

# Sequence-specific recognition and cleavage of DNA by metallobleomycin: Minor groove binding and possible interaction mode

(bleomycin/groove binder/guanine recognition/anthramycin/DNase cleavage-inhibition patterns)

JUNE KUWAHARA AND YUKIO SUGIURA\*

Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606, Japan

Communicated by George H. Büchi, December 4, 1987

**ABSTRACT** The DNase I cleavage-inhibition analysis shows binding sites of approximately 2 or 3 base pairs—in particular, 5' N-G-C sequences—for the green-colored Co<sup>III</sup> and fully oxidized Fe<sup>III</sup> complexes of bleomycin. The apparent binding constant of the bleomycin-Co<sup>III</sup> complex is quite similar for glucosylated and nonglucosylated phage T4 DNAs, whereas poly[d(I-C)] clearly gives a smaller binding constant than does poly[d(G-C)]. In contrast to the covalent attachment of the guanine N-7 with aflatoxin B<sub>1</sub>, the modification of the guanine 2-amino group with anthramycin remarkably inhibits the DNA cleavages at 5' G-C and 5' G-T sites by the Fe<sup>III</sup> and Co<sup>III</sup> complex systems of bleomycin. These results strongly indicate that metallobleomycin binds in the minor groove of B-DNA and that the 2-amino group of guanine adjacent to the 5' side of the cleaved pyrimidine base is one key element of the specific 5' G-C or G-T recognition by the bleomycin-metal complex. A possible binding mode of metallobleomycin in the DNA helix has been proposed by computer-constructed model building.

Bleomycin (BIM) is clinically used in the treatment of squamous cell carcinoma, malignant lymphoma, and testis tumor (1). DNA strand scission by BIM is believed to be responsible for its therapeutic effect and requires certain metal ions as a cofactor (2). DNA cleavage by the BIM-Fe<sup>III</sup> complex occurs preferentially at guanine-pyrimidine (5' → 3') sequences, in particular at G-C sites (3). Some metallobleomycin systems such as Fe<sup>III</sup>, Co<sup>III</sup>, and Mn<sup>II</sup> give similar DNA breakage patterns (4, 5). The antitumor antibiotic consists of a linear hexapeptide and a disaccharide. In the BIM molecule, a difference in the terminal amine side chain and an absence of the gulose-mannose sugar portion have negligible effect on the sequence specificity of its DNA cutting (6, 7). Pretreatment of DNA with distamycin A or *cis*-diamminedichloroplatinum(II) clearly alters the sequence-specific cleavage by the BIM-Fe<sup>III</sup> complex (8, 9). In contrast with the recognition of A + T-rich sites in the minor groove of B-DNA by small molecules such as netropsin and distamycin, G-C recognition elements have not been well understood (10). Recently, it was shown that protooncogene promoters of human Harvey *ras* epidermal growth factor receptor have an extremely high G + C content and contain multiple G-C boxes (11). The molecular recognition for G-C sequences is also of interest in connection with the expression of such "growth control" genes, in addition to understanding of DNA-drug specific interaction.

The present experiments have been carried out to address the question of preferential binding of the 5' G-C sequence and cleavage of DNA by Fe<sup>III</sup> and Co<sup>III</sup> complexes of BIM.

The chemical structures of BIM and anthramycin (AnM) antibiotics used in this study are given in Fig. 1.

## MATERIALS AND METHODS

**Drugs and Chemicals.** Peplomycin (PeM) was supplied by Nippon Kayaku and AnM was a gift of L. H. Hurley (University of Texas), DNase I and T4 phage DNA were obtained from Sigma, and T4dC DNA was purchased from Takara Shuzo (Kyoto, Japan). All other chemicals used were of commercial reagent grade. After equimolar Co(NO<sub>3</sub>)<sub>2</sub> and PeM were mixed, the green-colored PeM-Co<sup>III</sup> complex species was isolated by preparative HPLC (12) and used in the present experiments.

**Estimation of Binding Affinity of the PeM-Co<sup>III</sup> Complex to DNAs.** Apparent binding constants between the green PeM-Co<sup>III</sup> complex and DNAs were evaluated in 10 mM Tris-HCl buffer (pH 7.5) by a fluorescence-quenching method similar to that of Chien *et al.* (13). The experimental data were analyzed by the neighbor-exclusion model of McGhee and von Hippel (14). Binding parameters,  $K$  (M<sup>-1</sup>) and  $n$  (base), obtained from nonlinear least-squares fit, indicate the apparent binding constant and number of bases per binding site, respectively. The assay reflects bithiazole fluorescence quenching rather than direct binding by the metallobleomycin.

