Enantiospecific Recognition of DNA by Bleomycin

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Abstract: The specific double-stranded DNA recognition mechanism of bleomycin was investigated using L-d(CGCGCG), the enantiomer of natural D-d(CGCGCG). The L-enantiomer was clearly shown not to be cleaved at all by bleomycin under the same conditions that the corresponding D-enantiomer was cleaved, but the conventional DNA-binding domain of bleomycin was able to bind to the L-enantiomer to essentially the same extent as the natural one, as shown by ^{1}H NMR titration experiments. However, other protons in the metal-binding domain and linker moiety showed different behaviors depending on the chirality of DNA. The DNA-binding domain thus binds to DNA with a nonenantiospecific manner, and the primary determinant for specific DNA recognition of bleomycin is the linker–metal-binding region. After binding of the DNA binding domain to a right-handed B-form DNA, the remaining moiety should recognize specifically the shape of the DNA ("induced-fit"). Thus, L-oligonucleotides are a powerful tool for discriminating specific interactions from nonspecific association for DNA–drug interaction studies.

Introduction

DNA is thought to be a critical target for bleomycins (BLMs), which are a family of glycopeptide antitumor antibiotics discovered by Umezawa *et al.* in 1966.[1] This drug specifically cleaves double-stranded DNA at 5′-GT-3′ and 5′-GC-3′ sites.[2] BLM consists of the metal-binding domain, the DNA-binding domain,[3] and the linker moiety joining both functional domains (Figure 1). The metal-binding domain of BLM, which chelates ferrous ions and activates oxygen molecules, cleaves DNA strands[4] by abstraction of the H4′ atom of the deoxyribose moiety in pyrimidine residues.[5] It has been suggested that the bithiazole moiety is responsible for the binding with target DNA and sequence selectivity (GC and GT) of BLM[6] with an intercalation model[7] or a groove binding model.[8] This functionally independent feature of the BLM molecule led to the development of BLM-mimetic DNA-cleaving agents.[9] Recently, participation of the metal-binding domain of BLM in DNA interactions was suggested.[10] However, X-ray crystallography of BLM or its DNA complex has not been reported. Neither has the intermolecular NOE of DNA–BLM complexes been observed in NMR studies.[11] Thus, the mechanism of DNA recognition by BLMs has yet to be determined in detail.

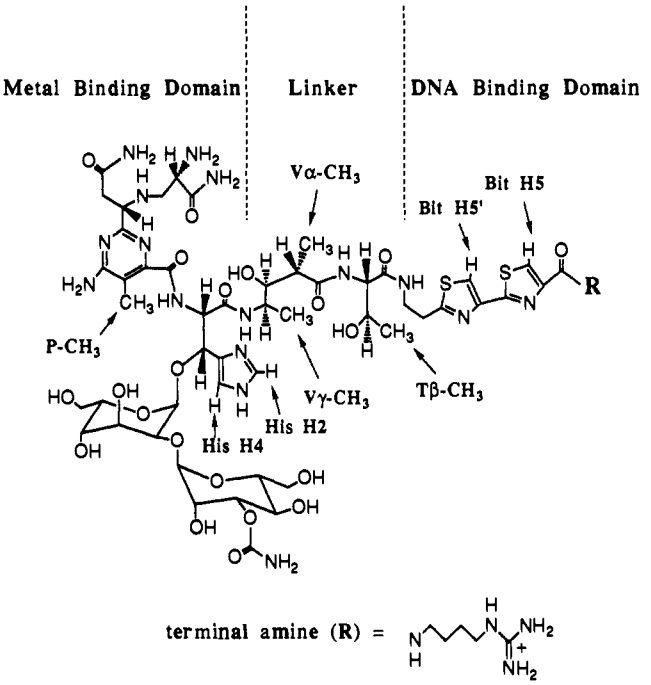

Figure 1. Structure of bleomycin B_2.

This paper reports the interactions of BLM with L-d(CGCGCG), a mirror-image nucleotide analog of natural D-d(CGCGCG). The conformations and dynamic properties of L-d(CGCGCG) were previously shown to be the same as those of the natural one except for chirality.[12] Enantiomers can discriminate specific binding interactions from nonspecific as-

(1) Umezawa, H.; Maeda, K.; Takeuchi, T.; Okami, Y. *J. Antibiot., Ser. A* 1966, *19*, 201–209.

