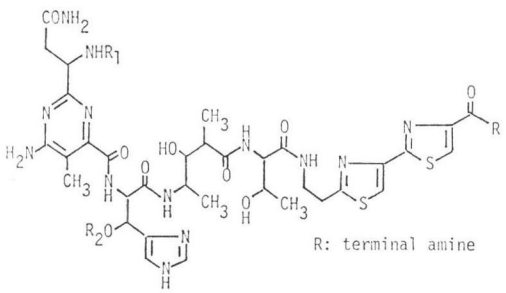
DEGLYCO-BLEOMYCIN-IRON COMPLEXES: IMPLICATIONS FOR IRON-BINDING SITE AND ROLE OF THE SUGAR PORTION IN BLEOMYCIN ANTIBIOTICS

Sir:

The oxidation of bleomycin (BLM)-Fe-DNA complex by molecular oxygen leads to damage of DNA.[1,2] This activity is related to the bifunctionality of BLM, namely DNA- and iron-binding. The bithiazole and terminal amine residues contribute toward the binding to DNA[3,4] and the pyrimidoblamyl-β-hydroxyhistidyl portion (the amino acid residue containing pyrimidine is called pyrimidoblamic acid) is involved in chelation with metal ions.[2,5,6] From ^{1}H NMR analysis of a diamagnetic BLM-Fe(II)-CO adduct, the carbamoyl group on the 3-position of mannose was proposed to be one coordination donor group toward the ferrous ion, in conjunction with carbon monoxide, the primary and secondary amine nitrogens, the pyrimidine N-1 and the imidazole N-1.[7] However, this model has several drawbacks. The proposed 5–5–9 chelate ring member and the coordination of the carbamoyl amide nitrogen are considered to be unlikely, because certain spectroscopic properties[8] and the binding of oxygen antagonists (CO, NO, and C_2H_5NC)[9] similar to hemoproteins strongly suggest a rigid chelate conformation of the BLM-iron complex. Our potentiometric study showed one proton dissociation (pK_e=5.5) from the peptide backbone group in the BLM-Fe(II)-complexation.[10] In addition, the ESR results of the BLM-Co(II) complex and its dioxygen adduct revealed that the primary amino nitrogen is the fifth axial donor atom.[11]

In this paper, deglyco-BLM was used to clarify the role of the sugar portion for the coordination and O_2-activation of "active BLM-Fe(II) complex species". Deglyco-BLM lacks the sugar moiety in BLM molecule.

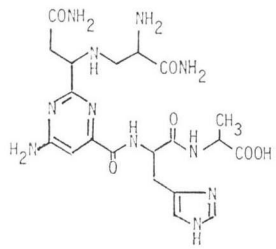
Deglyco-BLM was obtained from the culture broth of BLM fermentation and also from the mild acid hydrolysate of BLM.[12] Recently, the total chemical synthesis of deglyco-BLM-A_2 was achieved by us.[13] The 1 : 1 deglyco-BLM-Fe(II) complex was prepared by mixing deglyco-BLM and Fe(II) ion in aqueous solution (pH 6.9) containing a small amount of $NaBH_4$ under the condition of total deaeration. The Fe(II)-NO (or CO) adducts of deglyco-BLM and BLM were prepared by addition of a few milligrams of $NaNO_2$ (or CO gas) and sodium borohydride to the Fe(II) complexes. Spin-trapping experiments using *N-tert*-butyl-α-phenylnitrone were carried out according to the previously reported procedure.[8] X-Band ESR(sample conc.: 1 mM) and 220 MHz FT-^{1}H NMR (10 mM) spectra were



	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



P-3A

recorded using a JES-FE-3X and a Varian HR-220 spectrometer, respectively.