**Preparation of <sup>32</sup>P-Labeled DNA Fragments.** The restriction fragments used in this study were as follows: 3'-end-labeled fragments were 200 base pairs (bp; positions 1006–1205) and 99 bp (positions 1208–1306) of the 298-bp fragment of pBR322 DNA; 5'-end-labeled fragments were 200 bp (positions 1006–1207) and 99 bp (positions 1206–1306) of the 298-bp fragment and 122 bp (positions 1525–1646) of the 506-bp fragment of pBR322 DNA. The number is used from the genomic numbering system of pBR322 DNA (15). [ $\alpha$ -<sup>32</sup>P]dTTP and [ $\gamma$ -<sup>32</sup>P]ATP were utilized for 3'- and 5'-end-labelings, respectively. DNA sequencing was carried out by the Maxam-Gilbert method (16). Densitometric analysis of the autoradiogram was performed with a Bio-Rad 1650-II scanning densitometer.

**DNase I Cleavage-Protection Analysis ("Footprinting") for the PeM-Co<sup>III</sup> Complex.** The reaction mixture contained the singly end-labeled fragment, sonicated calf thymus DNA (500  $\mu$ M), and the PeM-Co<sup>III</sup> complex (50  $\mu$ M) in 10 mM Tris-HCl buffer (pH 7.5). After preincubation at 37°C for 30 min, the sample was digested with DNase I (final concentration, 0.5  $\mu$ g/ml) for 4 min. The reaction was stopped by adding NaOH-adjusted Na<sub>2</sub>EDTA, and then the sample was lyophilized. To avoid the DNA cleavage by the PeM-Co<sup>III</sup> complex itself, the reaction was done in dark conditions.

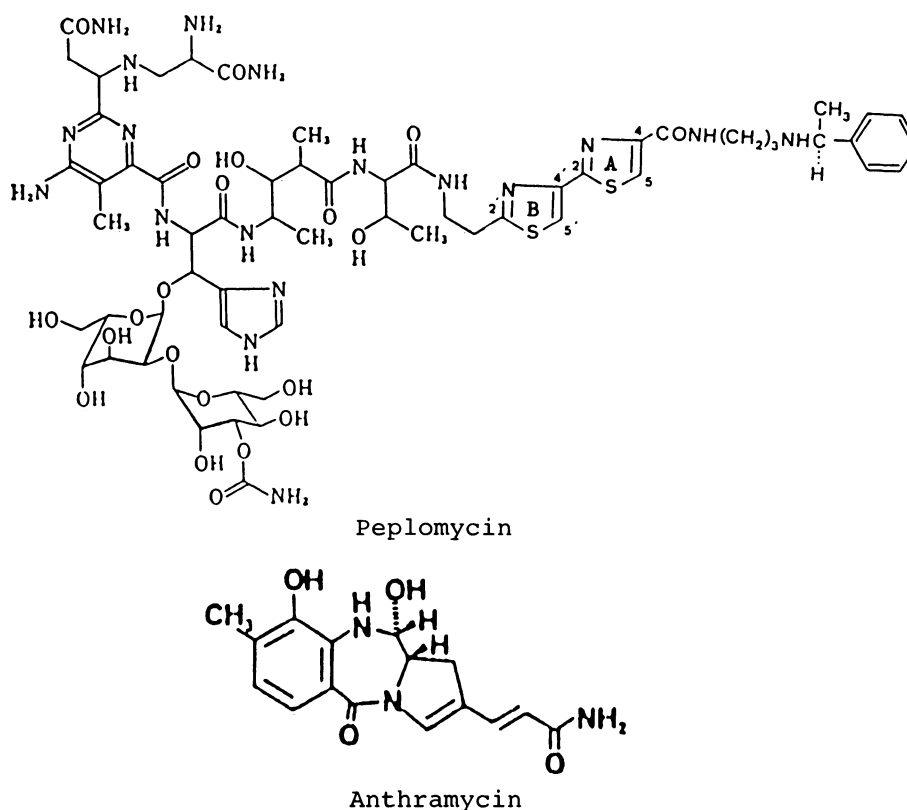


FIG. 1. Chemical structures of PeM and AnM.

**Cleavage of AnM-Modified DNA by PeM-Metal Complexes.** Modification of DNA with AnM was carried out by the method of Hurley and coworkers (17). The reaction mixture contained AnM-modified (or native) DNA (75  $\mu\text{M}$ ), a singly end-labeled DNA fragment (99 or 200 bp), and the PeM-Fe<sup>III</sup> [or -Co<sup>III</sup>] complex (5  $\mu\text{M}$ ) in 10 mM Tris-HCl buffer (pH 7.5). The DNA cleaving reaction was initiated by addition of dithiothreitol (final concentration, 200  $\mu\text{M}$ ) in the case of the PeM-Fe<sup>III</sup> complex or by irradiation of commercial germicidal lamp in the case of the PeM-Co<sup>III</sup> complex. After incubation at 37°C for 7 min or irradiation at 10°C for 10 min, the reaction was terminated by addition of Na<sub>2</sub>EDTA and subsequent cold ethanol. To alleviate the smearing of electrophoretic bands due to AnM, the DNA-bound AnM was thoroughly removed by extraction with 1-butanol and diethyl ether after heating at 90°C for 5 min.

