Tetrahedron Vol. 40, No. 3, pp. 501 to 509, 1984
Printed in Great Britain.

0040-4020/84 $3.00 + .00
Pergamon Press Ltd.

DNA–BLEOMYCIN INTERACTION

NUCLEOTIDE SEQUENCE-SPECIFIC BINDING AND CLEAVAGE OF DNA BY BLEOMYCIN

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(*Received in U.S.A. October* 1982)

Abstract—Biosynthetic intermediates and synthetic analogues of bleomycin (BLM) have been investigated for their metal binding, dioxygen activation, and DNA cleavage. Molecular O_2 was activated by the Fe(II) complex of a synthetic model ligand. Nucleotide sequence specificities in DNA cleavage by the BLM–Fe(II) and deglyco-BLM–Fe(II) complexes were almost identical. It has been shown that (1) the β-aminoalanine-pyrimidine-β-hydroxyhistidine portion of BLM is essential for the metal binding and dioxygen activation and (2) the bithiazole moiety contributes to the specific binding to guanine base of DNA.

Bleomycin (BLM) is a family of metalloglycopeptide antitumor antibiotics clinically used in the treatment of Hodgkin's lymphoma, carcinomas of the skin, head and neck, and tumors of the testis.[1] The primary action of BLM responsible for the bioactivity has been believed to be DNA cleavage in the presence of Fe(II) and molecular O_2. Indeed, the BLM–Fe(II)–O_2 complex system cleaves isolated DNA specifically at G-C(5′→3′) and G-T(5′→3′) sequences,[2] and also can preferentially degrade the DNA sequences in open chromatin within isolated nuclei.[3] The binding affinity of BLM toward ribohomopolymer is in decreasing order poly G($K = 5 \times 10^6$) > poly A(1×10^6) > poly U(1×10^5) > poly C, indicating higher affinity for guanine bases.[4] The similar binding parameters for the DNA complexes fo BLM and tripeptide S suggest the participation of the bithiazole-terminal amine portion in the DNA binding, because tripeptide S consists of the bithiazole and terminal amine portion of BLM. In the ^1H NMR spectra, indeed, the signals for bithiazole protons and dimethylsulfonium protons were selectively broadened in the presence of DNA.[5] Presumably, the base-specific DNA binding of the drug is responsible for the sequence specificity in DNA cleavage.

On the other hand, it is known that (1) ferrous ion and molecular O_2 are cofactors for DNA strand scission by BLM and (2) divalent metal ions such as Cu(II), Zn(II) and Co(II) inhibit BLM-induced damage to DNA.[6] The potentiometric results indicate that the stability of BLM-metal complexes is in the order Fe(II) < Co(II) < Ni(II) < Cu(II) > Zn(II), and that these divalent metal complexes have similar coordination environments.[7] The spectral features of various BLM–iron complex species with O_2–analogues were comparable to those of the corresponding hemoprotein complexes.[8] For the iron-coordination environment, various spectroscopic studies revealed that the secondary amine N, pyrimidine ring N, deprotonated peptide N bind as the basal planar ligand and α-amino N as axial donor, and that the iron site is substantially a square–pyramidal structure with four chelate ring of 5–5–5–6 ring members (Fig. 1).[9,10] The structure has been supported by X-ray crystallography of the Cu(II) complex of P-3A, a biosynthetic precursor of BLM,[11] and spectroscopic study of an acid hydrolysis product of the BLM–Co(III) complex.[12]

The pink colored BLM–Fe(II) complex is rapidly oxidized by molecular O_2, yielding a yellow-brown and stable species, the BLM-Fe(III)–OH^- complex. The addition of hydrogen peroxide induced the convertion from the inactive BLM–Fe(III)–OH^- complex ($g_1 = 1.893$, $g_2 = 2.185$, and $g_3 = 2.431$) to the active BLM–Fe(III)–O_2H^- species ($g_1 = 1.937$, $g_2 = 2.171$ and $g_3 = 2.254$) for DNA cleavage.[13–15] During the reversible redox reaction of the BLM–iron complex, production of reactive O_2-radical species such as $^{\cdot}OH$ was demonstrated by use of the ESR spin trapping technique for a mixture of BLM–Fe(III)–O_2 and appropriate spin traps such as N-t-butyl-α-phenylnitrone (BPN)[16] and 5,5-dimethyl-1-pyrroline-N-oxide (DMPO).[17] On the basis of these results, a hypothetical mechanism of O_2-activation process as shown in Fig. 2 has been proposed.[15] Herein, we describe the results on chelation chemistry and DNA cleavage using BLM and its related compounds.

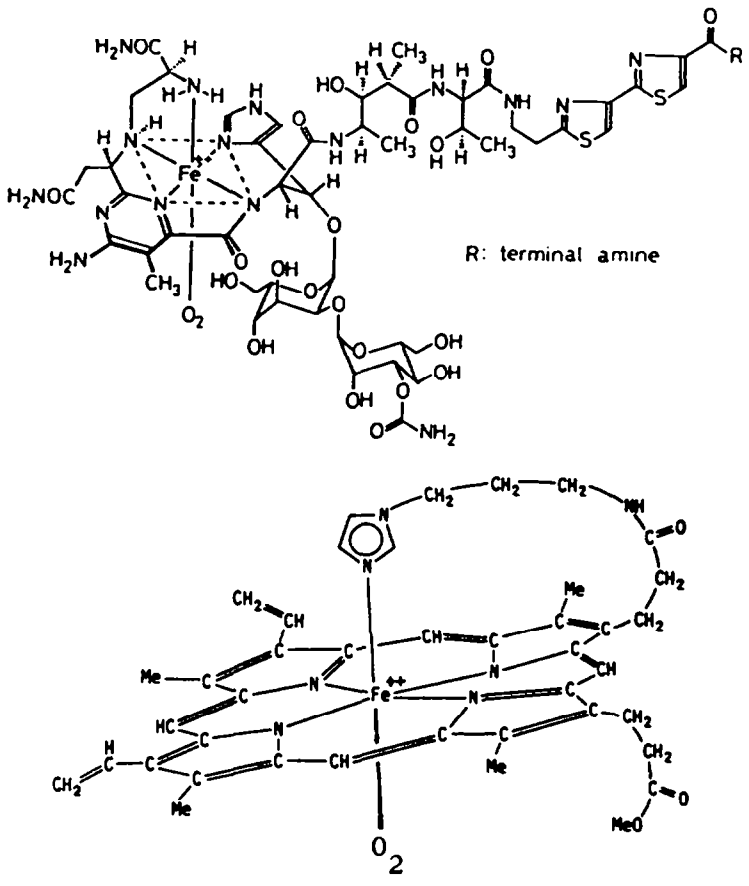


Fig. 1. Iron–bleomycin (upper) and chelated protoheme (lower).