(2) (a) Takeshita, M.; Grollman, A. P.; Ohtsubo, E.; Ohtsubo, H. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5983–5987. (b) D'Andrea, A. D.; Haseltine, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 3608–3612.

(3) Chien, M.; Grollman, A. P.; Horwitz, S. B. *Biochemistry* **1977**, *16*, 3641–3647.

(4) (a) Sugiura, Y. *J. Am. Chem. Soc.* **1980**, *102*, 5208–5215. (b) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107–1136. (c) Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1981**, *256*, 11636–11644.

(5) (a) Giloni, L.; Takeshita, M.; Johnson, F.; Iden, C.; Grollmam, A. P. *J. Biol. Chem.* **1981**, *256*, 8608–8615. (b) Wu, J. C.; Kozarich, J. W.; Stubbe, J. *J. Biol. Chem.* **1983**, *258*, 4694–4697. (c) Sugiyama, H.; Xu, C.; Murugesan, N.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *Biochemistry* **1988**, *27*, 58–67. (d) Kozarich, J. W.; Worth, L., Jr.; Frank, B. L.; Christner, D. F.; Vanderwall, D. E.; Stubbe, J. *Science* **1989**, *245*, 1396–1399.

(6) Hamamich, N.; Natrajan, A.; Hecht, S. M. *J. Am. Chem. Soc.* **1992**, *114*, 6278–6291.

(7) (a) Povirk, L. F.; Hogan, M.; Dattagupta, N. *Biochemistry* **1979**, *18*, 96–101. (b) Kuroda, R.; Neidle, S.; Riordan, J. M.; Sakai, T. T. *Nucleic Acids Res.* **1982**, *10*, 4753–4763. (c) Hénichart, J.-P.; Bernier, J.-L.; Helbecque, N.; Houssin, R. *Nucleic Acids Res.* **1985**, *13*, 6703–6716.

(8) (a) Kuwahara, J.; Sugiura, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 2459–2463. (b) Dickerson, R. E. In *Mechanism of DNA Damage and Repair: Implications for Carcinogenesis and Risk Assessment in Basic Life Sciences*; Smi, M. J., Grossman, L., Eds.; Plenum: New York, 1986; Vol. 38, pp 245–255.

(9) (a) Dervan, P. B. *Science* **1986**, *232*, 464–471. (b) Schultz, P. G.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 6834–6837. (c) Hertzberg, R. P.; Dervan, P. B. *J. Am. Chem. Soc.* **1982**, *104*, 313–315.

(10) (a) Levy, M. J.; Hecht, S. M. *Biochemistry* **1988**, *27*, 2647–2650. (b) Carter, B. J.; Murty, V. S.; Reddy, K. S.; Wang, S.-N.; Hecht, S. M. *J. Biol. Chem.* **1990**, *265*, 4193–4196. (c) Sugiyama, H.; Kilkuskie, R. E.; Chang, L.-H.; Ma, L.-T.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1986**, *108*, 3852–3854.

(11) (a) Booth, T. E.; Sakai, T. T.; Glickson, J. D. *Biochemistry* **1983**, *22*, 4211–4217. (b) Gamcsik, M. P.; Glickson, J. D.; Zon, G. *J. Biomol. Struct. Dyn.* **1990**, *7*, 1117–1133. (c) Hiroaki, H.; Nakayama, T.; Ikehara, M.; Uesugi, S. *Chem. Pharm. Bull.* **1991**, *39*, 2780–2786.

(12) Urata, H.; Shinohara, K.; Ogura, E.; Ueda, Y.; Akagi, M. *J. Am. Chem. Soc.* **1991**, *113*, 8174–8175.

(13) Mickus, D. E.; Levitt, D. G.; Rychnovsky, S. D. *J. Am. Chem. Soc.* **1992**, *114*, 359–360.

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sociation;[13] and thus this molecule was used as a probe in DNA–LM interactions. Of particular interest is how BLM interacts with the L-hexanucleotide, since the conventional DNA-binding domain of BLM has no asymmetric center.