## RESULTS

**Binding Affinity of the PeM-Co<sup>III</sup> Complex to DNAs.** Table 1 summarizes the DNA binding parameters of the green PeM-Co<sup>III</sup> complex. The binding constant for poly[d(G-C)] was clearly higher than that for poly[d(I-C)] or poly[d(A-T)]. On the other hand, wild-type T4 and its mutant (T4dC) DNAs gave quite similar binding constants for the PeM-Co<sup>III</sup> complex. The former DNA has glucose residues covalently attached to cytosine C-5 in the major groove of DNA (18). The sizes of the binding sites ( $n/2 = 2.3\text{--}2.5$  bp) of the

Table 1. Binding parameters of PeM-Co<sup>III</sup> complex to DNAs

DNA	$K \times 10^{-5}, \text{M}^{-1}$	$n$ (base)
Poly[d(G-C)]	$3.67 \pm 0.37$	$4.86 \pm 0.43$
Poly[d(I-C)]	$1.09 \pm 0.14$	$4.54 \pm 0.58$
Poly[d(A-T)]	$0.67 \pm 0.17$	$4.97 \pm 1.36$
T4 (glucosylated)	$3.81 \pm 0.87$	$2.14 \pm 0.77$
T4dC (nonglucosylated)	$3.95 \pm 0.66$	$1.34 \pm 0.47$

PeM-Co<sup>III</sup> complex with polydeoxynucleotides correspond well to results from DNase I footprinting. The distinction of  $n$  values between natural T4 DNAs and synthetic polymers may result from the difference between the average molecular sizes [the former = 166 kilobases (kb), and the latter = 0.3–2.4 kb] of DNAs used in this experiment.

**DNase I Footprinting for DNA Binding of the PeM-Co<sup>III</sup> Complex.** Fig. 2 shows a typical autoradiogram of DNase I footprinting for the green PeM-Co<sup>III</sup> complex in the 5'-end-labeled (lanes 1–5) and 3'-end-labeled (lanes 6–11) 200-bp fragments. The comparison of DNase I digestion with (lanes 4 and 7) and without (lanes 3 and 8) the Co<sup>III</sup> complex evidently reveals several DNA footprints. In particular, the strong protection region is observed at positions 1022–1026 containing the 5' G-C sequence. Similar 2- or 3-bp footprints involving 5' G-N sites were also detected in the 122-bp fragment. The single-band footprints shown may be rationalized as PeM inhibiting the DNase rather than blocking it. In addition, the footprinting sites of the fully oxidized PeM-Fe<sup>III</sup> complex corresponded well to those of the present Co<sup>III</sup> complex (data not shown). These DNase I digestions at different ratios (0.1–1.0) of drug/base concentrations caused no significant changes in the present footprinting results. Consensus sequence analysis of the footprinting data for the PeM-Co<sup>III</sup> complex showed that the highest inhibition ratio in the 5' N-N-G-C-N-N 3' sequences was observed at the central guanosine (92%), and the adjacent cytosine (67%) is the next blocking site. These inhibition frequencies are distinctively higher than those (*ca.* 30–45%) of other flanking bases (N). A similar tendency also was noted in the 5' N-N-G-T-N-N and 5' N-N-G-A-N-N sequences. Herein, the ratio (%) is represented as the number of bases inhibited by the PeM-Co<sup>III</sup> complex per total number of bases digested by DNase I in native DNA.

**Effect of Guanine Modification by AnM on DNA Cleavage by the PeM-Metal Complex.** Fig. 3 presents a typical autoradiographic result with the 5'-end-labeled 99-bp fragment in

