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COMPARISON OF METAL COMPLEXES BETWEEN DEPYRUVAMIDE BLEOMYCIN AND BLEOMYCIN : AN IMPORTANT EFFECT OF AXIAL DONOR ON METAL COORDINATION AND OXYGEN ACTIVATION

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Summary : The 1:1 Cu(II), Co(II), Co(II)-O_2, Fe(II)-NO, and Fe(III) complexes of depBLM have been investigated by ESR spectroscopy and compared with the corresponding metal complexes of BLM. DepBLM which lacks the α-amino group of β-aminoalanine portion in BLM molecule, forms the metal complexes different from BLM with regard to the fifth axial donor. In addition, the formation of hydroxyl radical by the depBLM-Fe(II) complex is remarkably lower than that by the BLM-Fe(II) complex. This study indicates an important effect of fifth axial nitrogen on metal coordination and oxygen activation of BLM.

Introduction

Cleavage of cellular DNA by BLM probably accounts for the antibiotic and antitumor activities of this drug. It has been proposed that ferrous ion enhances the rate of DNA cleavage by BLM[1] and that a labile BLM-Fe(II)-O_2 complex is involved in the BLM action on DNA degradation.[2] In fact, recent experiment demonstrated that BLM breaks DNA at TT(T=thymine), AT(A=adenine), and TA, as well as GC(G=guanine and C=cytosine) and GT sequences in the presence of ferrous ion.[3] On the other hand, the previous ESR result showed evidently that the 1:1 BLM-Co(II) complex with a square-pyramidal geometry incorporates dioxygen molecule into the vacant sixth coordination site.[4] In BLM-Fe(II)-O_2 system, the formation of reactive free-radicals such as $O_2^{\cdot-}$ and ·OH has been also detected by ESR spin trapping.[5] Herein, the metal complexes of depBLM have been compared with those of BLM, and a significant result found in this investigation is an important effect of fifth axial donor on metal coordination and oxygen activation by these antibiotics.

Abbreviations used : depBLM, depyruvamide bleomycin; BLM, bleomycin; ESR, electron spin resonance; BPN, N-tert-butyl-α-phenylnitrone; DNA, deoxyribonucleic acid.

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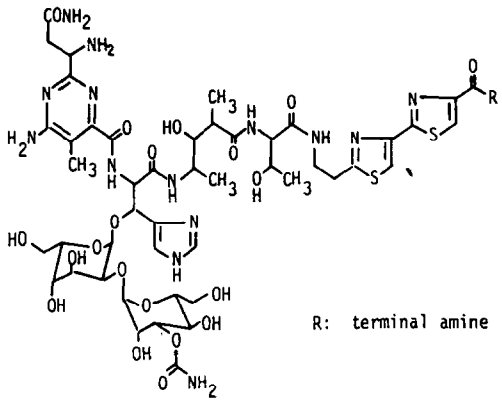
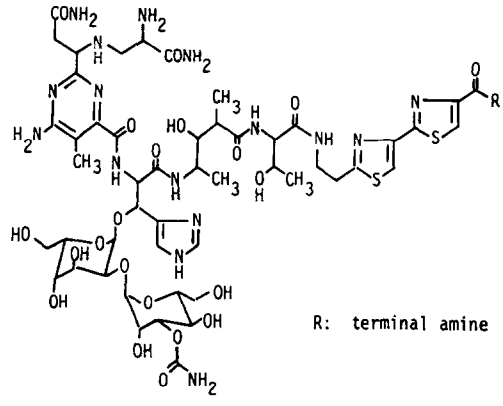


Depyruvamide Bleomycin (dep BLM)



Bleomycin (BLM)

Experimental

Purified BLM-A_2 and depBLM-A_2 which contain 3-aminopropyldimethylsulfonium residue, were used in this study. Bovine liver catalase(3000 units per mg) and BPN were obtained from P-L Biochemicals and Aldrich, respectively. X-Band ESR spectra of the depBLM(or BLM)-metal complexes(1.0 mM) were measured at pH 6.8 and 77 K with a JES-FE-3X spectrometer. The spin trapping experiment was performed according to the previous procedure.[5]

Results and Discussion

Figure 1 and Table I summarize the ESR spectral data for the Cu(II), Co(II), Co(II)-O_2, Fe(II)-NO, and Fe(III) complexes of depBLM and BLM. Although the spectral feature of the 1:1 depBLM-Cu(II) complex resembles that of the 1:1 BLM-Cu(II) complex, the larger $A_{\parallel}$ value of the former suggests a weaker axial coordination for the central Cu(II) ion. Of interest is the fact that depBLM also forms low-spin Co(II) and its oxygen adduct complexes similar to BLM.[4] Under the anaerobic condition, the 1:1 depBLM-Co(II) complex gave the ESR spectrum which has the relationship of $g_{\perp} > g_{\parallel} \simeq 2.0$ and is characteristic of a penta-coordinated square-pyramidal configuration. By oxygen-bubbling of the depBLM-Co(II) complex, the ESR spectrum clearly changed to that of typical mono-oxygenated low-spin Co(II) complex. However, the $A_{\parallel}$ and $A_{\perp}$ values of the depBLM

Figure 1 ESR spectra for Cu(II)(A), Co(II)(B), Co(II)-O_2(C), Fe(II)-NO(D), and Fe(III)(E) complexes of depBLM(left) and BLM(right) at 77 K.