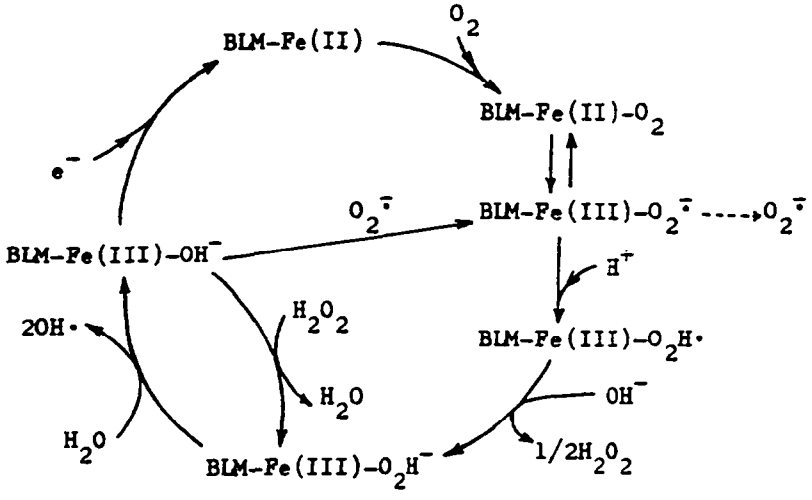


Fig. 2. Hypothetical mechanism for cyclic function of bleomycin–iron complex.

RESULTS AND DISCUSSION

Synthetic analogues and biosynthetic intermediates of bleomycin. Although the structure of the BLM-metal complex shown in Fig. 1 seems most reasonable, on the other hand, some possible transition metal binding sites have been proposed on the basis of theoretical and spectroscopic investigations.[18-20] To clarify the role of the respective functional groups, we have studied metal binding and dioxygen interaction of the biosynthetic intermediates and synthetic analogues of BLM shown in Fig. 3. PYML (Pyridine Model Ligand)-1 and -2 are synthetic analogues corresponding to the metal binding site shown in Fig. 1.[21] PEML (Pyridine Ethylenediamine Model Ligand) has a simple amino group instead of an imidazole group.

PYMLs were synthesized as follows (Scheme 1).

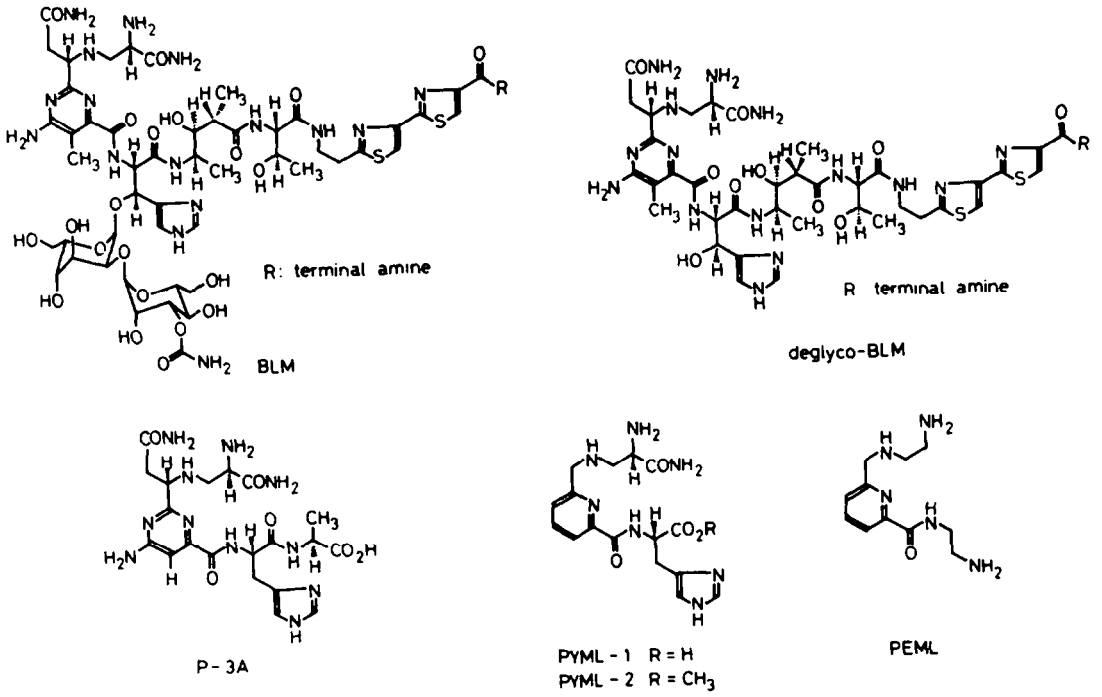
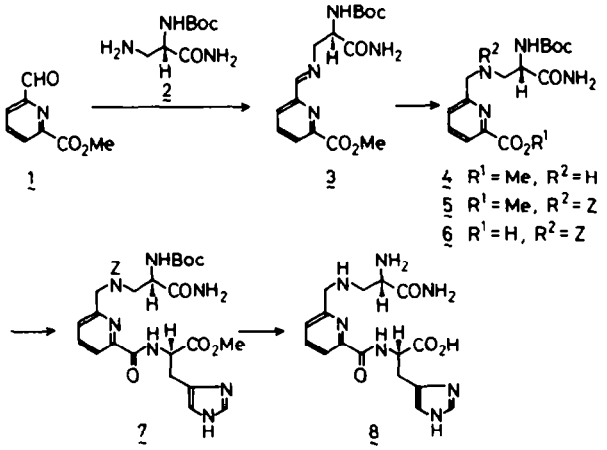