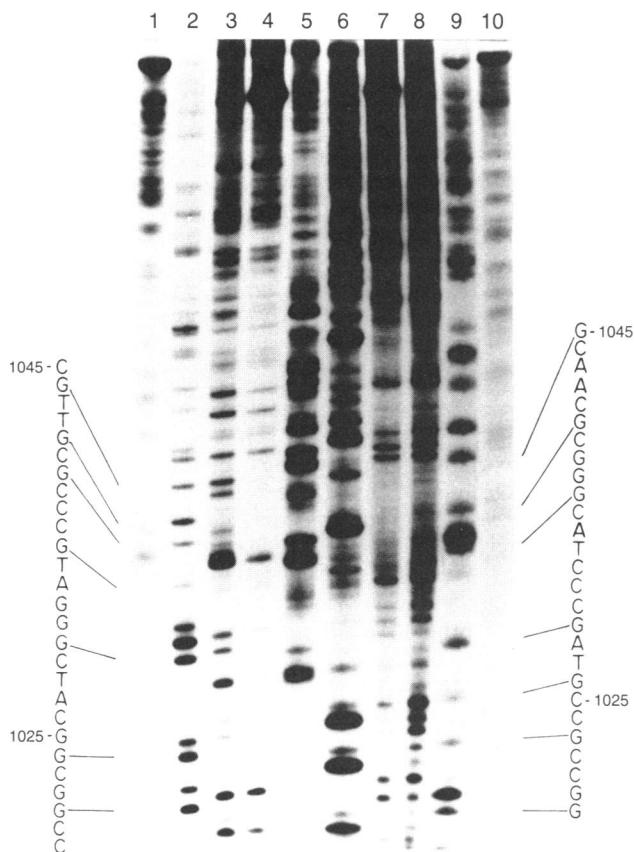


FIG. 2. Autoradiograms of DNase I footprinting with the  $\text{PeM-Co}^{\text{III}}$  complex and of DNA cleavage by  $\text{PeM-Co}^{\text{III}}$ /UV light system. The 5'-end-labeled (lanes 1-5) and 3'-end-labeled (lanes 6-10) 200-bp fragments used were  $^{32}\text{P}$ -labeled at the same end (position 1006). The DNA preincubated with the  $\text{PeM-Co}^{\text{III}}$  complex (lanes 4 and 7) and native DNA (lanes 3 and 8) were digested with DNase I. Lanes 5 and 6 show the cleavage of native DNA by the  $\text{PeM-Co}^{\text{III}}$ /UV light system; lanes 1 and 10 and 2 and 9 show the Maxam-Gilbert C+T and G reactions, respectively.

detecting the influence of selective guanine modification on DNA breakage by  $\text{PeM-Fe}^{\text{III}}$ /dithiothreitol or  $\text{PeM-Co}^{\text{III}}$ /UV light systems. AnM was chosen as the guanine modifier based on the following favorable properties: (i) selective covalent-binding to the guanine 2-amino group, (ii) small binding site of  $\approx 3$  bp, and (iii) no significant perturbation by AnM binding upon B-DNA structure. AnM strongly prevented the DNA cleavage by the  $\text{PeM-Fe}^{\text{III}}$  [or- $\text{Co}^{\text{III}}$ ] complex system with increase of incubation time (Fig. 3, lanes 6-8 and 10-12). From spectrophotometric measurements, the AnM binding ratio (AnM per DNA nucleotide) was estimated to be 0.04, 0.06, and 0.13 with 15-min-, 4.5-hr-, and 16-hr-treated samples, respectively. On the basis of consensus sequence analysis of the present AnM-induced blocking, the inhibition frequency at 5' N-G-C-N 3' cleavage sites was evaluated to be 66% and 69% for  $\text{PeM-Co}^{\text{III}}$  and  $\text{PeM-Fe}^{\text{III}}$  complexes, respectively. Taking into account that (i) the G+C-content of calf thymus DNA is 39% and (ii) the ratio of AnM binding to DNA nucleotide is 0.13 even at an incubation time of 16 hr, the present inhibition percentage at 5' N-G-C-N sequences suggests nearly complete blocking of the DNA breakage by metallobleomycin. Similar AnM blocking at 5' G-C cleavage sites was also detected in the 200-bp fragment. The AnM inhibition of the BIM cleavage can be interpreted mainly in terms of indirect structural effects because AnM blocking sites correspond well to the footprinting sites of metallobleomycin. However, the strong inhibition by AnM of DNA breakage by the  $\text{PeM-Co}^{\text{III}}$ /UV

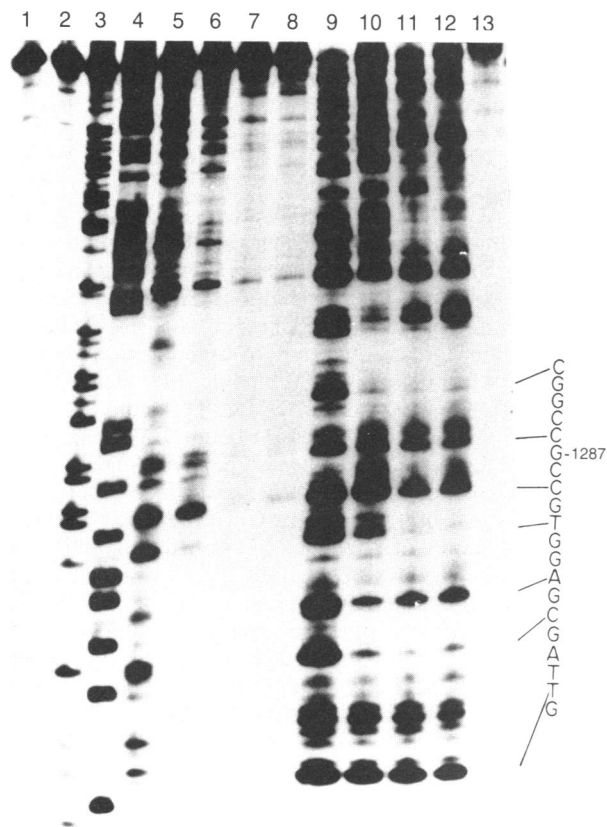


FIG. 3. Autoradiogram of strand scission of AnM-modified DNA by metallobleomycin. The 5'-end-labeled 99-bp fragment was incubated with AnM at 37°C for 15 min (lanes 6 and 10), 4.5 hr (lanes 7 and 11), and 16 hr (lanes 2, 8, 12, and 13). Native DNA (lanes 5 and 9) and the AnM-modified DNA (lanes 8 and 12) were cleaved by the  $\text{PeM-Co}^{\text{III}}$ /UV light (lanes 5-8) or by  $\text{PeM-Fe}^{\text{III}}$ /dithiothreitol systems (lanes 9-12). Lanes 2 and 13 show controls for the light irradiation and Fe/dithiothreitol systems, lane 1 shows intact DNA, and lanes 3 and 4 show the Maxam-Gilbert C+T and G reactions, respectively.

