Solution Structure of a Zn(II)·Bleomycin A_5-d(CGCTAGCG)$_2$ Complex

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The bleomycins (BLMs)[1] are clinically used antitumor antibiotics[2] that bind and degrade DNA[3] and RNA[4] selectively in the presence of O_2 and certain metals. Bleomycin contains at least two functional domains: a metal binding domain required for reductive activation of O_2[1] and DNA binding[5] and a DNA binding domain comprised of the bithiazole (Bit) and C-terminal substituent (spermidine (Sp) in BLM A_5, Figure 1).

NMR spectroscopy has been used to investigate the nature of BLM binding with various metal ions[6] and DNA.[7] However, the absence of intermolecular NOEs[7] and the continuing uncertainty concerning the exact metal ligands[6] have precluded definition of the nature of DNA binding by metallobleomycins. Presently, we use Zn(II)·BLM A_5 and a DNA octanucleotide (d(CGCTAGCG)$_2$) that is a particularly efficient substrate for BLM[8] to address the issue of DNA binding. The key observations of six intermolecular BLM−DNA NOEs and the lack of perturbation of DNA sequential connectivities upon BLM binding[9] have permitted us to utilize molecular dynamics calculations to develop a model of DNA binding by Zn(II)·BLM A_5.

Proton assignments for Zn(II)·BLM A_5 alone and free d(CGCTAGCG)$_2$[10] were established by means of DQF-COSY[11] and NOESY[12] experiments (supplementary material, Tables 1 and 2). Zn(II)·BLM A_5 binding did not disrupt the C_2 symmetry of the duplex, indicative of fast exchange; consistent with this, only one set of drug resonances was observed. In addition, it

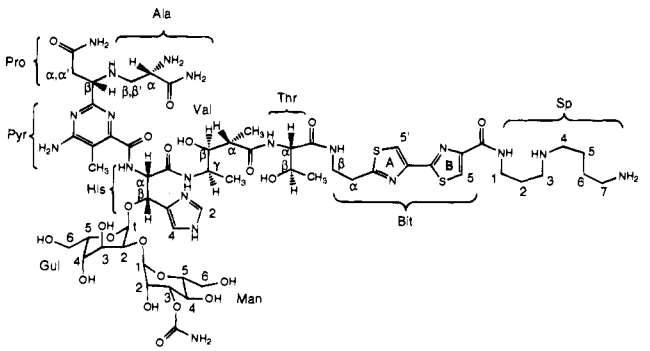

Figure 1. Structure of bleomycin A_5.

was clear from the DQF-COSY spectrum of the Zn·BLM complex that the octamer remained in a B-form conformation.[13]

Both minor groove binding and (partial) intercalation models have been proposed for DNA binding by the bithiazole and C-terminal substituent of BLM.[7c,14] We also obtained data that support both models. Consistent with intercalative binding, upon admixture of Zn·BLM and DNA, we observed broadening and upfield shifting of the aromatic bithiazole (Bit 5, 0.18 ppm; Bit 5′, 0.52 ppm) and base-paired thymidine$_4$ and guanosine$_2$ imino protons (0.1 ppm).[15,16] However, the data do not support a classical mode of intercalation, as the sequential connectivities of base and sugar protons in the NOESY spectrum were not disrupted (Figure 2).[17,18] In addition to the lack of disruption of the NOE walk,[15a,19] strong supportive evidence for groove binding derives from the finding of intermolecular NOEs (supplementary material, Figure 1 and Table 3) between Sp 3 and Bit 5 of BLM with adenosine$_5$ H2 in the minor groove (Figure 3).[20] Also, protons in the β-hydroxyhistidine (His α) and methyl valerate moieties (Val Me) had intermolecular NOEs to minor groove protons (cytidine$_7$ H4′ and H5′,5″) (Figure 3). Some protons in the BLM metal binding domain were also shifted downfield, consistent with minor groove binding.[15a,19]

Analysis of the intermolecular BLM−DNA NOEs[21] allows placement of a folded BLM structure[22] in the minor groove of d(CGCTAGCG)$_2$. Molecular dynamics calculations[23] suggest a possible structure for the Zn(II)·BLM A_5 complex that is fully consistent with the observed NMR-derived distance data (Figure

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(8) Fe·BLM cleaves d(CGCTAGCG)$_2$ with selectivity for cytidine$_3$ and cytidine$_7$. Van Atta, R. B.; Long, E. C.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1989**, *111*, 2722.

(9) NMR experiments used a General Electric Omega 500 spectrometer. The 1:1 Zn(II)·BLM A_5−d(CGCTAGCG)$_2$ complex (4.2 mM) was studied at 15 and 35 °C in D_2O and H_2O, pH 7.0, containing 20 mM NaCl.

(10) d(CGCTAGCG)$_2$ exists as a B-form duplex at concentrations similar to those utilized here (Pieters, J. M. L.; de Vroom, E.; van der Marel, G. A.; van Boom, J. H.; Altona, C. *Eur. J. Biochem.* **1989**, *184*, 415).

(11) Double quantum filtered ^{1}H−^{1}H correlated spectroscopy (Piantini, U.; Sorenson, O. W.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 6800).

(12) Two-dimensional nuclear Overhauser enhancement spectroscopy (Jeener, J.; Meier, B. H.; Bachmann, P.; Ernst, R. R. *J. Chem. Phys.* **1979**, *71*, 4546). Mixing times of 100 and 300 ms were used. NOESY cross peak volumes were converted to distances by the two spin approximation, using the cytidine H5−H6 distance of 2.45 Å for calibration.