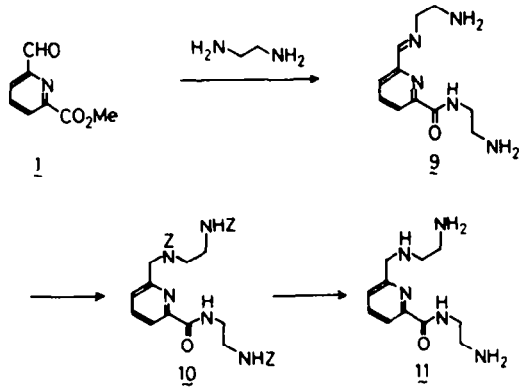
Fig. 3. Biosynthetic intermediates and synthetic analogues of bleomycin.



Scheme 1.

Condensation of formylpyridine **1**[22] with (S)-3-amino-2-[(t-butoxycarbonyl)-amino]propionamide **2**[23] afforded the Schiff base **3**, which was hydrogenated to give **4** in 60% yield based on **1**. Treatment of **4** with benzyl chloroformate gave benzyloxycarbonyl derivative **5** in 88% yield. Condensation of **5** with histidine was carried out smoothly. Free acid **6**, obtained by hydrolysis of **5**, was treated with N,N′-carbonyldiimidazole and condensed with histidine methyl ester,[24] affording peptide **7** in 83% yield from **5**. PYML-2 was obtained by deprotection of **7** with HBr–AcOH. The methyl ester of PYML-2 was hydrolyzed to give PYML-1 **8** in 87% yield.

PEML was synthesized as follows (Scheme 2). Schiff base **9**, obtained by treatment of **1** with a large excess of ethylenediamine, was hydrogenated. The crude product was treated with benzyl chloroformate (4 equiv) to afford tris(benzyloxycarbonyl) derivative



Scheme 2.

10 in 59% yield form **1**. The protective groups of **10** were removed to give PEML **11** quantitatively.

The electronic and CD spectral features and redox potential of the PYML-1–Cu(II) complex corresponds well to those of naturally occurring BLM–Cu(II) complex[25] (Table 1). The λ_{max} values indicate similar Cu-ligand field for PYML-1 and BLM, because the magnitude of the ligand field around the central Cu(II) is reflected in the $d_{xz,yz}$–$d_{x^2-y^2}$ transition.[26] The red shift of the ν_{d-d} for the P-3A and deglyco–BLM–Cu(II) complexes suggests a distortion from square planar configuration of the Cu(II) site. Their Cu(II)/Cu(I) potentials are clearly higher than those of the PYML-1 and BLM. Indeed, X-ray crystallographic result of the P-3A–Cu(II) complex demonstrated that Cu(II) site is a distorted square-pyramidal structure.[11] The high reduction potential of the PEML–Cu(II) complex is presumably attributed to the structural strain of 5-5-5-5 chelate ring members.

Table 2 summarizes the ESR parameters for the divalent metal complexes of PYMLs, PEML, P-3A and BLM. The ESR parameters for the Cu(II) complexes of PYMLs and BLM were comparable and characterized by axially symmetric g- and A- tensor components. The close similarity between ESR parameters of the metal complexes of PYML-1 and -2 indicates that the carboxyl group of PYML-1 does not participate to metal binding. Similar axially symmetric ESR feature was observed for the PEML–Cu(II) complex, although the increasing $g_{\parallel}$ value and the decreasing $A_{\parallel}$ value are noted in this case. While, the ESR spectra for the Cu(II) complexes of P-3A and deglyco-BLM showed the copper hyperfine structure with lower symmetric g-anisotropies, suggesting a rhombic distortion of the Cu(II) chromophore.

PYMLs, P-3A and deglyco-BLM formed the Co(II) complexes and their dioxygen adducts similar to those of BLM. These ESR features are typical of a low-spin square-pyramidal Co(II) complex with the electronic configuration $[(d_{xy,yz,zx})^6(d_{z^2})^1]$ and of monooxygenated low spin Co(II)–O_2 adduct complex. The

Table 1. Visible, circular dichroism spectral characteristics and redox potential for Cu(II) complexes of PYML-1, PEML, P-3A, deglyco–BLM and BLM

Complex	λ_{max}, nm(ε)	CD Extrema, nm(Δε)	$E_{\frac{1}{2}}$(V vs NHE)
PYML-1-Cu(II)	597(140)	545(+0.88) 655(-0.10)	-0.319
PEML-Cu(II)	588(110)	no CD signals	-0.061
P-3A-Cu(II)	625(125)	580(+0.56) 700(+0.21)	-0.136
deglyco-BLM-Cu(II)	610(130)	560(+0.70) 675(+0.13)	-0.176
BLM-Cu(II)	595(120)	555(+1.21) 665(-0.60)	-0.327

Table 2. ESR parameters for divalent metal complexes of PYMLs, PEML, P-3A deglyco–BLM and BLM