Experimental Procedures

Materials. Bleomycin B_2 was kindly provided by Nippon Kayaku, Co., Ltd. The synthesis of L-d(CGCGCG) was reported previously.[14]

Methods. (a) Strand Cleavage Reaction of D- and L-d(CGCGCG) with Fe(II)–BLM. The reaction mixture (total volume 100 μL) contained D- or L-d(CGCGCG) (200 μM duplex concentration), 200 μM BLM, 200 μM $Fe^{II}(NH_4)_2(SO_4)_2$, and 1 mM hydrogen peroxide[15b] in 50 mM sodium phosphate at pH 7.0. The reaction was performed at 0 °C for 30 min. After the addition of EDTA (final 20 mM), aliquots were analyzed by reversed-phase HPLC. Elution was performed on a μBondasphere C18 100-Å column (3.9 × 150 mm) with a Shimadzu LC-6A system and 260 nm detection using a linear gradient of acetonitrile (0–12.5%) over 20 min in 50 mM potassium phosphate, pH 4.0.

(b) ^{1}H NMR Titration Experiments. There were two sets of titration experiments.[16] For one set of high duplex/Zn(II)–BLM ratios, an equimolar aqueous solution of $Zn^{II}SO_4$ and BLM containing EDTA and sodium phosphate was lyophilized. The residue was dissolved in D_2O and the pD was adjusted with NaOD to 7.2; then the sample was lyophilized three times from D_2O and, finally, dissolved in 99.95% D_2O (final concentrations were 0.5 mM Zn(II)–BLM, 20 mM phosphate, and 50 μM EDTA, pD 7.2). This sample was titrated by adding aliquots of a 50 mM (duplex concentration) unbuffered stock solution (pD 7.2 adjusted by 30 mM phosphoric acid in D_2O, followed by lyophilizing and dissolving the sample in D_2O) of D- or L-d(CGCGCG) whose C8 protons of the guanine residues had been deuterated. For the second set of low duplex/Zn(II)–BLM ratios, D- or L-d(CGCGCG) containing the above salts was lyophilized three times from D_2O followed by dissolution in 99.95% D_2O (final concentrations were 0.3 mM DNA duplex, 20 mM sodium phosphate, and 50 μM EDTA, pD 7.2). Titration of this sample was performed by adding aliquots of a 100 mM stock solution (pD 7.2) of Zn(II)–BLM. Throughout all experiments, the hexanucleotides were maintained at more than 0.3 mM duplex concentration.[11b] ^{1}H NMR spectra were recorded with a Varian XL-300 spectrophotometer at 5 °C. Chemical shifts were referenced to internal 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid.

Results and Discussion

The strand cleavage reaction of D-d(CGCGCG) with BLM is well-established.[15] BLM-mediated DNA strand scission affords 3′-phosphoglycolate nucleotides and modified apyrimidinic nucleotides *via* 4′-hydrogen abstraction followed by peroxygenation or hydroxylation, respectively.[4b,5] Under the conditions that natural D-d(CGCGCG) was cleaved by BLM (Figure 2a),[17] the corresponding L-enantiomer was not cleaved at all (Figure 2b), which was not entirely unexpected. These results and an achiral feature of the DNA-binding domain allow us to consider two hypotheses: (i) BLM cannot bind to L-DNA or can bind to L-DNA with considerably reduced strength; (ii) BLM binds to L-DNA but cannot cleave it. The former indicates the presence of other important DNA-binding residues which interact with DNA in cooperation with the conventional DNA-binding domain; the latter indicates that regions other than the DNA-binding domain are essential to the specific DNA recognition of BLM for strand cleavage.