light system rather than by the  $\text{PeM-Fe}^{\text{III}}$ /reductant system suggests that the AnM molecule absorbs UV energy and partly disturbs the photo-induced DNA cleavage reaction by  $\text{PeM-Co}^{\text{III}}$ . Covalent binding of one AnM molecule to a 5' G-C sequence may additionally inhibit the DNA cleavage at its complementary site on the opposite strand because the phenolic hydroxyl group (9-OH) of guanine-bound AnM forms a hydrogen bond to the carbonyl group (O-2) of cytosine base-paired to the guanine (19). The blocking effect (13% and 25% inhibitions for the  $\text{PeM-Co}^{\text{III}}$  and  $\text{PeM-Fe}^{\text{III}}$  complexes, respectively) of AnM at 5' N-G-A-N sequences was remarkably smaller than those at 5' N-G-C-N (66% and 69%) and 5' N-G-T-N (50% and 67%) sequences. Indeed, the protecting effect of AnM on some trinucleotide sequences decreases in the order of 5' W-G-C > 5' G-C-W, G-G-C, G-C-C > 5' G-C-G > 5' C-G-C (W = A or T).

## DISCUSSION

**DNA Minor Groove Binding of Metallobleomycin.** The major or minor groove binding specificity of BIM is an important aspect of its interaction with DNA. Wild-type T4 DNA is a steric probe for the major groove because the uncharged glucose residues of this DNA do not significantly perturb the double-helix structure (18). The estimated binding constant of the  $\text{PeM-Co}^{\text{III}}$  complex to the glucosylated DNA ( $3.81 \times 10^5 \text{ M}^{-1}$ ) is remarkably close to that to the nonglucosylated T4 mutant DNA ( $3.95 \times 10^5 \text{ M}^{-1}$ ); hence,

it is clear that the glucose residues in the major groove have a negligible effect on metallobleomycin binding to DNA. AnM is an antitumor antibiotic that reacts covalently with the 2-amino group of guanine in the minor groove. It is known that AnM is topologically matched to the minor groove of DNA and covers  $\approx 3$  bp to produce an uncharged adduct with very little perturbation of the double-helix structure (17). In AnM modification of DNA, the present result exhibits a drastic change of the sequence-specific cleavage mode by metallobleomycin, even at a low binding ratio of AnM to DNA nucleotide. The strong inhibition seems to be attributed to AnM binding at a BIM cleavage site of the minor groove but not to significant alteration of B-DNA conformation by AnM binding. Indeed, the  $^1\text{H}$  NMR result with the AnM-d(A-T-G-C-A-T)<sub>2</sub> complex (20) and molecular simulation of the AnM-bound DNA complex (21) demonstrate that covalent attachment of AnM gives very minimal distortion in the DNA helix and does not disrupt any base pairs of DNA. By contrast, we previously found that the modification of guanine N-7 with aflatoxin B<sub>1</sub> or dimethyl sulfate shows no obvious alterations for the DNA cleavage mode by metallobleomycin (22). Certainly, the guanine N-7 atom is situated in the major groove of DNA, whereas a clear variation in BIM cleavage after *cis*-diamminedichloroplatinum(II) binding (9) could be attributed to a large alteration in the DNA structure induced by its major binding mode, the d(GpG) intrastrand crosslink (23). On the other hand, the DNA binding of distamycin A—namely, an A-T-specific minor groove binder—strongly masked the cleavage of G-T and G-A (5' → 3') sequences by the BIM-Fe<sup>III</sup> complex system (8). These results strongly suggest that metallobleomycin prefers the binding in the minor groove of B-DNA helix.

