have ESR values for $g_\perp$ of 2.30-2.45 and $g_\parallel$ of 2.01-2.02, while the (Por)Co(B) complexes have $g_\perp$ values of ~2.33 and $g_\parallel$ of 2.03.[1]

With oxygenation, the ESR spectrum of the low-spin BLM-Co(II) complex undergoes a drastic change, as shown in Figure 2B. The signal at $g = 2.272$ disappears, with a new symmetrical signal replacing it at $g \sim 2.0$. The original ^{59}Co hyperfine splittings of 92.5 G are replaced with much smaller splittings due to ^{59}Co of 20.2 G. The effective g values, the relationship of $g_\parallel > g_\perp \simeq 2.00$, and the relatively smaller A_{iso}^{Co} value[22] of the BLM-Co(II)-O_2 complex strongly suggest that the unpaired spin density no longer resides on the cobalt metal center, but instead resides on the dioxygen moiety. These ESR characteristics closely resemble those of various monooxygenated low-spin Co(II) complexes (see Table II). Hoffman et al. 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 the mo-

(17) The A_{iso}^{Co} value of the BLM-Co(II) complex is estimated to be 40 G from the equation $A_{iso}^{Co} = (2A_\perp^{Co} + A_\parallel^{Co})/3$.

Table I. ESR Parameters of Co(II) Complexes of Bleomycin and Its Related Ligands

complex	$g_\perp$	$g_\parallel$	$A_\parallel^{Co}$, G	$A_\parallel^{N}$, G	pK_a of base
(BLM)Co	2.272	2.025	92.5	13	7.72
(BLM)Co(DNA)	2.272	2.026	92.5	13	
(P-3A)Co	2.275	2.027	93.8	not detd	
(iso-BLM)Co	2.270	2.025	93.0	13	
(dep-BLM)Co(H_2O)	2.368	2.025	123.3		(15.74)
(dep-BLM)Co(H_2S)	2.420	2.022	131.8		(7.02)
(dep-BLM)Co(py)	2.303	2.023	112.1	14	5.17
(dep-BLM)Co(Im)	2.294	2.022	103.2	14	6.95
(dep-BLM)Co-(N-MeIm)	2.290	2.020	91.4	13	7.25
(dep-BLM)Co(NH_3)	2.285	2.020	89.7	13	9.25
(dep-BLM)Co-(CH_3NH_2)	2.270	2.025	86.9	13	10.62
(acacen)Co(py)[5a]	2.44	2.011	97.53	15.7	
(acacen)Co-(CN-py)[5a]	2.39	2.013	101.17	16.24	
(3-methoxy-salen)Co(py)$_2$[18]	2.33	2.038	78.0	12.5	
(Ts-phthalo-cyanine)Co[19]	2.27	2.068	107		
deoxy Co myoglobin[20]	2.32	2.03	75	17	
deoxy Co peroxidase[21]	2.34	2.03	78	18.3	

Table II. ESR Parameters of Co(II)-Oxygen Complexes of Bleomycin and Its Related Ligands

complex	$g_\perp$	$g_\parallel$	$A_\perp^{Co}$, G	$A_\parallel^{Co}$, G	A_{iso}^{Co}, G
(BLM)Co(O_2)	2.007	2.098	12.4	20.2	15.0
(BLM)Co(O_2)(DNA)	2.004	2.106	11.5	18.9	14.0
(P-3A)Co(O_2)	2.007	2.102	13.0	22.4	16.1
(iso-BLM)Co(O_2)	2.007	2.100	12.5	20.2	15.1
(dep-BLM)Co(H_2O)-(O_2)	2.012	2.122	16.2	28.7	20.4
(dep-BLM)Co(H_2S)-(O_2)	2.014	2.130	17.0	29.8	21.3
(dep-BLM)Co(py)-(O_2)	2.005	2.095	15.0	25.6	18.5
(dep-BLM)Co(Im)-(O_2)	2.006	2.098	13.5	23.3	16.8
(dep-BLM)Co-(N-MeIm)(O_2)	2.007	2.100	12.6	21.6	15.6
(dep-BLM)Co-(NH_3)(O_2)	2.008	2.096	11.9	20.0	14.6
(dep-BLM)Co-(CH_3NH_2)(O_2)	2.007	2.096	11.3	18.0	13.5
(acacen)Co(py)(O_2)	1.999	2.082	10.73	19.64	13.70
(acacen)Co(CN-py)-(O_2)	1.997	2.080	10.30	20.11	13.57
(3-methoxy-salen)Co(py)(O_2)	1.997	2.079	10.25	17.3	12.6
(Ts-phthalo-cyanine)(O_2)	2.004	2.075	7.9	14.9	10.2
oxy Co myoglobin	2.007	2.08	9	16	11.3
oxy Co peroxidase	2.01	2.10	13.6	23.2	16.8