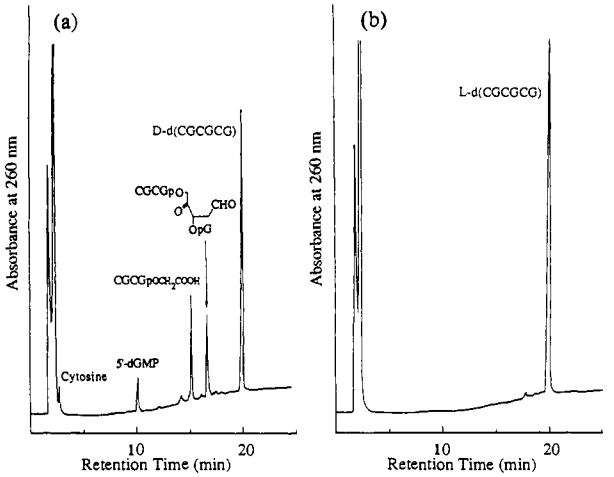

Figure 2. HPLC analysis of the cleavage reactions of (a) D-d(CGCGCG) and (b), its L-enantiomer with Fe(II)–BLM B_2. Elution was performed on a μBondasphere C18 100-Å column (3.9 × 150 mm) with a Shimadzu LC-6A system and 260-nm detection using a linear gradient of acetonitrile (0–12.5%) over 20 min in 50 mM potassium phosphate, pH 4.0.

In order to compare the binding capacity of metallo-BLM to natural DNA and mirror-image DNA, ^{1}H NMR titration experiments were conducted on Zn(II)–BLM, which is a diamagnetic metal–BLM complex,[11b,19] used for most NMR studies of metallo-BLM,[20] with the D- or L-hexamer as the titrant. With the addition of Zn(II) ions, protons derived from the metal-binding domain showed considerable chemical shift changes, indicating metal chelation in this region. However, protons of the DNA-binding domain were hardly affected. Figure 3 shows the titration spectra in the aromatic region. The bithiazole protons (Bit H5 and Bit H5′) of Zn(II)–BLM underwent a larger chemical shift change than the other protons with considerable line broadening upon titrating with either D- or L-d(CGCGCG), although the imidazole protons (His 2 and His 4) were not affected. The extent of the upfield shift of the bithiazole protons was essentially independent of the chirality of the hexanucleotides (Figure 4a) and was comparable with the results of Gamcsik *et al.*[11b] This indicates that the DNA-binding domain binds to both D- and L-DNA in essentially the same manner. It is quite likely that there is no significant cooperative assistance of the metal-binding–linker regions for the binding of the DNA-binding domain to DNA, and that this domain causes a BLM molecule to have affinity for both DNA enantiomers in a nonenantiospecific manner due to the achiral interactions of the bithiazole moiety with the G-C sites and the electrostatic interactions of the positively charged terminal amine moiety with negatively charged DNA phosphate groups.[21] The function of the DNA-binding domain is thus independent of the chirality of DNA, *i.e.*, the shape of DNA, in spite of the ability of BLM to degrade D-DNA only, and thus the binding of this domain to DNA does not necessarily imply DNA recognition by BLM. Since the extent of line broadening for the bithiazole proton signals is dependent on the chirality of the hexanucleotides (Figure 3), there will be some differences in the process or dynamics of the binding of BLM to D-and L-DNA, probably due to chiral interactions between BLM and DNA.[22] The primary determinant for stereospecific DNA recognition (not a base sequence selectivity) is thus a region or regions other than the DNA-binding domain.

(14) Urata, H.; Ogura, E.; Shinohara, K.; Ueda, Y.; Akagi, M. *Nucleic Acids Res.* **1992**, *20*, 3325–3332.

(15) (a) Rabow, L.; Stubbe, J.; Kozarich, J. W.; Gerlt, J. A. *J. Am. Chem. Soc.* **1986**, *108*, 7130–7131. (b) Uesugi, S.; Shida, T.; Ikehara, M.; Kobayashi, Y.; Kyogoku, Y. *Nucleic Acids Res.* **1984**, *12*, 1581–1592.

(16) This procedure is not applicable for the determination of binding constants, stoichiometries, *etc.* but is useful for the comparison of the bindings of BLM to both enantiomers under identical conditions.

(17) The peaks corresponding to cytosine and 5′-dGMP were identified by coinjection with authentic samples. d(CGCGpOCH$_2$COOH) was consistent with a sample obtained by independent synthesis.[18] The remaining major peak was assigned to the apyrimidinic nucleotide at the C5 residue since it was also produced effectively under anaerobic conditions and was degraded by alkali treatment to afford 5′-dGMP and unidentified materials (not shown). The major reaction site is thus the C5 residue.

(18) Urata, H.; Akagi, M. *Tetrahedron Lett.*, in press.

