INTERACTION OF BLEOMYCIN WITH NUCLEIC ACIDS AS PROBED BY ESR SPECTROMETRY OF IRON(II)-NITROSYL COMPLEX OF BLEOMYCIN

Sir:

Bleomycin (BLM) causes DNA strand scission in the presence of Fe(II) ion and molecular oxygen[1]. Cleavage of cellular DNA by BLM probably accounts for the antitumor activity of this drug. Fluorescence quenching study has demonstrated that (1) the bithiazole portion of BLM preferentially binds to guanine base in nucleic acids and (2) the positive charge at the terminal amine enhances BLM binding to nucleic acids[2]. However, the bindings of these groups to DNA as seen by the quenching effects are not sufficient to interprete the BLM-DNA reaction because BLM binds to RNA as well as to DNA[2], although BLM does not cleave RNA[3]. The electron spin resonance (ESR) studies have shown that the BLM-Fe(II)-NO complex is conformationally perturbed in the presence of calf thymus DNA[4,5]. This paper deals with the interaction of the Fe(II)-NO complex of BLM with DNA, denatured DNA, ribohomopolymers and RNA.

BLM-A2, BLM-B2, iso BLM-A2[6], and depyruvamide (dep) BLM-A2[7] were used in this experiment. Calf thymus DNA, bakers yeast RNA, polyguanylic acid (poly G), and polyuridylic acid (poly U) were purchased from P-L Biochemicals. The hyperchromicity of heat-denatured DNA used in this study was 30%. In the presence or absence of nucleic acid, the Fe(II)-NO complex of BLM was prepared by addition of a few milligrams of $Na^{14}NO_2$ and sodium dithionite to the 1:1 BLM-Fe(II) complex solution at pH 6.9 according to the previous procedure[4]. ESR spectrum was measured at the concentration of 0.2 mM BLM-Fe(II)-NO complex in the presence of 2~10 mg/ml nucleic acids. X-Band ESR spectrum was recorded using a JES-FE-3X spectrometer.

Fig. 1 (A) shows the ESR spectrum of BLM-A2-Fe(II)-NO complex. This ESR feature exhibits rhombic symmetry with a triplet nitrogen-hyperfine interaction in the central g_z signal, which is typical of six-coordinated type. We interpreted that the three-line N-splittings on g_{mid} are due to the strong NO-to-iron bonding with the concomitantly weakened fifth axial ligand-

Fig. 1. ESR Spectra of BLM-A2-Fe(II)-NO (A), BLM-A2-Fe(II)-NO+DNA (B), BLM-A2-Fe(II)-NO+denatured DNA (C), and BLM-A2-Fe(II)-NO+RNA (D) at 77 K.

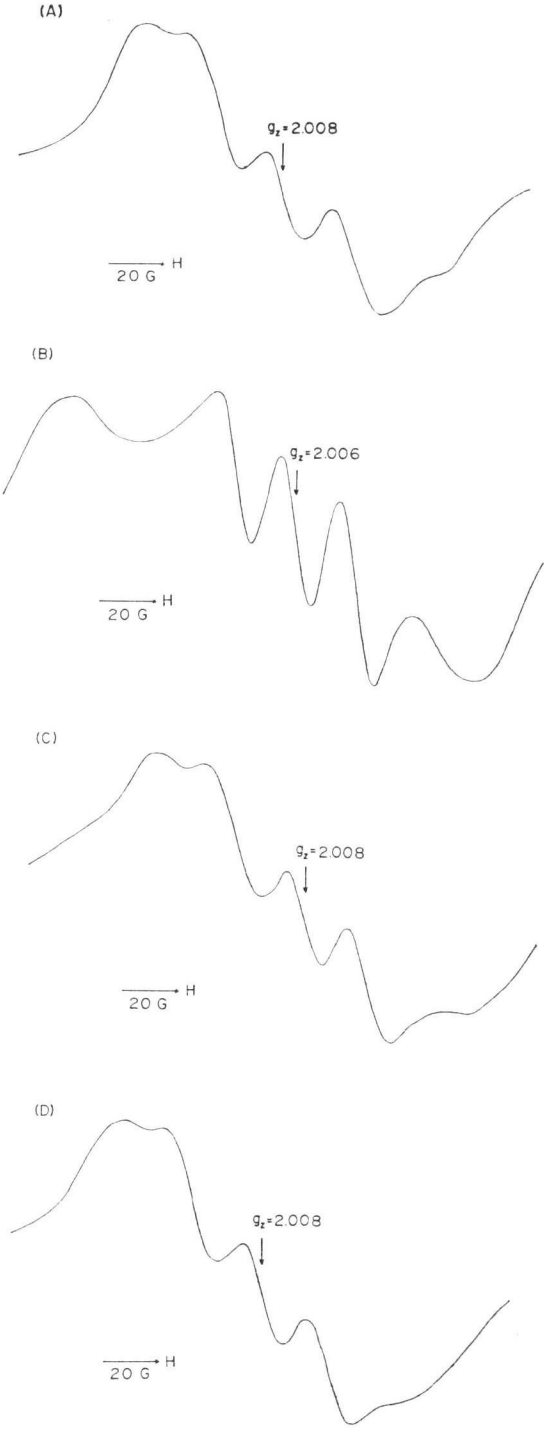

Table 1. ESR Parameters for iron(II)-nitrosyl complex of bleomycin in the presence or absence of nucleic acid.