**Possible DNA Interaction Mode of Metallobleomycin.** Fig. 4 summarizes the results of DNase I footprinting for the PeM-Co<sup>III</sup> complex, together with those of DNA cleavage inhibi-

tion by AnM modification. The DNase I footprinting results reveal that (i) the DNA binding site of metallobleomycin occupies  $\approx 2$  or 3 bp involving a 5' G-N sequence, and (ii) the PeM-Co<sup>III</sup> complex highly blocks the guanosine base adjacent to the 5' side of the cleaved base (N). The selective guanine N-2 binding by AnM covering approximately 3 bp inhibits the DNA breakage at 5' guanosine-pyrimidine sites by metallobleomycin. The Co<sup>III</sup> and Fe<sup>III</sup> complex systems of PeM preferentially degrade the DNA fragments in the order of 5' G-C > 5' G-T > 5' G-A. Therefore, metallobleomycin appears to recognize at least the guanosine base at 5' guanosine-pyrimidine sequences. In addition, the binding affinity of the PeM-Co<sup>III</sup> complex to poly[d(G-C)] is higher than that to poly[d(I-C)], which lacks the 2-amino group in the purine ring. The fact suggests an important contribution of the guanine 2-amino group to DNA binding of metallobleomycin. Stereo-models show that the conformational situation composed of the 2-amino group of guanine, the 2-carbonyl oxygen of the pyrimidine base in the 5' guanosine-pyrimidine sequence, and the 2-carbonyl oxygen of the cytosine base complementary to guanosine on the opposite strand exists in the minor groove of 5' G-C and 5' G-T sequences. This convex environment appears to be profitable for the primary binding of metallobleomycin and is entirely different from the situation in 5' C-G and 5' T-G sequences. The guanine 2-amino group projects significantly above the floor of the minor groove of B-DNA. To achieve specific guanosine recognition in the minor groove, therefore, steric space and a hydrogen-bond acceptor for the guanine 2-amino group are probably required in the drug molecule.

BIM consists of three components—namely (i) a metal-chelating site of  $\beta$ -aminoalanine residue-pyrimidine base- $\beta$ -hydroxyhistidine residue portion, (ii) a DNA interaction site of bithiazole terminal-amine moiety, and (iii) a gulose-mannose sugar region (2). The  $\approx 2$  or 3 bp binding size of the PeM-Co<sup>III</sup> complex suggests a compactly folded configura-

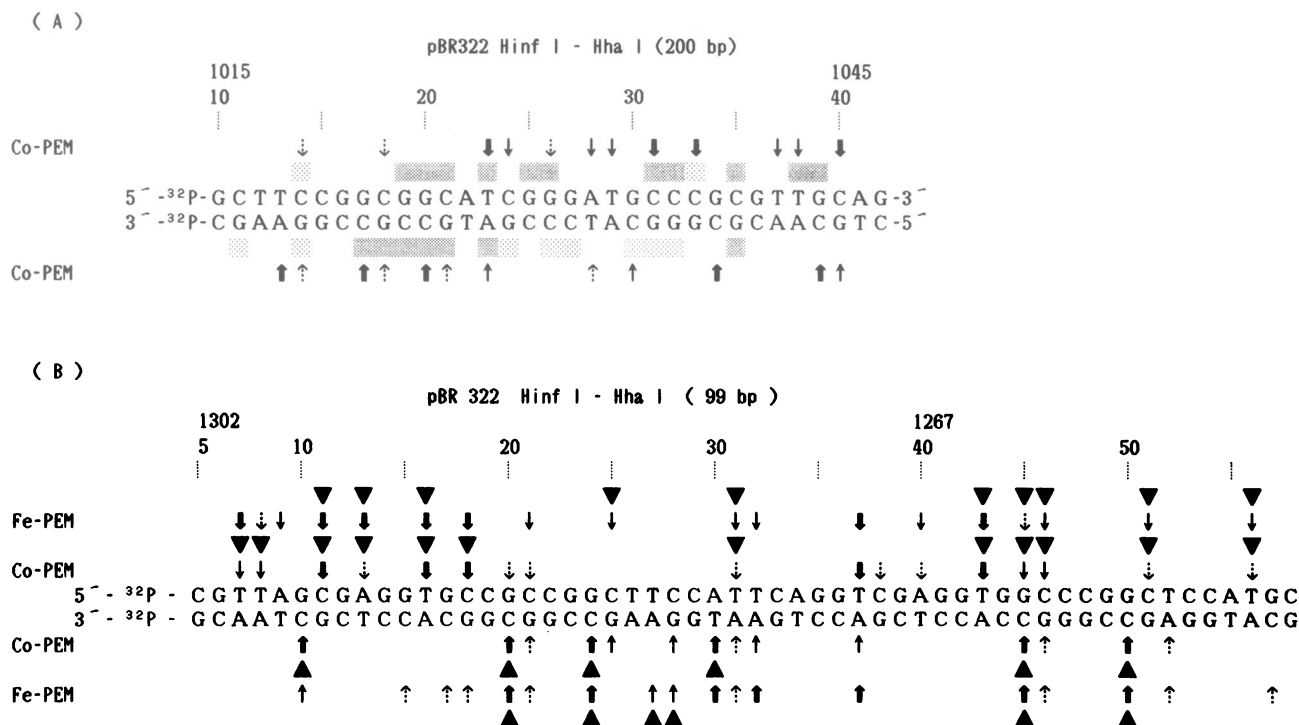


FIG. 4. Footprint with the PeM-Co<sup>III</sup> complex and DNA cleavage by the PeM-Co<sup>III</sup>/UV light system (A) and illustration of the change in DNA cleavage by the PeM-metal complex in AnM-modified DNA (B). The shaded areas display the footprint with the PeM-Co<sup>III</sup> complex, and the density of the shaded areas represents the extent of protection against DNase I digestion. The arrows indicate the cleavage site of native DNA by the PeM-Co<sup>III</sup>/UV light or PeM-Fe<sup>III</sup>/dithiothreitol systems, and the thickness reflects the relative intensity of the band on the autoradiogram. Solid triangles show the sites of AnM modification causing inhibition of DNA breakage by the PeM-metal complex.