Complex	$g_{\parallel}(g_z)$	$g_{\perp}(g_x, g_y)$	$A_{\parallel}$, G	A_N, G	N-hfs (line)
PYML-1-Cu(II)	2.206	2.048	179.4		
PYML-1-Co(II)	2.022	2.255	92.5	13	3
PYML-1-Co(II)-O_2	2.093	2.005	22.5		
PYML-1-Fe(II)	no ESR signals				
PYML-1-Fe(II)-^{14}NO	2.009	2.036 1.972		25.6	3
PYML-1-Fe(II)-^{15}NO	2.009	2.036 1.972		35.6	2
PYML-1-Fe(II)-^{14}NO+DNA	2.009	2.036 1.972		25.6	3
PYML-2-Cu(II)	2.204	2.052	179.8		
PYML-2-Co(II)	2.020	2.252	92.0	13	3
PYML-2-Co(II)-O_2	2.093	2.005	22.5		
PYML-2-Fe(II)	no ESR signals				
PYML-2-Fe(II)-^{14}NO	2.009	2.036 1.970		25.5	3
PEML-Cu(II)	2.237	2.250	170.8		
PEML-Co(II)*	2.018	2.260	84.3	13	3
PEML-Co(II)-O_2	not detected				
PEML-Fe(II)*	no ESR signals				
PEML-Fe(II)-^{14}NO	not detected				
P-3A-Cu(II)	2.214	2.133 2.078	167.3		
P-3A-Co(II)	2.027	2.275	93.8	13	3
P-3A-Co(II)-O_2	2.102	2.007	22.4		
P-3A-Fe(II)	no ESR signals				
P-3A-Fe(II)-^{14}NO	2.007	2.038 1.968		24.8	3
P-3A-Fe(II)-^{15}NO	2.007	2.038 1.969		35.0	2
P-3A-Fe(II)-^{14}NO+DNA	2.007	2.038 1.968		24.8	3
deglyco-BLM-Cu(II)	2.214	2.131 2.077	167.0		
deglyco-BLM-Co(II)	2.027	2.277	95.0	13	3
deglyco-BLM-Co(II)-O_2	2.100	2.009	22.5		
deglyco-BLM-Fe(II)	no ESR signals				
deglyco-BLM-Fe(II)-^{14}NO	2.007	2.038 1.969		24.8	3
deglyco-BLM-Fe(II)-^{15}NO	2.007	2.038 1.970		33.0	2
deglyco-BLM-Fe(II)-^{14}NO+DNA	2.007	2.046 1.963		25.1	3
BLM-Cu(II)	2.211	2.055	183.0		
BLM-Co(II)	2.025	2.272	92.5	13	3
BLM-Co(II)-O_2	2.098	2.007	20.2		
BLM-Fe(II)	no ESR signals				
BLM-Fe(II)-^{14}NO	2.008	2.041 1.976		23.6	3
BLM-Fe(II)-^{15}NO	2.008	2.040 1.976		31.6	2
BLM-Fe(II)-^{14}NO+DNA	2.006	2.060 1.962		24.0	3

* The complex was detected only under the fully anaerobic condition.

PEML–Co(II) complex was detected only under fully anaerobic condition, and its $A_{\parallel}^{Co}$ value was smaller than those of the Co(II) complexes of PYMLs, P-3A, deglyco–BLM, and BLM. This is due to the higher pK_a of the axial α-amino base in PEML ligand, which lacks the electron-withdrawing $CONH_2$ group. Repeated and careful experiments demonstrated no formation of monooxygenated adduct for the PEML–Co(II) complex.

The nitric oxide adduct complexes were easily obtained by addition of a few milligrams of $Na^{14}NO_2$ (or $Na^{15}NO_2$) and sodium dithionite (or $NaBH_4$) to the Fe(II) complexes of PYMLs, P-3A, deglyco–BLM, and BLM. In contrast, the PEML–Fe(II)–NO complex was not detected even under fully anaerobic condition. The ESR features exhibited rhombic symmetry with a triplet ^{14}N (or doublet ^{15}N) hyperfine splitting in the central g_z signal, and typical of six-coordination type.[10] The changes of the N-hfs lines and the A_N values by the substitution of ^{14}NO by ^{15}NO were in accord with the nuclear spin and magnetogyric ratio of $^{14}N(I = 1$ and $\gamma_N = 1.934)$ and $^{15}N(I = 1/2$ and $\gamma_N = 2.712)$ nuclei.

Except for the PEML–Fe(II) complex, the Fe(II) complexes of PYML-1, P-3A, deglyco-BLM and BLM reacted with carbon monoxide, ethyl isocyanide, and nitric oxide to form these adduct complexes. As shown in Table 3, the visible absorption spectra of these adduct complexes were characterized by their large extinction coefficients which are due to iron-ligand charge transfer transition, and the absorption maxima shifted to longer wavelength in the order $C_2H_5NC > NO > CO$.

^{57}Fe-Mössbauer spectra of the PYML-1–Fe(II) complex and its CO adduct, measured at 110 K in zero magnetic field, were characterized by a single quadrupole doublet. The quadrupole splitting ($\Delta E_Q = 3.00$ mm/s) and the isomer shift ($\delta_{Fe} = +1.05$ mm/s) of the PYML-1–Fe(II) complex are typical of a high spin ferrous ion ($S = 2$) and remarkably close to those of the BLM–Fe(II) complex ($\Delta E_Q = 2.99$ mm/s and $\delta_{Fe} = +1.10$ mm/s). The PYML-1–Fe(II)–CO complex showed Mössbauer characteristic ($\Delta E_Q = 0.51$ mm/s and $\delta_{Fe} = +0.18$ mm/s) which are similar to those of the BLM–Fe(II)–CO complex ($\Delta E_Q = 0.66$ mm/s and $\delta_{Fe} = +0.19$ mm/s) and consistent with as $S = 0$ ferrous assignment. These are in accordance with the results of ^{1}H NMR spectra of the PYML-1–Fe(II) complexes, which indicate that high-spin Fe(II) ion ($S = 2$) and diamagnetic Fe(II) ion ($S = 0$) are present in the PYML-1–Fe(II) and its CO adduct complexes, respectively.[21]