(19) (a) Oppenheimer, N. J.; Rodriguez, L. O.; Hecht, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 5616–5620. (b) Glickson, J. D.; Pillai, R. P.; Sakai, T. T. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 2967–2971. (c) Akkerman, M. A. J.; Haasnoot, C. A. G.; Hilbers, C. W. *Eur. J. Biochem.* **1988**, *173*, 211–225.

(20) These and present studies are based on the assumption that Zn(II)–BLM forms the same complex with DNA as activated Fe(II)–BLM. Other metal complexes such as CO–Fe(II)–BLM may be more suitable for the experiments. However, the same assumption has not yet been proven.

(21) Sakai, T. T.; Riordan, J. M.; Glickson, J. D. *Biochim. Biophys. Acta* **1983**, *758*, 176–180.

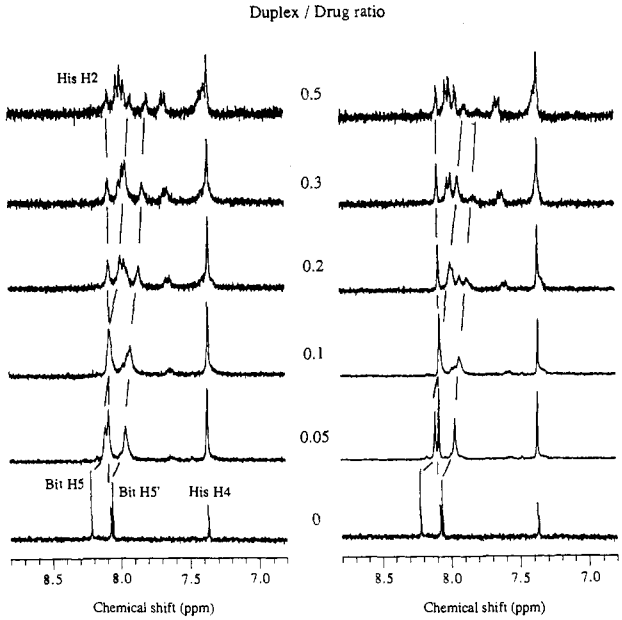

Figure 3. ^{1}H NMR titration spectra of the aromatic region of Zn(II)–BLM B_2 upon titration by D- (left) and L-d(CGCGCG) (right) in 20 mM phosphate, 50 μM EDTA, pD 7.2 at 5 °C. The three singlet peaks around 8.1 ppm are the H8 protons of the guanine residues, which are not deuterated in the experiments of low duplex/drug ratios. The chemical shifts are referenced to internal 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid.

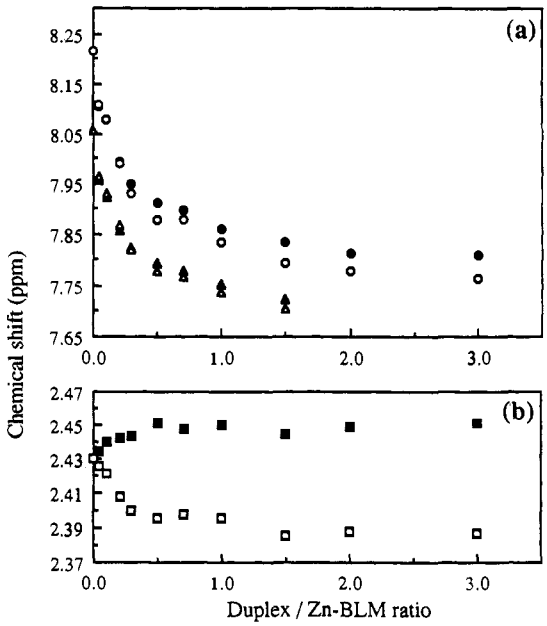

Figure 4. Dependence of the chemical shifts of (a) the bithiazole C5 proton (Bit H5, circles) and the bithiazole C5′ proton (Bit H5′, triangles) and (b) the pyrimidine ring methyl protons (P-CH_3, squares) on the ratio of the hexanucleotide duplex to Zn(II)–bleomycin B_2 (Duplex/Zn(II)–BLM). The conditions are the same as for Figure 3. The closed and open symbols denote titration with D- and L-d(CGCGCG), respectively.