nooxygenated Co(II)–Schiff base complexes.[18] Magnetic data using oxygen-17 also showed that in the (bzacen)Co(py)(O_2) complex there is present only one unparied electron, and that over 90% of the residual electron spin resides on the dioxygen.[23] On the other hand, Tovrog et al.[24] suggested that the reduced ESR anisotropy results from a spin polarization mechanism with the unpaired electron residing mainly on O_2, which does not require a formal electron transfer to form O_2^-. However, the observed g values for these monomeric Co(II)–dioxygen complexes are close to those ($g_\perp$ = 2.006 and $g_\parallel$ = 2.080–2.103) of the typical superoxide anion radical ($O_2^-\cdot$) which is formed by the reaction in the xanthine oxidase–xanthine–oxygen system[25] or by the photolysis of oxygen-saturated formate solution at pH 11.7.[26]

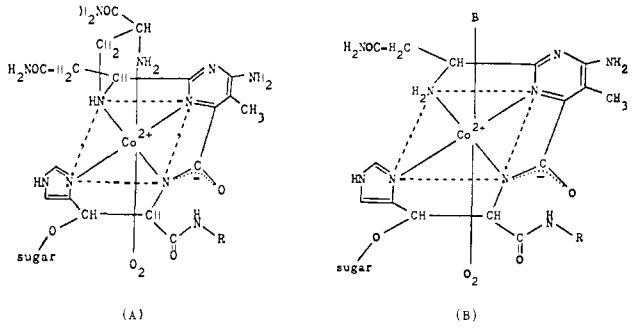


Figure 3. BLM–Co(II)–O_2 (A) and dep-BLM–Co(II)–O_2 (B) complexes.

Iso-BLM is the product of carbamoyl migration in the sugar of the BLM molecule.[15] The 1:1 iso-BLM–Co(II) complex and its dioxygen adduct have ESR parameters which are almost the same as far as the corresponding BLM complex species, indicating no direct participation of the carbamoyl group in the sugar portion toward Co(II) coordination (see Tables I and II).

Recent potentiometric titrations revealed that the 1:1 BLM–Co(II) complex has an equilibrium constant, log K = 9.74 and pK_c = 4.55, and that BLM behaves at least as a tetradentate ligand by the coordinations of its α-amino, secondary amine, deprotonated peptide, and histidine imidazole groups.[14] Further, it was indicated that the BLM-divalent metal complexes have the stability order of Fe(II) < Co(II) < Ni(II) < Cu(II) > Zn(II) and are in a similar coordination environment. Spectroscopic and X-ray crystallographic analyses clarified that the 1:1 Cu(II) complexes of BLM and P-3A have a substantially distorted square-pyramidal structure with N_5—[$N_{amine}N_{pyrimidine}N_{peptide}N_{imidazole}$]$N_{\alpha\text{-amino}}$—amino$^-$ donor sets.[13,14] The present ESR results strongly indicate that the 1:1 BLM–Co(II) complex has a square-pyramidal configuration with an axial nitrogen donor, and that dioxygen is incorporated into the vacant sixth axial coordination position. Therefore, the rigid BLM–Co(II) complex, which probably consists of a square-pyramidal structure with N_5 donor sets and four chelate rings of 5–5–5–6 numbers, gives the electronic configuration [$(d_{xz,yz})^4(d_{xy})^2(d_{z^2})^1$], which is similar to that of the corresponding porphyrin and Schiff-base complexes (see Figure 3). Of special interest is the fact that the BLM ligand environment is significantly different from these π-electron-rich ligand environments. In the BLM–Co(II) complex, dπ–pπ interaction between the cobalt and the fifth axial nitrogen ligand is not expected, because the α-amino ligand clearly lacks a π electron. On the other hand, the binding of O_2 to the prophyrin–Co(II)–base complexes is sensitive to the π-donation (or π-acceptor) ability of the axial base ligand. Good π donors such as imidazole will promote oxygenation by increasing the electron density available for back-bonding.