The ESR spin trapping experiments using BPN and DMPO at pH 6.9 showed that OH radicals are generated from the PYML-1–Fe(II)–O_2 complex system. The ESR pattern and the parameter were as follows: BPN adduct (triplet of doublet, $g = 2.0057$ and $a^N = 15.3$ G) and DMPO adduct (quartet, $g = 2.0058$ and $a^N = A_\beta^H = 15.2$ G). Carbon monoxide competitively interfered the dioxygen activation by PYML-1–Fe(II) complex (Fig. 4). Of special importance is the fact that (1) the relative radical spin concentration of the PYML-1 (or P-3A)–Fe(II) and deglyco–BLM–Fe(II) complex system was estimated to be approx. 20 and 40% of that of the corresponding BLM-Fe(II) complex system, respectively,[21,27] and (2) the dioxygen activating ability of the PEML–Fe(II) complex system was negligibly small. Consequently, in the total structure of BLM, the portion corresponding to PYML-1 is considered to be responsible for metal binding and dioxygen activa-

Table 3. Visible absorption characteristics for Fe(II) complexes of PYML-1, PEML, P-3A, deglyco–BLM and BLM with dioxygen analogues

Complex	λ_{max}, nm(ε)
PYML-1-Fe(II)	465(300)
PYML-1-Fe(II)-CO	390(2000)
PYML-1-Fe(II)-C_2H_5NC	490(1800)
PYML-1-Fe(II)-NO	470(1650)
PEML-Fe(II)	470(300)
PEML-Fe(II)-CO*	not detected
PEML-Fe(II)-C_2H_5NC*	not detected
PEML-Fe(II)-NO*	not detected
P-3A-Fe(II)	470(320)
P-3A-Fe(II)-CO	385(2300)
P-3A-Fe(II)-C_2H_5NC	490(2000)
P-3A-Fe(II)-NO	470(1800)
deglyco-BLM-Fe(II)	472(300)
deglyco-BLM-Fe(II)-CO	380(2800)
deglyco-BLM-Fe(II)-C_2H_5NC	495(2700)
deglyco-BLM-Fe(II)-NO	475(2200)
BLM-Fe(II)	476(380)
BLM-Fe(II)-CO	380(3000)
BLM-Fe(II)-C_2H_5NC	495(2700)
BLM-Fe(II)-NO	470(2300)

* The formation of these dioxygen analogous adducts was not observed even under the fully anaerobic condition.

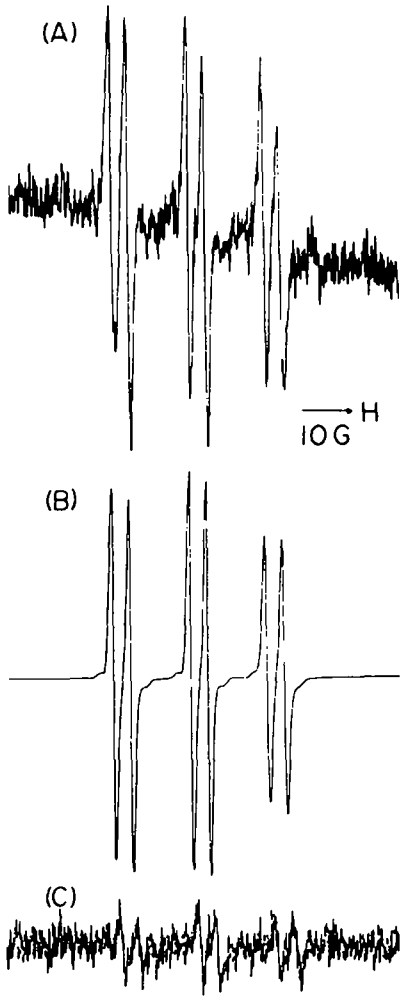


Fig. 4. ESR spin trapping by PYML-1-Fe(II) complex systems in the presence of BPN. The ESR spectra were obtained by oxygen bubbling of 0.5 mM PYML-Fe(II) complex (A), 0.5 mM PYML-Fe(II) complex plus 5.0 mM $Na_2S_2O_4$ (B), and 0.5 mM PYML-Fe(II)-CO complex (C) in the presence of 0.08 M BPN. Conditions of ESR spectroscopy: microwave power, 10 mW; modulation amplitude, 0.5 G; time constant, 0.03 sec; and scan time, 4 min.

tion, and the gulose-mannose moiety seems to play an important role as an environmental factor in the effective dioxygen activation, just as the pivalimidophenyl groups in the picket-fence porphyrins.[28,29]

Sequence specificity in DNA cleavage of bleomycin. Fig. 5 shows the nucleotide sequences at specific sites on a pBR 322 DNA fragment cleaved by the BLM-FE(II) complex system, together with the deglyco-BLM-Fe(II) complex system. Cleavage by the BLM-iron system evidently occurred preferentially at G-C(5′→3′) and G-T(5′→3′) sequences, and in particular the pyrimidine bases at positions 30, 33, 41 and 59 were the most preferred cleavage sites. The present guanine-pyrimidine (5′→3′) specificity is consistent with the observations by D'Andrea and Haseltine[30] and Takeshita *et al.*,[2] who used lactose operon pL J3 and bacteriophage ϕX 174 DNA fragments, respectively. Interestingly, the Fe(II) complex systems of BLM and deglyco-BLM showed almost identical nucleotide sequence-specific mode for the DNA cleavages. Therefore, it appears that the sugar moiety does not produce a noticeable effect on the specificity for the binding and cleavage of DNA by BLM antibiotics. Recently, the binding specificity of the four BLM congeners was compared, and the result also revealed no significant contribution of the terminal amine to the nucleotide sequence specificity.[31] On the other hand, various bithiazole derivatives were tested for their ability to inhibit the BLM-DNA interaction.[32] It was found that, although DNA degradation was diminished, the specificity of DNA cleavage by BLM was not altered by any of the bithiazoles rested. In contrast, we observed that certain DNA binding agents such as distamycin A and actinomycin D clearly alter the sequence specificity of DNA breakage by Fe-BLM.[33] Distamycin A masked the cleavage at certain G-T and G-A sequences, and produced higher specificity for G-C sequences than that of Fe-BLM only. This is reasonably explained by the specific A and T binding of distamycin A, having planar pyrrole rings connected each other through peptide bonds. Actinomycin D also influenced the nucleotide sequence specificity of DNA cleavage. In this case, the pre-