The visible spectral constants of the deglyco-BLM-Fe(II) complexes with oxygen analogues are very similar to those of the corresponding BLM-Fe(II) complexes[9]: deglyco-BLM-Fe(II) complex, λ_{max} 472 nm (ε 300); deglyco-BLM-Fe-(II)-CO adduct, 380 nm(2800); and deglyco-BLM-Fe(II)-NO adduct, 475 nm(2200). Fig. 1 shows the ESR spectra of the deglyco-BLM-Fe(II)-NO adduct(A) and the deglyco-BLM-Fe(III) complex (B). The ESR parameters obtained for these iron complexes are close to those for the corresponding BLM complexes. Table 1 summarizes the ESR parameters for the Fe(II)-NO and Fe (III) complexes of deglyco-BLM, BLM, *iso*-BLM, P-3A(a biosynthetic intermediate of BLM), and depyruvamide(dep)-BLM.[8] The results strongly indicate that (1) the pyrimidoblamyl-β-hydroxyhistidyl portion is substantially important for Fe(II) and Fe(III) coordination to BLM ligands and (2) the sugar portion is not participating as a direct ligand in the iron binding. As demonstrated in BLM-Fe(II)-NO complex,[14,15] the addition of DNA to deglyco-BLM-Fe(II)-NO complex also induced a greater separation of the g_x and g_y absorptions in comparison with the original ESR spectrum, suggesting increased in-plane anisotropy in the Fe(II) site by the interaction between deglyco-BLM and DNA. Of special interest is the fact that the g_x value (2.060) of BLM-Fe(II)-NO-DNA complex is larger than that (2.046) of the corresponding deglyco-BLM complex. An interaction such as the hydrogen bond between the sugar of BLM and DNA, in this case, may be responsible for the larger g_x

Fig. 1. ESR spectra of deglyco-BLM-Fe(II)-^{14}NO complex(A) and deglyco-BLM-Fe(III) complex(B) at 77 K.

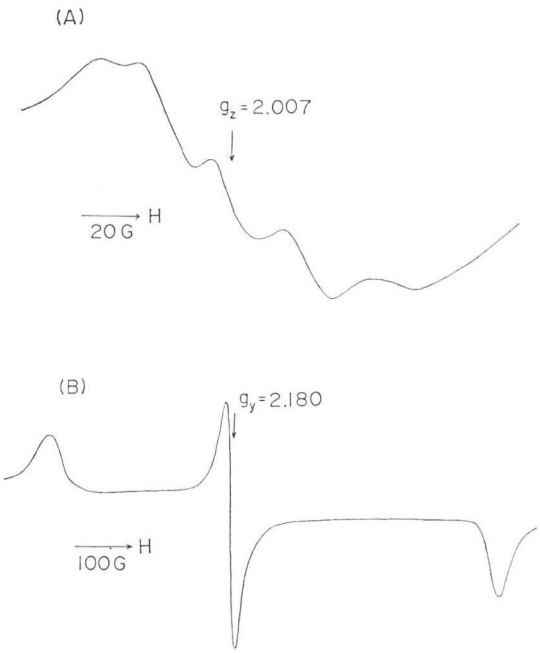


Table 1. ESR parameters for iron(II)-nitrosyl and iron(III) complexes of deglyco-bleomycin and its related compounds.

Complex	g_x	g_y	g_z	A^N, G	Nitrogen hfs
Deglyco-BLM-Fe(II)-^{14}NO	2.038	1.969	2.007	24.8	3
Deglyco-BLM-Fe(II)-^{15}NO	2.038	1.970	2.007	33.0	2
BLM-Fe(II)-^{14}NO	2.041	1.976	2.008	23.6	3
BLM-Fe(II)-^{15}NO	2.041	1.976	2.008	31.6	2
Iso-BLM-Fe(II)-^{14}NO	2.040	1.976	2.008	23.6	3
P-3A-Fe(II)-^{14}NO	2.038	1.968	2.007	24.8	3
Dep-BLM-Fe(II)-^{14}NO	2.052	2.016	1.999	17.5	3
Deglyco-BLM-Fe(II)-^{14}NO-DNA	2.046	1.963	2.007	25.1	3
BLM-Fe(II)-^{14}NO-DNA	2.060	1.962	2.006	24.0	3
Deglyco-BLM-Fe(III)	1.887	2.180	2.432		
BLM-Fe(III)	1.893	2.185	2.431		
Iso-BLM-Fe(III)	1.892	2.185	2.430		
P-3A-Fe(III)	1.887	2.179	2.434		
Dep-BLM-Fe(III)		g=4.28			

Table 2. Proton paramagnetic shifts for iron (II) complexes of bleomycin and related compounds.