Effect of DNA Interaction Site and Sugar Portion of BLM on Oxygen Binding of BLM Antibiotic–Co(II) Complexes. Recent fluorescence studies demonstrated that the bithiazole portion of BLM preferentially binds to guanine residue of DNA and that the positive charge at the terminal amine portion facilitates BLM

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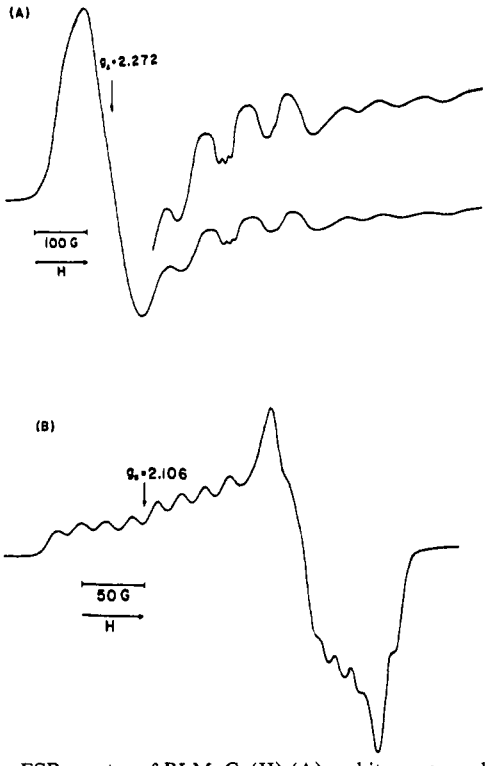

Figure 4. ESR spectra of BLM-Co(II) (A) and its oxygen adduct (B) complexes in the presence of DNA at 77 K.

binding to DNA.[27] Therefore, it was of interest to investigate the effect of DNA on the metal coordination of BLM. Figure 4 shows the ESR spectra of the BLM-Co(II) and its dioxygen adduct in the presence of calf thymus DNA (0.1-0.02 mM). The effective g values and A tensors obtained indicate that the Co(II) coordination mode and the electron spin delocalization are substantially similar in both the presence and absence of DNA. Nevertheless, the addition of DNA had a distinct effect on the ESR features of the BLM-Co(II)-O_2 complex. The $g_{\parallel}$ value (2.106) in the presence of DNA is larger by 0.008 units than that in the absence of DNA, the $g_{\perp}$ value (2.004) is smaller by 0.003, and the hyperfine structure is better resolved. The hyperfine splittings, namely, A^{Co} values ($A_{\parallel}^{Co}$ = 18.9 and $A_{\perp}^{Co}$ = 11.5 G), were clearly observed in the presence of DNA. Repeated experiments have established that the ESR spectrum is reproducible under similar conditions. The present result suggests that the interaction of DNA with the bithiazole ring and terminal groups of BLM has an appreciable effect on the orientation of the bound O_2 molecules relative to the Co(II) plane. Similar effects of DNA have been demonstrated for NO orientation in the BLM-Fe(II)-NO complex.[28]