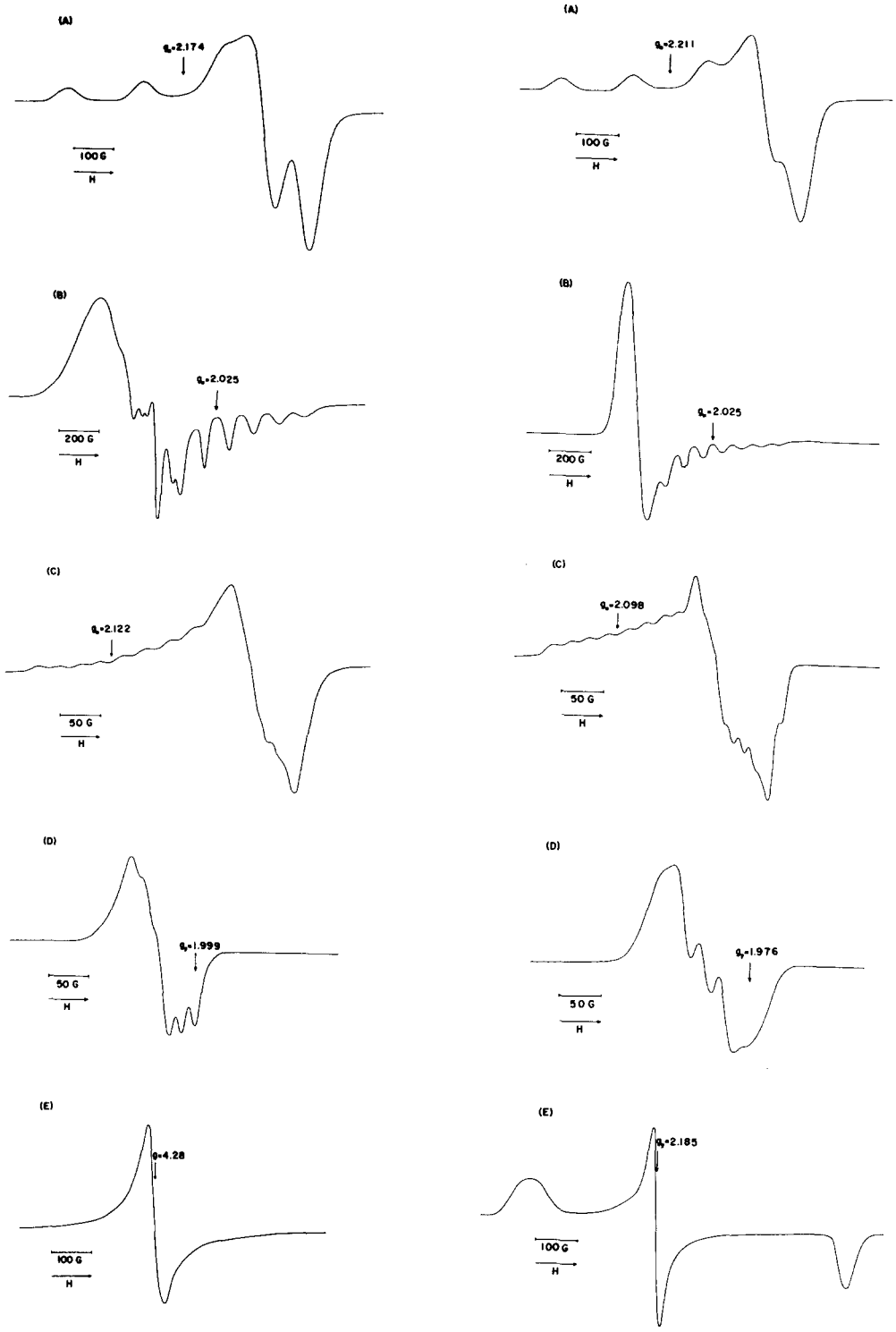

(A)
g=2.174
100 G
H
(A)
g=2.211
100 G
H
(B)
g=2.025
200 G
H
(B)
g=2.025
200 G
H
(C)
g=2.122
50 G
H
(C)
g=2.098
50 G
H
(D)
g=1.999
50 G
H
(D)
g=1.976
50 G
H
(E)
g=4.28
100 G
H
(E)
g=2.185
100 G
H

Table I ESR Parameters for Cu(II), Co(II), Co(II)-O_2, Fe(II)-NO, and Fe(III) Complexes of depBLM and BLM