Complex	Proton chemical shift (ppm relative to TSP)						
BLM-Fe(II)	47.4	45.1	42.9	34.7	23.8	20.6	15.1
Iso-BLM-Fe(II)	48.0	45.6	43.7	35.3	24.6	21.2	14.2
Deglyco-BLM-Fe(II)	47.7	40.6	22.4	17.0	11.6		
Dep-BLM-Fe(II)	38.0	34.8	28.6	27.0	17.7		
Dep-BLM-Fe(II)-2·MeIm	52.9	49.1	35.4	32.4	20.4		
Dep-BLM-Fe(II)-NH_3	46.7	40.8	33.8	31.1	14.9		

TSP: Sodium 3-(trimethylsilyl)propionate-d_4.

Table 3. Proton chemical shifts for Fe(II)-CO adduct complexes of deglyco-bleomycin and bleomycin.

	Proton chemical shifts (ppm relative to TSP)			
	Deglyco-BLM (pD 7.7)	Deglyco-BLM-Fe(II)-CO (pD 7.7)	BLM (pD 7.2)	BLM-Fe(II)-CO (pD 7.2)
Bithiazole protons	8.20, 7.99	8.19, 8.01	8.18, 7.99	8.18, 8.01
Hydroxyhistidine				
C2–H	7.71	8.47	7.82	8.55
C4–H	7.15	7.26	7.30	7.43
β-Aminoalanine				
CH	3.98	3.88	3.98	3.84
CH_2	2.97	2.93, 2.62	2.99	2.93, 2.64
Pyrimidine CH_3	2.02	2.39	1.99	2.38
α-Methylvalerate α,δ-CH_3	1.14	1.12	1.15	1.11

separation. The ESR features of deglyco-BLM-Fe(III) complex are typical of a rhombic low-spin type and the estimated g-values were much the same as those of the BLM-Fe(III) complex. The similarity of the ESR parameters for the low-spin Fe(III) complexes between the BLM ligand and hemoprotein indicates that the iron ligand donors in BLM are arranged in a rigid conformation, as in the case of heme. Indeed, it has been proposed that BLM-Fe(III) complex has a rigid square-pyramidal arrangement with 5–5–5–6 ring member.[8] Macrocyclic ferric thiolate Fe(III)N_4SR also possesses low-spin(S=1/2) for 6565 ring size complex and inermediate-spin(S=3/2) for 6566 complex.[16]

Deglyco-BLM-Co(II) complex showed a typical low-spin ESR spectrum ($g_\perp$=2.277, $g_\parallel$=2.027, $A_\parallel^{Co}$=95.0 G, and $A_\parallel^{N}$=14 G) and the ESR feature closely resembles that ($g_\perp$=2.272, $g_\parallel$=2.025, $A_\parallel^{Co}$=92.5 G, and $A_\parallel^{N}$=13 G)[11] of the corresponding BLM complex. As well as BLM-Co(II) complex, deglyco-BLM-Co(II) complex reacted with dioxygen to form a monomeric dioxygen adduct. The ESR characteristics of the Co(II)-O_2 complex for deglyco-BLM and BLM are similar: the former ($g_\perp$=2.009, $g_\parallel$=2.100, $A_\parallel^{Co}$=22.5 G, and $A_\perp^{Co}$=13.2 G) and the later ($g_\perp$=2.007, $g_\parallel$=2.098, $A_\parallel^{Co}$=20.2 G, and $A_\perp^{Co}$=12.4 G).[11]