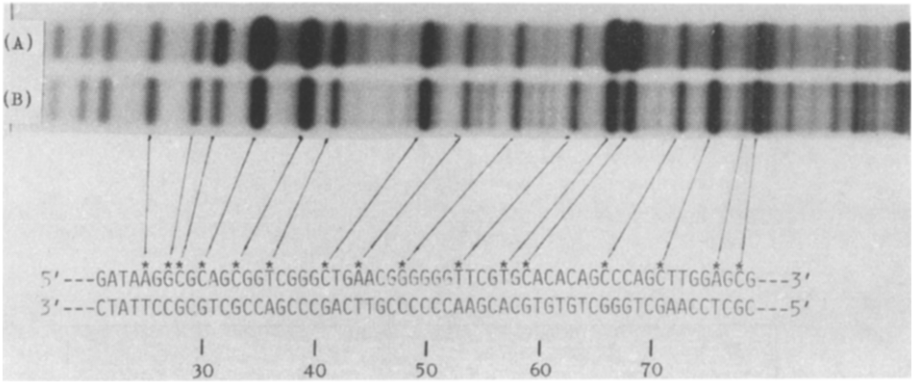


Fig. 5. Release of bases from double-strand restriction fragment plasmid pBR 322 DNA by iron complex systems of bleomycin (A) and deglyco-bleomycin (B).

ferred cleavage sites in Fe–BLM were shifted from G-C sequences to G-A and G-T sequences. Partial wobble of the DNA duplex by the phenoxazone ring and/or the peptide loop of actinomycin D may induce a delicate change for DNA binding by the bithiazole group of BLM. Certainly, the preferred "recognition site" of BLM in DNA involves GpC and GpT sequences and the sequences with reversed polarity (CpG and TpG) are not recognized. The guanine–pyrimidine (5′→3′) specificity of BLM-Fe(II) appears to be mainly due to the interaction between the BLM bithiazole group and DNA guanine base.

CONCLUSION

BLM molecule is divided into two regions showing different functions, metal chelation site and DNA binding. We have succeeded in separating these two functionalities. Synthetic models corresponding to the metal binding site mimicked the property and function of BLM as a metal complex. The physicochemical property of the PYML-1-divalent metal complexes are remarkably similar to those of the BLM–metal complexes. Moreover, molecular oxygen was catalytically activated by the PYML-1–Fe(II) complex, as demonstrated by ESR spin trapping experiments.

As seen between PYMLs and PEML, the replacement of the imidazole group by the amino group gives significant influence on dioxygen-binding and -activation by the Fe(II) complexes. The Co(II)–O_2, Fe(II)–CO, Fe(II)–C_2H_5NC, and Fe(II)–NO adduct complexes of PEML were not observed by all means. Therefore, the presence of the imidazole group, in particular, the *trans*-position of pyrimidine (or pyridine) and imidazole groups in the Fe(II)-coordination appears to be essential for the effective binding and activation of molecular O_2. The remarkable similarity of the divalent metal complexes between deglyco–BLM and BLM definitely indicates that the carbamoyl group of the sugar portion in BLM does not coordinate directly to the metal ions. Thus, PYML-1 is indeed the metal binding site of BLM and thought to be the "minimum requirement" for the dioxygen activation.

On the other hand, bithiazole–terminal amine moiety is thought to be DNA binding site. As clearly demonstrated in the DNA cleavage by the deglyco–BLM–Fe(II) complex system, the sugar moiety does not contribute significantly to the specificity of DNA binding, which is mainly due to the intercalation with the bithiazole group. The synthetic analogue PYML-1–Fe(II) complex was remarkably less active than the corresponding BLM complex in the DNA cleavage reaction despite its fairly effective dioxygen activation. As shown in Table 2, although separation of the g_x and g_y was observed by addition of DNA to the BLM–Fe(II)–NO complex, ESR parameters of PYML–Fe–II)–NO complexes were not influenced by addition of DNA, suggesting almost negligible PYML–DNA interaction. In contrast, both dioxygen activation and sequence-specific DNA cleavage were caused by deglyco–BLM, having bithiazole–terminal amine portion. On the other hand, Dervan *et al.* recently reported interesting DNA cleaving molecules, methidiumpropyl–EDTA–Fe(II)[34] and distamycin–EDTA–Fe(II).[35] These results demonstrated that, for the effective DNA strand scission, a DNA binding molecule must deliver a metal ion to the site of the DNA helix attacked by the activated molecular O_2. Consequently, it is considered that guanine base portion of DNA molecule plays a typical role as a receptor having a specific interaction with the bithiazole moiety of BLM.

EXPERIMENTAL

Materials. Purified BLM-A2 and deglyco–BLM–A2 were the gift of Nippon Kayaku Co. Ltd. The isotope $^{57}Fe(Fe_2O_3$, 90.92%) was obtained from Oak Ridge National Laboratory. The air-unstable Fe(II) complexes of BLM, deglyco–BLM, and the synthetic ligands with CO, C_2H_5NC and NO were prepared anaerobically at pH 6.9 in a Thunberg cuvette.

Spectroscopic measurements. Electronic absorption spectra were obtained on a Shimadzu Double-40R recording spectrophotometer. FT-^{1}H NMR (220 MHz) and X-band ESR spectra were recorded at 25° and 77 K with a Varian HR-220 and a JES-FE-3X spectrometers, respectively. ^{57}Fe–Mössbauer spectra in the zero magnetic field were obtained at 110 K with a conventional constant-acceleration type. The radiation was detected by a proportional counter and multichannel analyzer system. All the given isomer shifts are relative to the iron metal at 300 K and all workups were carried out in an oxygen-free environment.