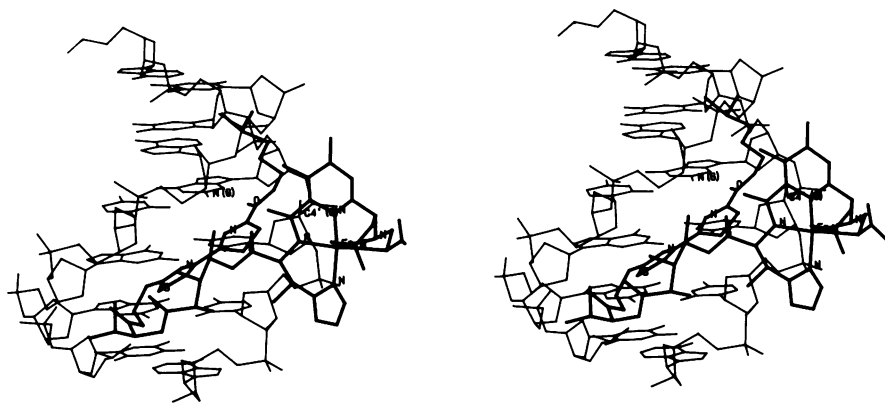


FIG. 5. Possible binding mode of deglycobleomycin  $A_2$  with self-complementary  $d(A-T-G-C-C-A)_2$ . Thin and thick lines represent the deoxyribonucleotide and drug, respectively. The distances are as follows: G(N2)-thiazole A(N), 3.86 Å; G(N2)-thiazole B(N), 5.18 Å; G(N2)-carboxamide(O), 2.60 Å; and C(C4')-Fe, 7.32 Å.

tion (U-form) in metallobleomycin. Indeed, a  $^1\text{H}$  nuclear magnetic relaxation study of the BIM-Mn<sup>II</sup> complex indicates that the bithiazole and terminus protons (5.4 and 7.4 Å) are considerably close to the metal center (24). We previously indicated that the BIM-Fe<sup>III</sup> complex involves as many as five nitrogenous ligands of the  $\beta$ -aminoalanine-pyrimidine- $\beta$ -hydroxyhistidine portion, with a geometry similar to heme complexes (25). In addition, it is proposed that the BIM-Fe<sup>III</sup>-O<sub>2</sub><sup>2-</sup> complex species primarily attacks the C4' of cytosine ribose at the 5' G-C sequence (26). On the basis of these observations, we examined the stereochemical fit of metallobleomycin in duplex DNA by inspection of a computer-constructed molecular model using a FRODO program (27) on an Evans and Sutherlands multipicture system. Fig. 5 presents a preliminary stereoview of the interaction mode of the deglycobleomycin-metal complex to self-complementary  $d(A-T-G-C-C-A)_2$ . Herein, 5' T-G-C-C was chosen as the best sequence from the present consensus sequence analysis of the DNA breakage by metallobleomycin, and deglycobleomycin is known to have the nucleotide-sequence cleavage pattern almost identical to BIM (7). The configurations of the nucleic acid fragment and metal coordination core were taken from the standard B-DNA proposed by Arnott *et al.* (28) and x-ray crystallographic data of the Cu<sup>II</sup> complex of P-3A, a putative biosynthetic precursor of BIM (29). In our opinion, the guanine recognition by compactly folded metallobleomycin in the minor groove of the 5' G-C sequence can be achieved by a snug fit of the concave face of the bithiazole-carboxamide moiety and by hydrogen bonds of the guanine 2-amino group with two ring nitrogens of the bithiazole group and/or carboxamide oxygen of the terminal amine group. Indeed, our recent study on phototransformed BIMs suggests that nitrogens rather than sulfurs of the bithiazole group may participate in the sequence specificity of BIM (30). In the case of pyridine-2-carboxamide, Wade and Dervan (31) have pointed out the similar key feature of a nitrogen position for recognition of the G-C base pair. The present results reveal that metallobleomycin interacts with DNA as a groove binder rather than as an intercalator. It is difficult for metallobleomycin to intercalate its bithiazole moiety between DNA base pairs because of the structural bulkiness. This situation is clearly different from the proposed intercalative DNA binding of the simple bithiazole model compound alone (32). To understand well the flexibility of interaction between metallobleomycin and the DNA fragment, further detailed study of the model-building of metallobleomycin-bound DNA is needed.