Under anaerobic conditions, the 1:1 P-3A-Co(II) complex also exhibits an ESR spectrum which can be analyzed in terms of nearly axial symmetry ($g_{\perp}$ = 2.275 and $g_{\parallel}$ = 2.027), with $g_{\parallel}$ split into eight lines due to ^{59}Co hyperfine interaction ($A_{\parallel}^{Co}$ = 93.8 G). The ESR parameters of the monooxygenated low-spin P-3A-Co(II)-O_2 complex also closely resemble those of the corresponding BLM complex, though the $A_{\parallel}^{Co}$ value (20.2 G) of the latter is somewhat smaller than that ($A_{\parallel}^{Co}$ = 22.4 G) of the former (see Table II). However, it is interesting to note that the oxygenated P-3A-Co(II) complex species is more unstable than the BLM-Co(II)-O_2 complex and undergoes further oxidation, to a marked degree. P-3A and BLM have the same metal-coordination donors, that is, the secondary amine, pyrimidine ring, deprotonated peptide of histidine residue, and histidine imidazole nitrogens as planar donors and the α-amino nitrogen as axial donor.[29] Accordingly, the sugar and bithiazole portions of BLM molecule presumably contribute to stabilization of the monomeric Co(II)-dioxygen adduct complex.

Effect of External Axial Bases on Dep-BLM-Co(II) and Its Dioxygen Adduct Complexes. Since the dep-BLM lacks the β-aminoalanine group, it is expected to form a Co(II) and a cobalt-dioxygen complex which is different from the parent BLM with regard to the fifth axial donor ligand (see Figure 3). In fact, it was found that dep-BLM could form a low-spin Co(II) complex and a dioxygen adduct, probably with water as the base in the fifth axial coordination position (see Figure 5A). Furthermore, the ESR spectra in Figures 5B and 5C show significant changes in the magnitude and anisotropy of g and A^{Co} values caused by the addition of external nitrogen bases. The formation of dep-BLM-Co(II) and its dioxygen complexes with *N*-methylimidazole or ammonia in the fifth axial ligand was therefore suggested. On the other hand, trimethylamine, $(CH_3O)_3P$, CH_3CN, and CO did not coordinate to the dep-BLM-Co(II) complex, because they exhibited the same ESR spectra as that of the original dep-BLM-Co(II) complex.[30] Spin Hamiltonian values for these dep-BLM-Co(II) complexes are listed in Tables I and II and are compared with those for the BLM-Co(II) complexes. In the dep-BLM-Co(II)-base (B) complexes as well as in the BLM-Co(II) complex, the order of g anisotropy ($g_{\parallel} < g_{\perp}$) reveals that the unpaired electron resides mostly in the d_{z^2} orbital of Co(II) in a low-spin d^7 configuration, namely, a well-defined $[(d_{xz})^2(d_{yz})^2(d_{xy})^2(d_{z^2})^1]$ ground configuration. The appropriate relationships between electronic and magnetic parameters for this case are

$$g_{\parallel} = 2.002,\ g_{\perp} = 2.002 - 6\lambda/\Delta E_{xz,yz-z^2}$$

where λ is the spin-orbit coupling constant of Co(II).[31] The observed $g_{\parallel}$ values in all of Co(II) complexes are slightly larger than the theoretical value (2.002), which may result from a small contribution of the $d_{x^2-y^2}$ orbital to the ground state.[32] The $g_{\perp}$ and A^{Co} values in the dep-BLM-Co(II)-B complexes are reduced to the order pyridine > imidazole > *N*-methylimidazole > ammonia > methylamine and $H_2S > H_2O$, consistent with the order of pK_a of these bases.