DNA cleavage experiments. Plasmid pBR 322 DNA was digested with Hinf I, and the restriction fragment (396 bp) was isolated from a 6% polyacrylamide gel. Terminal phosphates were removed by treatment with bacterial alkaline phosphatese, and the 5′ ends were labeled with ^{32}P by using T4 polynucleotide kinase and [γ-^{32}P]ATP. This doubly end-labeled molecule was digested with Hae III and the singly end-labeled 327 bp fragment was isolated by electrophoresis on a 5% polyacrylamide gel. The reaction sample containing ^{32}P-labeled DNA fragment, sonicated calf thymus DNA (20 μg/ml), the BLM (or deglyco–BLM)–Fe(II) complex (4 μM), and 2-mercaptoethanol (20 mM) were incubated at 37° for 5 min. EDTA (final conc. 10 mM) was added to stop the reaction, and the DNA was recovered as precipitate by the addition of cold EtOH. A 20% polyacrylamide-7 M urea slab gel was used for nucleotide sequence analysis. Nucleotide sequences of the restriction fragments were determined by the method of Maxam and Gilbert.[36]

Synthesis of the model ligands. M.ps were measured on a Yamato MP-21 apparatus and uncorrected. NMR spectra were obtained on a JEOL FX-100 spectrometer and chemical shifts are expressed in ppm downfield from TMS. Abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. IR spectra were recorded on a JASCO DS-402G spectrometer. Mass spectra were measured with a JEOL JMS DX-300 mass spectrometer. Optical rotations were measured with a JASCO DIP-181 digital polarimeter. The preparations of PYML-1, -2, and PEML are described below.

Methyl 6 - [[[(S) - 2 - [(*t* - *butoxycarbonyl*)*amino*] - 2 - (*carbamoyl*) - *ethyl*]*amino*]*methyl*]*pyridine* - 2 - *carboxylate* (**4**). To a mixture of activated molecular sieve 3A (5 g), **1**[22] (826 mg, 5 mmol) and MeCN (20 ml), a soln of **2**[23] (1016 mg, 5 mmol) in MeCN was added under argon. The mixture was stirred overnight at room temp. Crude base **3** (1.73 g, 4.94 mmol, 99% yield) was obtained by removal of the molecular sieve and the solvent. Pd–C (5%, 345 mg) was added to a soln of **3** (1.73 g, 4.94 mmol) in MeOH (100 ml). The mixture was stirred overnight under H_2 at room temp. Yellow foam obtained by removal of the catalyst and the solvent was chromatographed on silica gel (eluted with CH_2Cl_2 : MeOH = 9 : 1) to give yellow foam **4** in 58% yield (1.001 g, 2.84 mmol).

$[\alpha]_D^{20}$ + 36.2°, c = 1, $CHCl_3$. MS, m/e = 353, M^+ + 1.

IR($CHCl_3$), 3420, 2980, 1725, 1688, 1592, 1490 cm^{-1}. ^1H NMR ($CDCl_3$, TMS), δ 1.45 (9H, s), 2.35 (1H, br. s), 2.80 (1H, dd, J = 7.0, 12.0 Hz), 3.17 (1H, dd, J = 5.0, 12.0 Hz), 4.02 (3H, s), 4.09 (2H, s), 4.25 (1H, dd, J = 5.0, 7.0 Hz), 5.84 (2H, br. d), 5.98 (1H, br. s), 7.44–8.16 (3H, m).

Methyl 6 - [[N - *benzyloxycarbonyl* - N - [(S) - 2 - [(*t* - *butoxycarbonyl*) - *amino*] - 2 - (*carbamoyl*) *ethyl*]*amino*]*methyl*]*pyridine* - 2 - *carboxylate* (**5**). Benzyl chloroformate (68 mg, 0.40 mmol) in CH_2Cl_2 (2 ml) and 0.1 N NaOH (3.4 ml) were added to a soln of **4** (119 mg, 0.34 mmol) in CH_2Cl_2 (3.4 ml). The mixture was vigorously stirred for 3 hr at room temp. The organic layer was separated, washed with water, dried over Na_2SO_4, and concentrated. White foam obtained was chromatographed on silica gel (eluted with CH_2Cl_2 : MeOH = 9 : 1) to give white foam of **5** in 88% yield (114 mg, 0.30 mmol).

$[\alpha]_D^{20} + 77.7°$, $c = 1$, $CHCl_3$. MS, $m/e = 486$, M^+. IR ($CHCl_3$), 3470, 3420, 3000, 2980, 1720, 1697, 1688, 1592, 1495 cm^{-1}. ^1H NMR ($CDCl_3$, TMS), δ 1.40 (9H, s), 3.42–5.20 (7H, br. m), 3.94 (3H, s), 5.68 (1H, br. d), 6.19 (1H, br. d), 6.84–8.32 (8H, br. m).

N - [6 - [[N - *Benzyloxycarbonyl* - N - [(S) - 2 - [(*t* - *butoxycarbonyl*) - *amino*] - 2 - (*carbamoyl*) *ethyl*]*amino*]*methyl*]*pyridine* - 2 - *carbonyl*] - L - *histidine methyl ester* (**7**). To a soln of **5** (259 mg, 0.53 mmol) in MeOH (5 ml), 0.1 N LiOH (5.3 ml) was added at 0°. After stirring for 30 min at 0° and 1 hr at room temp., the soln was neutralized by adding 0.1 N HCl (5.3 ml) at 0°. MeOH of the soln was evaporated under reduced pressure. The remaining aqueous soln was extracted with CH_2Cl_2. The extract was dried over Na_2SO_4, and the solvent was removed to give almost pure **6** (261 mg). N,N'-Carbonyldiimidazole (86 mg, 0.53 mmol) in THF (5 ml) was added to a soln of **6** in THF at 0° under argon. After stirring for 1 hr at room temp., THF (5 ml) soln of histidine methyl ester, prepared according to the known procedure[24] from histidine methyl ester dihydrochloride (484 mg, 2 mmol), was added. After stirring overnight at room temp., the soln was concentrated to give oily residue which was carefully chromatographed on silica gel (eluted with CH_2Cl_2 : MeOH = 9 : 1). Colorless oil of **7** was obtained in 83% yield based on **5** (273 mg, 0.44 mmol).