On the other hand, the pyrimidine ring methyl proton resonance (P-CH_3) in the metal-binding domain slightly shifts in the opposite direction depending on the chirality of the complexed hexanucleotide (Figure 4b) and in spite of the basically similar association of the DNA-binding domain with both DNA enantiomers. This result indicates that the pyrimidine ring methyl group is placed in quite different environments in diastereomeric complexes formed with D- and L-DNA and/or that conformational alteration of the metal-binding domain induced by the binding with DNA is dependent on the chirality of DNA. Consequently, the metal center may not be directed toward the 4′-position of the deoxyribose moiety when the DNA-binding domain binds to

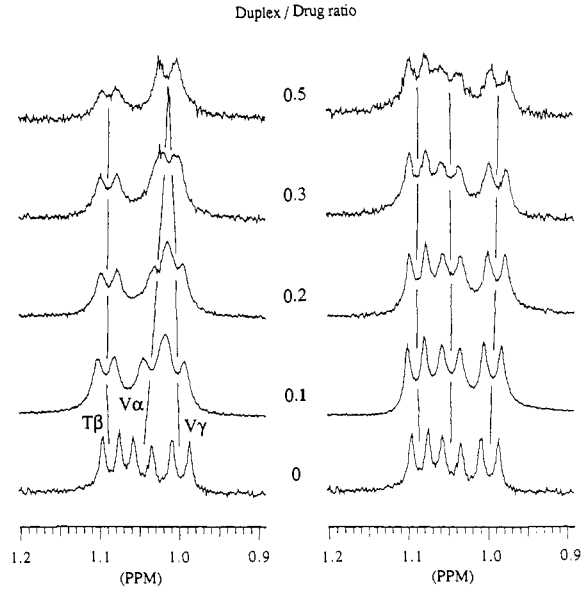

Figure 5. ^{1}H NMR spectra of the aliphatic methyl region of Zn(II)–BLM B_2 upon titration by D- (left) and L-d(CGCGCG) (right) in 20 mM phosphate, 50 μM EDTA, pD 7.2 at 5 °C. These methyl proton signals were assigned from downfield to T_β-CH_3, V_α-CH_3, and V_γ-CH_3, respectively, by 2D-COSY at the same temperature.

L-DNA. This is consistent with the results of chemical reactivity experiments. These features of the metal-binding domain may be explained by the direct binding of this domain to DNA[10b,23] or by a structure of this domain and linker moiety restricted by steric interactions induced by the binding of the DNA-binding domain to a right-handed B-form DNA. Presumably, both processes are necessary for stereospecific DNA recognition (cleavage) by BLM.

Not only both functional domains but also the linker moiety may possibly be essential for DNA cleavage and antitumor activity of BLM. The two methyl groups of the methylvalerate residue (V) showed very interesting behavior upon titration with D-or L-d(CGCGCG). The V_γ-CH_3 proton signal shifted in the opposite direction depending on the chirality of DNA although the chemical shift change was quite small (Figure 5 and 6). This is of particular interest since the epimer at this position of BLM analogs hardly has DNA-cleaving or biological activity.[24] Although the chemical shifts of the V_α-CH_3 signal were not affected by complexation with the L-hexanucleotide, this signal shifted upfield significantly upon complexation with natural D-d(CGCGCG). Conformational alteration around the V_γ position induced by complexation with DNA would thus appear dependent on the chirality or conformation of DNA, and the resulting conformation induced by specific interactions with a right-handed natural DNA would change the magnetic environment of the V_α-methyl group, while that with L-DNA would not. This would support the restricted structure of the linker region after binding of the DNA-binding domain to DNA, as described above. The chemical shifts of methyl protons of the threonine residue (T_β-CH_3) were not affected

(22) Our conclusion that the bithiazole group binds similarly to both DNA enantiomers is based on the similarity of the chemical shift changes in the Bit H5 and H5′ resonances upon complexation. These shifts are, however, largely determined by ring current effects emanating from the adjacent DNA base pairs. The observation that the complexes produce different extents of bithiazole line broadening suggests that there may be differences in the structure and/or dynamic properties of the complexes. It is thus likely that the extents of line broadening rather than the chemical shift changes of Bit H5 and H5′ reflects the specific recognition of DNA by BLM.