In conclusion, metallobleomycin binds in the minor groove of B-DNA helix and covers  $\approx 2$  or 3 bp on DNA. The 2-amino group of the guanosine base adjacent to the 5' side of the cleaved pyrimidine base is one key element of specific

5' G-C or G-T recognition by metallobleomycin, and the bithiazole group probably plays an important role as an anchor on B-DNA.

We thank L. H. Hurley (University of Texas) for a generous gift of anM and S. Imajo and M. Ishiguro (Suntory Biomedical Institute) for computer graphic modeling. This study was financially supported in part by a Grant-in-Aid for Special Project Research and Scientific Research B from the Ministry of Education, Science, and Culture, Japan.

1. Umezawa, H. (1977) *Lloydia* **40**, 67-81.
2. Sugiura, Y., Takita, T. & Umezawa, H. (1985) in *Metal Ions in Biological Systems*, ed. Sigel, H. (Dekker, New York), Vol. 19, pp. 81-108.
3. D'Andere, A. D. & Haseltine, W. A. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3608-3612.
4. Chang, C.-H. & Meares, C. F. (1984) *Biochemistry* **23**, 2268-2274.
5. Suzuki, T., Kuwahara, J., Goto, M. & Sugiura, Y. (1985) *Biochim. Biophys. Acta* **824**, 330-335.
6. Kross, J., Henner, W. D., Haseltine, W. A., Rodriguez, L., Levin, M. D. & Hecht, S. M. (1982) *Biochemistry* **21**, 3711-3721.
7. Sugiura, Y., Suzuki, T., Otsuka, M., Kobayashi, S., Ohno, M., Takita, T. & Umezawa, H. (1983) *J. Biol. Chem.* **258**, 1328-1336.
8. Sugiura, Y. & Suzuki, T. (1982) *J. Biol. Chem.* **257**, 10544-10546.
9. Mascharak, P. K., Sugiura, Y., Kuwahara, J., Suzuki, T. & Lippard, S. J. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6795-6798.
10. Dervan, P. B. (1986) *Science* **232**, 464-471.
11. Ishii, S., Merlino, G. T. & Pastan, I. (1985) *Science* **230**, 1378-1381.
12. Chang, C.-H., Dallas, J. L. & Meares, C. F. (1983) *Biochem. Biophys. Res. Commun.* **110**, 959-966.
13. Chien, H., Grollman, A. P. & Horwitz, S. B. (1977) *Biochemistry* **16**, 3641-3646.
14. McGhee, J. D. & von Hippel, P. H. (1974) *J. Mol. Biol.* **86**, 469-489.
15. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 479-487.
16. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499-560.
17. Petrussek, R. L., Anderson, G. L., Garner, T. F., Fannin, Q. L., Kaplan, D. J. & Hurley, L. H. (1981) *Biochemistry* **20**, 1111-1119.
18. Revel, H. R. & Luria, S. E. (1970) *Annu. Rev. Genet.* **4**, 177-210.
19. Rao, S. N., Singh, U. C. & Kollman, P. A. (1986) *J. Med. Chem.* **29**, 2484-2492.
20. Graves, D. E., Stone, M. P. & Krugh, T. R. (1985) *Biochemistry* **24**, 7573-7581.
21. Mirau, P. A. & Kearns, D. R. (1984) *Biochemistry* **23**, 5439-5446.
22. Suzuki, T., Kuwahara, J. & Sugiura, Y. (1983) *Biochem. Biophys. Res. Commun.* **117**, 916-922.
23. Sherman, S. E., Gibson, D., Wang, A. H.-J. & Lippard, S. J. (1985) *Science* **230**, 412-417.
24. Sheridan, R. P. & Gupta, R. K. (1981) *J. Biol. Chem.* **256**, 1242-1247.
25. Sugiura, Y. (1980) *J. Am. Chem. Soc.* **102**, 5208-5215.
26. Hecht, S. M. (1986) *Acc. Chem. Res.* **19**, 383-391.
27. Jones, T. A. (1978) *J. Appl. Crystallogr.* **11**, 268-272.
28. Arnott, S., Campbell-Smith, P. & Chandrasekaran, R. (1976) in *CRC Handbook of Biochemistry*, ed. Fasman, C. D. (CRC, Cleveland), Vol. 2, pp. 411-422.
29. Iitaka, Y., Nakamura, H., Nakatani, T., Muraoka, Y., Fujii, A., Takita, T. & Umezawa, H. (1978) *J. Antibiot.* **31**, 1070-1072.
30. Morii, T., Saito, I., Matsuura, T., Kuwahara, J. & Sugiura, Y. (1987) *J. Am. Chem. Soc.* **109**, 938-939.
31. Wade, W. S. & Dervan, P. B. (1987) *J. Am. Chem. Soc.* **109**, 1574-1575.
32. Kuroda, R., Neidle, S., Riordan, J. N. & Sakai, T. T. (1982) *Nucleic Acids Res.* **10**, 4753-4763.