Complex	g_x	g_z	g_y	A^N, G	N-hfs (line)
BLM-A2-Fe(II)-NO	2.041	2.008	1.976	23.6	3
BLM-B2-Fe(II)-NO	2.041	2.008	1.976	23.6	3
iso BLM-A2-Fe(II)-NO	2.040	2.008	1.976	23.6	3
dep BLM-A2-Fe(II)-NO	2.052	1.999	2.016	17.5	3
BLM-A2-Fe(II)-NO+DNA	2.060	2.006	1.962	24.0	3
BLM-A2-Fe(II)-NO+denatured DNA	2.045	2.008	1.971	23.9	3
BLM-A2-Fe(II)-NO+poly G	2.043	2.008	1.973	23.6	3
BLM-A2-Fe(II)-NO+poly U	2.041	2.008	1.976	23.6	3
BLM-A2-Fe(II)-NO+RNA	2.041	2.008	1.976	23.6	3

to-iron bonding[4]. BLM-B2-Fe(II)-NO and iso BLM-A2-Fe(II)-NO give the same ESR parameters as those of BLM-A2 (see Table 1). While the Fe(II)-NO complex of dep BLM-A2 which lacks the fifth axial amino nitrogen gives an ESR spectrum characteristic to the five-coordinated type (three-line N-splittings on g_{min})[8].

Fig. 1 also shows the ESR spectra of the BLM-A2-Fe(II)-^{14}NO complex in the presence of DNA (B), denatured DNA (C), and RNA (D). Table 1 summarizes the effect of several nucleic acids on the ESR parameters of the Fe(II)-NO complex of BLM-A2. Of special interest is the remarkable difference between DNA and RNA. DNA induced a large shift of the g_x and g_y values which were attributed to the in-plane anisotropy in the iron site. In contrast, RNA did not entirely affect the original ESR parameters. In addition, the effects of denatured DNA and poly G were significantly smaller than that of DNA. On the other hand, the fluorescence study has indicated that (1) BLM shows stronger interaction with heat-denatured calf thymus DNA (quenching, 65%) and poly G (77%) rather than with the intact double-strand DNA (56%) and (2) among ribohomopolymers the interaction decreases in the order of poly G>poly A (39%)>poly U (14%)>poly C (0%).[2] These results indicate that there is no apparent correlation between the extent of the BLM-nucleic acid interaction measured by fluorescence quenching and the structural alteration of the BLM-Fe(II)-NO complex in the presence of nucleic acids detected by ESR spectroscopy. The apparently different BLM-nucleic acid interaction observed by the two methods can be reasonably explained by the fact that the fluorescence quenching is the probe for the binding site of BLM to DNA while the ESR is the probe for the reaction site of BLM with DNA causing the DNA fragmentation. The binding site of BLM is the bithiazole-terminal amine portion and the reaction site is the β-aminoalanine-pyrimidine-β-hydroxyhistidine portion which is involved in the Fe(II)-complex formation[2,8,9].

In conclusion, the conformation of the BLM-Fe(II)-NO complex is not perturbed with RNA in contrast with DNA, although BLM can bind to RNA as well as to DNA. This will be an account for the selective strand scission of DNA but not of RNA by BLM.

YUKIO SUGIURA
*TOMOHISA TAKITA
*HAMAO UMEZAWA

Faculty of Pharmaceutical Sciences
Kyoto University, Kyoto 606, Japan
*Institute of Microbial Chemistry
Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

(Received October 15, 1980)

References

1) SAUSVILLE, E. A.; R. W. STEIN, J. PEISACH & S. B. HORWITZ: Properties and products of the degradation of DNA by bleomycin and iron (II). Biochemistry 17: 2746~2754, 1978

2) KASAI, H.; H. NAGANAWA, T. TAKITA & H. UMEZAWA: Chemistry of bleomycin. XXII. Interaction of bleomycin with nucleic acids, preferential binding to guanine base and electrostatic effect of the terminal amine. J. Antibiotics 31: 1316~1320, 1978

3) SUZUKI, H.; K. NAGAI, E. AKUTSU, H. YAMAKI, N. TANAKA & H. UMEZAWA: On the mechanism of action of bleomycin. Strand scission of DNA caused by bleomycin and its binding to DNA *in vitro*. J. Antibiotics 23: 473~480, 1970

4) SUGIURA, Y. & K. ISHIZU: Metallobleomycins. Electron spin resonance study on nitrosyl complex of iron(II)-bleomycin. J. Inorg. Biochem. 11: 171~180, 1979

5) ANTHOLINE, W. E. & D. H. PETERING: Reaction of FeBlm with DNA. Fe(II)Blm-NO. Biochem. Biophys. Res. Commun. 91: 529~533, 1979

6) NAKAYAMA, Y.; M. KUNISHIMA, S. OMOTO, T. TAKITA & H. UMEZAWA: Chemistry of bleomycin. XII. Iso-bleomycin A2, a product of carbamoyl group migration. J. Antibiotics 26: 400~401, 1973

7) NAGANAWA, H.; Y. MURAOKA, T. TAKITA & H. UMEZAWA: Chemistry of bleomycin. XVIII. Carbon-13 NMR studies. J. Antibiotics 30: 388~396, 1977

8) SUGIURA, Y.: Bleomycin-iron complexes. Electron spin resonance study, ligand effect, and implication for action mechanism. J. Am. Chem. Soc. 102: 5208~5215, 1980

9) TAKITA, T.; Y. MURAOKA, T. NAKATANI, A. FUJII, Y. IITAKA & H. UMEZAWA: Chemistry of bleomycin. XXI. Metal-complex of bleomycin and its implication for the mechanism of bleomycin action. J. Antibiotics 31: 1073~1077, 1978