Four-coordinate Co(II) complexes are known to be very poor oxygen binders.[1] The binding of a fifth axial ligand raises the d_{z^2} orbital above the d_{xy}, and leads to an advantageous configuration $[(d_{xz,yz},d_{xy})^6(d_{z^2})^1]$ for oxygenation. Of special interest is the fact that even the dep-BLM-Co(II) complex with hydrogen sulfide in the fifth axial position can form a monomeric dioxygen complex. The (dep-BLM)Co(H_2S)(O_2) complex has larger A^{Co} values than the (dep-BLM)Co(H_2O)(O_2) complex, consistent with the poorer σ-donor property of H_2S (see Table II). Recently, Chen et al. reported that the thioiminato (N_2S_2)-Co(II) complexes show less oxygen affinity than the corresponding ketoiminato (N_2O_2)-Co(II) complexes, and that in-planar sulfur has a destabilizing influence.[5c] In addition to the weaker σ interaction of the sulfur, they proposed that the sulfur donors can drain electron density from the Co(II) via a π-back-bonding interaction between filled d_{xz} and d_{yz} orbitals on the metal and empty d orbitals on the sulfurs. The ^{59}Co hyperfine splitting ($A_{\parallel}^{Co}$ = 28.7 G) of the present (dep-BLM)Co(H_2O)(O_2) complex corresponds well to that ($A_{\parallel}^{Co}$ = 28.85 G) of the previously reported (acacen)Co(H_2O)(O_2) complex.[5a] However, the dioxygen adduct of dep-BLM-Co(II)-H_2O complex has larger A^{Co} values than the dioxygen adduct of BLM-Co(II) complex, indicating a weaker localization of the unpaired electron to O_2. On the other hand, the ESR parameters listed in Table II suggest similar electronic and conformational

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(29) The proton paramagentic shifts indicate that the BLM-Co(II) complex has a coordination structure similar to the BLM-Fe(II) complex[11] (unpublished result).

(30) In the dep-BLM-Co(II) complex, a water ligand as axial ligand is inferred from the g values and $A_{\parallel}^{Co}$ value.

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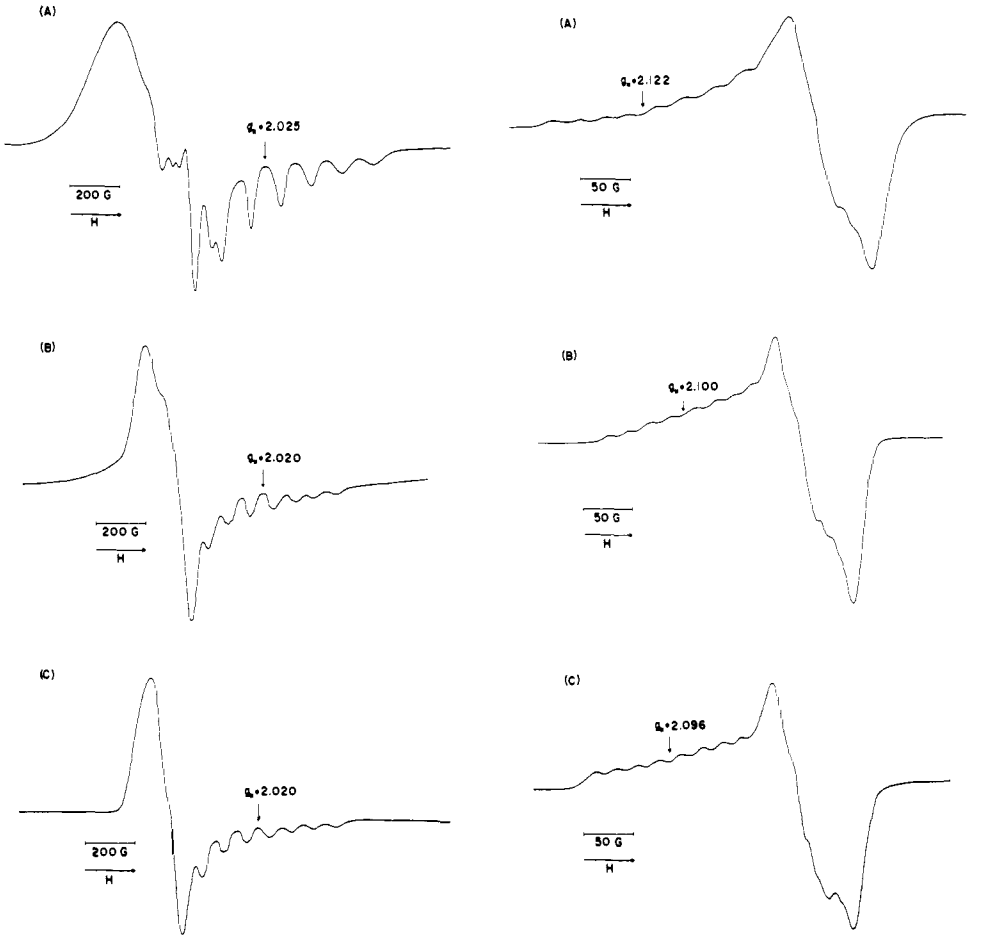