M.p., 168.5–170.0° (CH_2Cl_2). $[\alpha]_D^{20} + 60.1°$, $c = 1$, $CHCl_3$. MS, $m/e = 623$, M^+. (Found: N 15.50, C 57.70, H 6.01. Calc. for N 15.72, C 57.76, H 5.98%). IR (KBr), 3415, 3370, 2940, 1735, 1673, 1665, 1655, 1648, 1550, 1492 cm^{-1}. ^1H NMR ($CDCl_3$, TMS), δ 1.41 (9H, s), 3.07–3.28 (2H, m), 3.59–3.81 (1H, m), 3.69 (3H, s), 4.41–4.67 (2H, m), 4.88–5.03 (1H, m), 5.08 (2H, s), 5.13 (2H, s), 5.87–6.26 (3H, br. m), 6.74 (1H, s), 6.98–8.09 (8H, m), 7.61 (1H, s), 8.71 (1H, d).

N - [6 - [[[(S) - 2 - *Amino* - 2 - (*carbamoyl*)*ethyl*]*amino*]*methyl*]*pyridine* - 2 - *carbonyl*] - L - *histidine* (PYML-1, **8**) *and its methyl ester* (PYML-2). To a soln of **7** (109 mg, 0.175 mmol) in AcOH (2 ml), 30% HBr–AcOH (2 ml) was added at 0°. The soln was stirred at room temp. for 2.5 hr and concentrated to dryness. The remaining solid was washed with dry Et_2O and dried under a reduced pressure. PYML-2, obtained quantitatively, was dissolved in MeOH (2 ml) and 1 N NaOH was added to adjust the soln at pH 9 to 10. The soln was stirred at room temp. for 30 min, neutralized with 1 N HCl, and the solvent was evaporated. The residue was purified by Amberlite CG-50, H^+ form (eluted with 1% aqueous NH_3). Yellow solid of **8** was obtained in 87% yield (61 mg, 0.15 mmol).

PYML-1. m.p., 120.0–122.0°. $[\alpha]_D^{20} + 2.85°$, $c = 1$, H_2O. MS, $m/z = 376$, $M^+ + 1$. IR (KBr), 3390, 1710, 1655 cm^{-1}. ^1H NMR (D_2O, TSP), δ 2.77–3.43 (3H, dd × 3), 3.77 (1 H, dd, J = 2.0, 5.5 Hz), 3.91 (1H, dd, J = 2.0, 7.2 Hz), 4.18 (2H, s), 4.66 (1H, dd, J = 5.5, 7.2 Hz), 6.94 (1H, s), 7.25 (1H, s), 7.36–7.99 (3H, m).

PYML-2. $[\alpha]_D^{20} - 17.0°$, $c = 1$, H_2O. MS, $m/z = 390$, $M^+ + 1$. IR (KBr), 3400, 1730, 1700, 1655 cm^{-1}. ^1H NMR (D_2O, TSP), δ 3.20–4.12 (6H, m), 3.86 (3H, s), 5.00–5.28 (1H, m), 7.33 (1H, m), 7.59–7.81 (1H, m), 7.95–8.22 (1H, m), 8.04 (1H, s), 8.54–8.67 (1H, m).

N - [2 - [(*Benzyloxycarbonyl*)*amino*]*ethyl*] - 6 - [[*benzyloxycarbonyl* - [2 - [(*benzyloxycarbonyl*)*amino*]*ethyl*] *amino*]*methyl*]*pyridine* - 2 - *carboxamide* (**10**). A mixture of **1** (1.65 g, 10 mmol), ethylenediamine (10 ml), molecular sieve 3A (10 g), and EtOH (25 ml) was stirred overnight at room temp. After filtration of the molecular sieve, the soln of base **9** was hydrogenated at room temp. over Pd–C (5%, 450 mg) for 20 hr. The catalyst was removed by filtration, and the filtrate was concentrated. To a soln of the residue in CH_2Cl_2 (50 ml) was added benzyl chloroformate (6.82 g, 40 mmol) and 1 N NaOH (40 ml). The mixture was vigorously stirred for 1 hr at room temp. The organic layer was separated, washed with H_2O, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel (eluted with AcOEt : hexane = 4 : 1) to give colorless powder **10** in 59% overall yield (3.773 g, 5.9 mmol).

M.p. 106.0–107.0° (CH_2Cl_2–hexane). (Found N 10.72, C 65.87, H 5.93%. Calc. for N 10.95, C 65.71, H 5.83%. IR (KBr), 3320, 3030, 2940, 1718, 1700, 1695, 1685, 1645 cm^{-1}. ^1H NMR ($CDCl_3$, TMS), δ 3.11–3.71 (8H, m), 4.54 (2H, s), 4.91–5.16 (6H, m), 5.80 (1H, br. s), 6.92–8.50 (20H, m).

N - (2 - *Aminoethyl*) - 6 - [[(2 - *aminoethyl*)*amino*]*methyl*]*pyridine* - 2 - *carboxamide* (PEML, **11**). To a soln of **10** (300 mg, 0.469 mmol) in AcOH (3 ml), 30% HBr–AcOH (3 ml) was added. The soln was stirred for 1 hr at room temp. and concentrated to dryness. The solid thus obtained was washed with dry Et_2O, dried under a reduced pressure, and purified by an ion-exchange resin using DIAION WK 10, H^+ form (eluted with 1% aqueous NH_3). PEML **11** was obtained in 99.6% yield (97.7 mg, 0.467 mmol).

MS, $m/e = 237$, M^+. IR (KBr), 3410, 1705 cm^{-1}. ^1H NMR (CD_3OD, TMS), δ 2.60–3.00 (6H, m), 3.37–3.59 (2H, m), 3.96 (1H, s), 7.37–8.11 (3H, m).

Acknowledgements—We are grateful to Drs. M. Takanami and H. Sugisaki for pertinent advice of DNA cleavage experiments, Drs. S. Ogawa and I. Morishima for ^1H NMR measurements, and Dr. K. Watanabe for ^{57}Fe–Mössbauer measurements. This study was supported in part by a grant from the Ministry of Education, Science, and Culture of Japan.

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