(23) Chikara, M; Sato, T.; Antholine, W. E.; Petering, D. H. *J. Biol. Chem.* **1991**, *266*, 2859–2863.

(24) (a) Umezawa, H.; Takita, T.; Saito, S.; Muraoka, Y.; Takahashi, K.; Ekimoto, H.; Minamide, S.; Nishikawa, K.; Fukuoka, T.; Nakatani, T.; Fujii, A.; Matsuda, A. In *Bleomycin Chemotherapy*; Sikic, B. I., Rozenweig, M., Carter, S. K., Eds.; Academic Press: Orlando, FL, 1985; pp 289–301. (b) Owa, T.; Haupt, A.; Otsuka, M.; Kobayashi, S.; Tomioka, N.; Itai, A.; Ohno, M.; Shiraki, T.; Uesugi, M.; Sugiura, Y.; Maeda, K. *Tetrahedron* **1992**, *48*, 1193–1208.

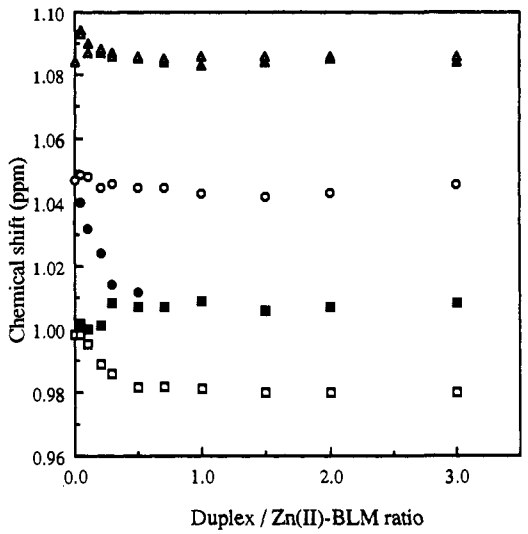

Figure 6. Dependence of the chemical shifts of V_α-CH_3 (circles), V_γ-CH_3 (squares), and T_β-CH_3 (triangles) on the ratio of the hexanucleotide duplex to Zn(II)–bleomycin B_2 (Duplex/Zn(II)–BLM). The conditions are the same as for Figure 5. The closed and open symbols denote titration with D- and L-d(CGCGCG), respectively.

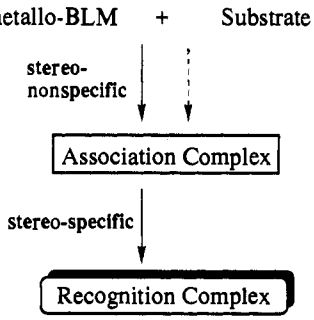

Figure 7. Schematic presentation of the proposed DNA recognition mechanism of BLM. The arrows indicate the pathways of D-DNA (solid line) and L-DNA (dashed line) recognition by BLM.

by DNA or its chirality. It is thus quite likely that the asymmetry of the T_β position and possibly the entire threonine residue is not related to the specific DNA recognition by BLM; this would be consistent with the fact that BLM analogs modified at this residue have substantial DNA-cleaving ability and the same sequence specificity.[10b]

On the basis of the present results, a model for DNA recognition by BLM was proposed (Figure 7) such that the DNA-binding domain causes BLM to take on greater affinity for DNA in a nonenantiospecific manner, although this domain adheres to a base sequence selectivity for G-Py sites.[6] This nonspecific association of BLM with the DNA enantiomers induces stereospecific conformational alteration and/or fixation in the metal-binding domain and linker moiety of BLM (induced-fit mechanism). Only a BLM refolded by association with a right-handed B-form DNA recognizes the shape (conformation) of natural DNA (recognition complex). The primary determinant for stereospecific and conformation-specific DNA recognition (not a base sequence recognition) by BLM appears to be the metal-binding domain and linker moiety, since the recognition complex would be formed by specific interactions of these domains with a natural right-handed DNA complexed with the DNA-binding domain. Although our experimental results give no indication of the mechanism for base sequence selectivity of BLM, there is the possibility that not only the DNA-binding domain but also the metal-binding domain are responsible for the selectivity of BLM.[10]