Figure 5. ESR spectra of dep-BLM-Co(II) (left) and dep-BLM-Co(II)-O_2 (right) complexes with H_2O (A), N-MeIm (B), and NH_3 (C) as axial donor at 77 K.

structures around Co for the (dep-BLM)Co(B)(O_2) (B = ammonia and methylamine) complexes and the (BLM)Co(O_2) complex. In the dioxygen adducts of dep-BLM-Co(II)-B complexes, there is also a good correlation between the ^{59}Co hyperfine coupling constants and the pK_a of bases. Therefore, the effect of varying axial bases on Co hyperfine splittings is rationalized in terms of increasing donation to the axial dioxygen through central Co. In general, the ^{59}Co hyperfine interaction in these dioxygen adduct complexes arises primarily from dπ population in the predominantly oxygen π^* odd-electron orbital. The reduction of the A^{Co} values in the (dep-BLM)Co(B)(O_2) complexes is attributed mainly to σ-donor ligand interaction. The formation of base adduct elevates the Co d_{z^2} orbital and would tend to localize Co-O_2 σ-bonding electrons on oxygen, thus causing both further bending of the Co-O_2 unit and a decline in Co dπ-O_2 π^* bonding. Direct correlations between the pK_a of structurally related axial bases and O_2 uptake have also been observed in the (PPIXDME)Co(B) complexes.[33]

pH-Dependent Axial Donor Change in Deamido-BLM-Co(II) Complexes. Deamido-BLM is a product of BLM inactivated by BLM hydrolase, a kind of aminopeptidase, which hydrolyzes the amido group of β-aminoalanine portion in the BLM molecule ($-CH_2CH(NH_2)CONH_2$).[16] In fact, deamido-BLM ($-CH_2CH(NH_2)COOH$) has the pK_a value of 9.4 for its α-amino group and this ionization constant is considerably higher than that (p$K_{NH_3^+}$ = 7.72) of BLM. In this respect, we looked at the axial donor coordination in the deamido-BLM-Co(II) and its dioxygen adduct complexes. Figure 6 shows the ESR spectra of the 1:1 deamido-BLM-Co(II) complex at pH 9.6 and 6.8. At pH 9.6, the ESR parameters ($g_\perp$ = 2.276, $g_\parallel$ = 2.023, and $A_\parallel^{Co}$ = 91.1 G) of the deamido-BLM-Co(II) complex closely resemble those ($g_\perp$ = 2.272, $g_\parallel$ = 2.025, and $A_\parallel^{Co}$ = 92.5 G) of the corresponding