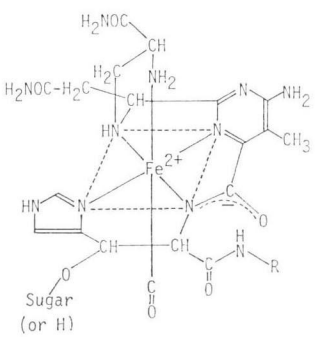
Fe(II) complexes of BLM[7] and deglyco-BLM showed large proton paramagnetic shifts as a result of contact and pseudo-contact effects by the central Fe(II) ion. Table 2 summarizes the proton chemical shifts at 25°C and pD 7.3 in 50 ppm ranges for the Fe(II) complexes of the BLM antibiotics. The present ^{1}H NMR results provide the following information: (1) the magnitude of the chemical shifts suggests the presence of a high-spin Fe(II) ion(S=2) in the Fe(II) complexes of deglyco-BLM and BLM,[17] (2) the similarity of the resonances between the BLM-Fe(II) and *iso*-BLM-Fe(II) complexes appears to reject the *O*-carbamoyl group of mannose as a direct ligand toward the Fe(II), (3) the addition of the external base as the fifth coordination ligand to the de-pyruvamide(dep)-BLM-Fe(II) complex induces proton chemical shifts which are close to those of the BLM-Fe(II) complex, and (4) the magnitude of the chemical shifts in the deglyco-BLM-Fe(II) complex is comparable to that in the BLM-Fe(II)

complex, but the resonance lines of the former are fewer than those of the latter. These observations suggest that the sugar moieties of BLM, *iso*-BLM, and dep-BLM are located near the Fe (II)-binding site spatially and the sugar protons experience the paramagnetic effect of the Fe(II). A complete assignment of these resonances will be published elsewhere.

Upon CO binding to deglyco-BLM-Fe(II) and BLM-Fe(II) complexes, the remarkable proton paramagnetic shifts completely disappeared, indicating the presence of a diamagnetic Fe(II) ion(S=0) in these CO adducts. Table 3 shows the proton chemical shifts for selected resonances of deglyco-BLM-Fe(II)-CO complex, together with those of the corresponding BLM complex. The result for BLM-Fe(II)-CO adduct is consistent with those published,[7] except for the value of the β-hydroxyhistidine C2-H. The present results indicate that (1) deglyco-BLM and BLM form a similar Fe(II)-CO complex and (2) in both the ligands, the primary and secondary amine groups of the β-aminoalanine portion, the pyrimidine ring nitrogen, and the imidazole N-1 of the β-hydroxyhistidine moiety at least participate as coordination groups. It is known that the chemical shifts of the imidazole protons are quite sensitive to any charge present on the ring.[18,19] Therefore, it is reasonable that the C2-H values of deglyco-BLM-Fe(II)-CO complex (Im_2=8.47 ppm) and BLM-Fe(II)-CO complex (Im_2=8.55 ppm) should be closer to that of the protonated ligands (Im_2=*ca.* 8.7 ppm) than to that of the neutral ligands (Im_2=*ca.* 7.7 ppm).

The spin-trapping experiments of deglyco-BLM-Fe(II) complex system at pH 6.9 revealed that the production of hydroxyl radical was clearly lower than that by the corresponding BLM system and that the radical spin concentration of the former was estimated to be approximately 43% of that of the latter.

Fig. 2. Probable structure for Fe(II)-CO adduct complexes of BLM and deglyco-BLM.



The present results suggest that the carbamoyl group of the sugar portion in BLM does not directly coordinate to the ferrous ion and that the iron-binding site consists of a rigid 5–5–5–6 ring member chelate coordinated by the nitrogens of the primary and secondary amines, the pyrimidine, the deprotonated peptide and imidazole of the β-hydroxyhistidine. Presumably, the sugar moiety is situated near the Fe(II)-coordination site and plays a role as an environmental factor in the effective oxygen activation and site-specificity of the oxygen radical.

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