It is well-known that BLM binds to single-stranded DNA, according to the results of fluorescence quenching[25] and NMR experiments.[11a,19b] However, BLM seems not to cleave single-stranded DNA. Although several investigators report the cleavage of single-stranded DNAs by BLM, these substrates may have a local double-stranded structure. Indeed, careful analysis of the DNA sequences indicated the cleavage of single-stranded DNAs to occur in possible stem-loop regions.[26] For the BLM-mediated strand cleavage reaction of a short DNA fragment, a duplex structure was shown to be necessary.[27] Thus, single-stranded DNA is a poor substrate for BLM. Our model may explain the inability of BLM to degrade single-stranded DNA in spite of the binding of BLM (DNA-binding domain) to it. In the case of RNA, BLM-mediated strand scission has also been reported.[28] Hecht *et al.* reported the cleavage of RNA by BLM to occur in a clearly different manner, the cleavage being at least 10 times more sequence selective than DNA cleavage and occurring at junctions between single- and double-stranded regions. As they described: "it seems likely that RNA conformation is the primary determinant of the position(s) of RNA degradation".[28a] The model also appears consistent with reports that conformational alteration in DNA induced by DNA-binding drugs changes the base sequence selectivity of BLM-mediated DNA strand scission.[29] Refolding of BLM induced by binding with a right-handed B-form DNA may enable BLM to achieve stringent sequence specificity, stereospecificity, and regiospecificity for DNA strand cleavage.

In conclusion, the application of L-oligonucleotides to DNA–drug interaction studies should facilitate clarification of the mechanisms involved. Apparently, the enantiomer of DNA accurately discriminates very small chemical shift differences induced by specific interactions from those induced by nonspecific interactions. The results clearly indicate that the binding of the DNA-binding domain of BLM to DNA does not necessarily mean DNA recognition by BLM. It seems very likely that BLM should be able to recognize the shape of a right-handed B-form DNA by the "induced-fit" mechanism. Thus, BLM is intrinsically different from BLM-mimetic DNA-cleaving agents.[9] Clarification of the mechanism in detail as to how BLM recognizes the conformation of natural B-DNA may provide a new concept for the design of DNA- and RNA-cleaving agents that recognize specific shapes or conformation of substrates.[30]

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(25) Kasai, H.; Naganawa, H.; Takita, T.; Umezawa, H. *J. Antibiot.* **1978**, *31*, 1316–1320.
(26) (a) Kross, J.; Henner, W. D.; Hecht, S. M.; Haseltine, W. A. *Biochemistry* **1982**, *21*, 4310–4318. (b) Ueda, K.; Kobayashi, S.; Sakai, H.; Komano, T. *J. Biol. Chem.* **1985**, *260*, 5804–5807.
(27) Sugiyama, H.; Sera, T.; Dannoue, Y.; Marumoto, R.; Saito, I. *J. Am. Chem. Soc.* **1991**, *113*, 2290–2295.
(28) (a) Carter, B. J.; de Vroom, E.; Long, E. C.; van der Marel, G. A.; van Boom, J. H.; Hecht, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9373–9377. (b) Carter, B. J.; Reddy, K. S.; Hecht, S. M. *Tetrahedron* **1991**, *47*, 2463–2474. (c) Magliozzo, R. S.; Peisach, J.; Ciriolo, M. R. *Mol. Pharmacol.* **1989**, *35*, 428–432.
(29) (a) Sugiura, Y.; Suzuki, T. *J. Biol. Chem.* **1982**, *257*, 10544–10546. (b) Gold, B.; Dange, V.; Moore, M. A.; Eastman, A.; van der Marel, G. A.; van Boom, J. H.; Hecht, S. M. *J. Am. Chem. Soc.* **1988**, *110*, 2347–2349. (c) Mascharak, P. K.; Sugiura, Y.; Kuwahara, J.; Suzuki, T.; Lippard, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 6795–6798.
(30) (a) Barton, J. K. *Science* **1986**, *233*, 727–734. (b) Mei, H.-Y.; Barton, J. K. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 1339–1343. (c) Pyle, A. M.; Long, E. C.; Barton, J. K. *J. Am. Chem. Soc.* **1989**, *111*, 4520–4522.