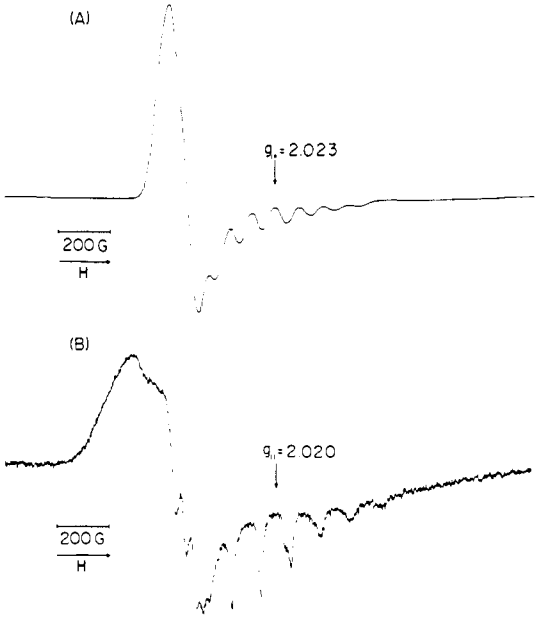

Figure 6. ESR spectra of deamido-BLM-Co(II) complex at pH 9.6 (A) and 6.8 (B).

BLM-Co(II) complex.[34] The ESR feature is characteristic of a square-pyramidal configuration with an axial primary amino nitrogen coordination. At pH 6.8, the ESR spectrum of the deamido-BLM-Co(II) complex is distinctly different from that

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(34) BLM forms the same low-spin Co(II) complex and its dioxygen adduct complex species in the pH region from 6 to 10.

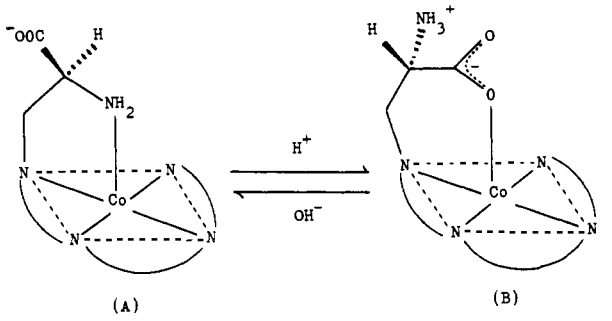

Figure 7. Change of axial donor in deamido-BLM–Co(II) complex.

Table III. ESR Parameters for Co(II) and Co(II)–O_2 Complexes of Deamidobleomycin and Its Related Ligands

complex	$g_\perp$	$g_\parallel$	$A_\parallel^{Co}$, G	$A_\perp^{Co}$, G	pK_a of base
(deamdio-BLM)Co (pH 6.8)	2.301	2.020	112.1		
(deamido-BLM)Co (pH 9.6)	2.276	2.023	91.1		9.4
(dep-BLM)Co(H_2O)	2.368	2.025	123.3		
(dep-BLM)Co(glycinamide)	2.290	2.023	93.0		8.06
(BLM)Co	2.272	2.025	92.5		7.72
(deamido-BLM)Co(O_2) (pH 6.8)	2.009	2.101	26.2	15.3	
(deamido-BLM)Co(O_2) (pH 9.6)	2.007	2.097	20.0	12.4	
(dep-BLM)Co(H_2O)(O_2)	2.012	2.122	28.7	16.2	
(dep-BLM)Co(glycinamide)-(O_2)	2.008	2.098	20.4	12.5	
(BLM)Co(O_2)	2.007	2.098	20.2	12.4	