Complex	ESR Parameters				
	$g_{\parallel}$	$g_{\perp}$	$A_{\parallel}$,G	$A_{\perp}$,G	A^N,G
(depBLM)Cu(II)	2.174	2.055	190.6		
(BLM)Cu(II)	2.211	2.055	183.0		
(depBLM)Co(II)(H_2O)	2.025	2.368	123.3		
(BLM)Co(II)	2.025	2.272	92.5		13.0
(depBLM)Co(II)(H_2O)(O_2)	2.122	2.012	28.7	16.2	
(BLM)Co(II)(O_2)	2.098	2.007	20.2	12.4	
(depBLM)Fe(II)(H_2O)(NO)	2.016	2.052 1.999			17.5
(BLM)Fe(II)(NO)	2.008	2.041 1.976			23.6
(depBLM)Fe(III)	g=4.28				
(BLM)Fe(III)	2.431	2.185 1.893			

complex are larger than those of the BLM complex, indicating a weaker delocalization of the unpaired electron from the central Co(II) ion. The ESR spectra for the 1:1 Fe(II)-nitrosyl complexes of depBLM and BLM showed a rhombic symmetry with a triplet hyperfine interaction in the g_z region. The present three-line g_z signal is due to the coordination of NO molecule, and the smaller A^N value of the depBLM-Fe(II)-NO complex is suggestive of weaker fifth axial ligand to iron bonding with concomitantly stronger NO to iron bonding. On the other hand, the ESR features for the Fe(III) complexes of depBLM and BLM are typical of high-spin(S=5/2) and low-spin(S=1/2) ferric types, respectively. The metal complexes of BLM have substantially a square-pyramidal structure which involves the secondary amine nitrogen, pyrimidine ring nitrogen, deprotonated peptide nitrogen of histidine residue, and histidine imidazole nitrogen as basal planar donors, and the α-amino nitrogen as axial donor.[2,4,7] DepBLM lacks the α-amino group of β-aminoalanine portion in BLM molecule, and hence forms the metal

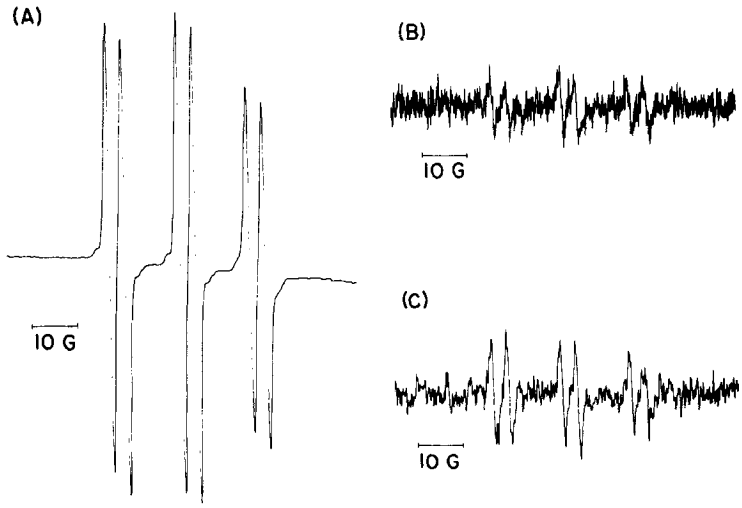


Figure 2 ESR spectra obtained by oxygen bubbling of BLM-Fe(II) and depBLM-Fe(II) complexes in the presence of BPN

(A) 1.0 mM BLM-Fe(II) complex and 0.08 M BPN
(B) 1.0 mM BLM-Fe(II) complex, 10 mg catalase, and 0.08 M BPN
(C) 1.0 mM depBLM-Fe(II) complex and 0.08 M BPN

Condition of ESR spectroscopy : microwave power, 10(A,C) and 20(B) mW; modulation amplitude, 0.5 G; time constant, 0.01(A) and 0.1(B,C) second; scan time, 4(A,C) and 8(B) minutes.

complexes different from BLM with regard to the fifth axial donor. Presumably, the oxygen and nitrosyl adducts of depBLM complexes bind aquo molecule in the fifth coordination position. The oxygen adduct of Schiff base with water as axial ligand, (acacen)Co(II)(H_2O)(O_2) complex, is known to have the large eight ^{59}Co hyperfine splittings($A_{\parallel}^{Co}$=28.85 G).[8]

The experimental result of the spin trapping showed the highly efficient production of ·OH radical at high concentration(1.0 mM) of the BLM-Fe(II) complex, and this reaction was strongly inhibited by catalase which is scavenger of H_2O_2(see Figures 2A & 2B). On the other hand, it has been reported that the degradation of DNA by the BLM-Fe(II) complex is not inhibited by catalase or superoxide dismutase at high BLM concentration.[1] The contradiction between this observation and the present interference of radical formation by catalase may be interpreted by the presumption that only oxygen radical species formed at the

binding site of the BLM-Fe(II) complex to DNA participates to the sequence specific cleavage of DNA by BLM.

In contrast with the BLM complex, the formation of hydroxyl radical by the depBLM-Fe(II) complex(1.0 mM) was remarkably low(see Figure 2C). The radical spin concentration of the latter is approximately estimated to 1/100 of that of the former. Here, it is important to note that a rigid square-pyramidal coordination environment and fifth axial nitrogen donor are essential for effective oxygen binding and oxygen reduction by the iron complexes of BLM antibiotics. In fact, the biological activity of depBLM is significant lower than that of BLM.[9]

Acknowledgment

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