at pH 9.6 and the ESR parameters ($g_\perp$ = 2.301, $g_\parallel$ = 2.020, and $A_\parallel^{Co}$ = 112.1 G) are close to those ($g_\perp$ = 2.368, $g_\parallel$ = 2.025, and $A_\parallel^{Co}$ = 123.3 G) of the dep-BLM–Co(II)–H_2O complex rather than those of the BLM–Co(II) complex. The ESR result strongly indicates that at physiological pH a weak axial donor, most probably the carboxyl group, occupies the fifth axial position in the deamido-BLM–Co(II) complex, as shown in Figure 7. The same pH-dependent axial donor change was also detected in the dioxygen adduct of the deamido-BLM–Co(II) complex (see Table III). At pH 6.8, the deamido-BLM–Co(II)–O_2 complex has Co hyperfine constants similar to those of the dep-BLM–Co(II)–O_2 complex rather than those of the BLM–Co(II)–O_2 complex. On the other hand, the higher pH (9.6) reverses this situation. At both pH 6.8 and 9.6, the formation of the dep-BLM–Co(II)–glycinamide and its dioxyen adduct complexes was demonstrated by their ESR spectra (see Table III). In contrast with glycinamide, glycine did not coordinate under the same conditions to the dep-BLM–Co(II) complex, because the addition of excess glycine gave an ESR spectrum which was the same as that of the dep-BLM–Co(II)–H_2O complex. The noncoordination of external glycine to the dep-BLM–Co(II) complex is presumably due to the higher p$K_{NH_3^+}$ value (9.9) and the zwitterion of the glycine molecule.

Recent experiments revealed that the activity of deamido-BLM for DNA cleavage reaction corresponds to approximately 1% of that of BLM.[35] The present result also supports our previous findings that the lower biological activity of deamido-BLM is attributed to the less effective oxygen activation of its Fe(II) complex, of which the fifth axial coordination donor is not occupied by the α-amino group, but predominantly by the carboxyl or aquo group at physiological pH.[11]

It appears likely that DNA degradation proceeds from the formation of an oxygenated BLM–Fe(II) complex, and that it is followed by the decomposition of a BLM–Fe(II)–O_2 complex to generate BLM–Fe(III) complex and reactive oxygen radicals such as $O_2^{-}\cdot$ and and The BLM–Co(II) complex also gave rise to the ESR signal of the hydroxyl spin adduct with *N-tert*-butyl α-phenyl nitrone, α-phenyl nitrone, though its signal was not so strong as that of the corresponding Fe(II) complex.[36] The inactivity of the BLM–Co(II) complex against DNA cleavage[37] may be due to the irreversibility of Co(II)–Co(III) redox reaction, in contrast with the reversible redox reaction of the "active" BLM–Fe(II) complex.[38] In addition, Co(II) inhibits the reaction with Fe(II).[37] This is explained by a higher binding affinity of Co(II) than Fe(II) toward BLM.[14] The present ESR study indicates that the 1:1 BLM–Co(II) complex has a rigid square-pyramidal arrangement with an axial nitrogen donor and that the five-coordinate chelate of the $[(d_{xy,yz},d_{xy})^6(d_{z^2})^1]$ electronic configuration readily binds dioxygen to form a monomeric Co(II)–dioxygen adduct. Dep-BLM, which lacks the β-aminoalanine portion in the BLM molecule, also forms the low-spin Co(II) complex and a dioxygen adduct with the external base in the fifth axial coordination position. In both the Co(II) and Co(II)–O_2 complexes of deamido-BLM, a product of BLM inactivated by enzyme, the unique pH-dependent axial donor change was detected, and at physiological pH the terminal carboxyl group was presumed to occupy the fifth axial position. These results clearly indicate that the α-amino nitrogen of the β-aminoalanine portion in BLM is the fifth axial donor. In this respect, the current model for the α-amino group as an equatorial ligand[39] should be reinvestigated. Recently, the NO and CO bindings of the BLM–Fe(II) complex have been demonstrated by ESR and ^1H NMR spectroscopies, respectively.[28,39] The BLM–Fe(II)–NO complex is isoelectronic with the BLM–Co(II)–O_2 complex and CO is a typical O_2 antagonist. The present BLM–Co(II) complex has clearly demonstrated the formation of such an oxygenated complex, which is important as it is related to the biological action of BLM. In addition, BLM ligands promote an environment for the formation of a typical monomeric Co(II)–dioxygen complex in aqueous solution.

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(38) Our cyclic voltammogram (CV) of the BLM–Fe complex showed a reversible redox reaction and E_p (pH 6.9) = +165 mV, whereas the BLM–Co complex showed an irreversible CV pattern and E_p < −1.5 V.
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