(13) The DQF-COSY spectrum had $^3J_{H1'-H2'} > J_{H1'-H2''}$ and readily apparent H2′−H3′ couplings but weak H2″−H3′ couplings. These features are typical of the C2′-endo sugar pucker in B-DNA (Kim, S. G.; Lin, L.; Reid, B. R. *Biochemistry* **1992**, *31*, 3564).

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(16) Upfield shifting (0.1 ppm) of the T_4 and G_2 imino protons was noted in spectra acquired in H_2O; this supports intercalation, since groove binders typically cause downfield shifts of imino protons (Feigon, J.; Denny, W. A.; Leupin, W.; Kearns, D. R. *J. Med. Chem.* **1984**, *27*, 450.

(17) In B-DNA, the base (H8, H6) exhibits NOEs to its own and 5′-flanking sugar H1′ and H2′,2″ protons, permitting the sequence connectivities to be defined (Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986). Intercalators disrupt this walk by causing helical unwinding that increases the distance between base steps to ≥5 Å. See, e.g.: Liu, X. L.; Chen, H.; Patel, D. J. *J. Biomol. NMR* **1991**, *1*, 323.

(18) In fact, unlike Zn·BLM A_5, a (2-aminoethyl)bithiazole A_5 derivative shown previously to bind strongly to DNA (Kross, J.; Henner, W. D.; Haseltine, W. A.; Rodriguez, L.; Levin, M. D.; Hecht, S. M. *Biochemistry* **1982**, *21*, 3711) did disrupt the NOE walk at the thymidine$_4$−adenosine$_5$ step of the same octamer, consistent with intercalation.

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(20) The Sp 3−A_5 H2 NOE was very weak at 35 °C but more intense at 15 °C. In contrast, the Bit 5−A_5 H2 cross peak was relatively strong at 35 °C but was not resolved at 15 °C due to line broadening. Line broadening is consistent with the existence of more than one BLM conformation.

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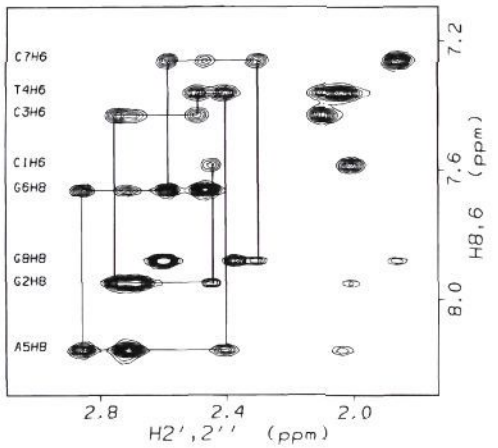

Figure 2. Expanded NOESY contour plot (300 ms mixing time, 35 °C in D_2O, pD 7.0, containing 20 mM NaCl) for Zn·BLM A_5–d(CGCTAGCG)$_2$, correlating distance connectivities between base (H8,6) and sugar (H2′,2″) protons. The tracing outlines connectivities between base protons and 5′-flanking sugar H2″ protons.

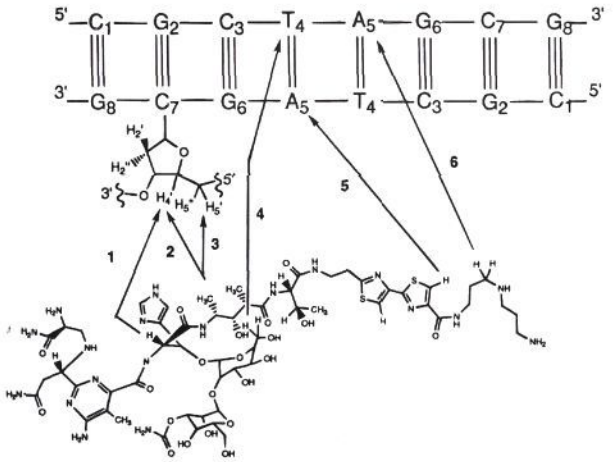

Figure 3. Schematic diagram showing the six intermolecular NOEs. Assignments: 1, C_7 H4′–His Hα; 2 C_7 H4′–Val Me; 3, C_7 H5′,5″–Val Me; 4, T_4 H3′–Gul H6, H6′; 5, A_5 H2–Bit 5; 6, A_5 H2–Sp 3. It was not possible to distinguish between the two CH_3 groups of the methyl valerate moiety.

4), although this structure is almost certainly not unique.[24] BLM (light blue) is positioned in the minor groove of the octamer (red), with Zn(II) (gold) at a distance of 3.3 Å from cytidine$_7$ H4′ (red ball), i.e., the atom abstracted to initiate DNA degradation.[1,8] Hydrogen-bonding interactions (yellow arrows) are present between the cationic spermidine C-terminus and the phosphate and ribose oxygens and between the A-ring bithiazole N (Figure 1) and guanosine$_6$ NH_2.[14] The metal binding domain occupies a widened minor groove[25] in the B-form duplex; Ala NH_2 is H-bonded to a DNA backbone oxygen.

Further studies employing other DNAs and BLMs are underway to allow us to test and refine this model.

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Supplementary Material Available: 2-D NMR spectrum highlighting the intermolecular BLM–DNA NOEs; tables showing ^{1}H chemical shifts for Zn·BLM A_5, d(CGCTAGCG)$_2$, and the Zn·BLM A_5–d(CGCTAGCG)$_2$ complex; and a correlation of the model to the NOE distance data (7 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

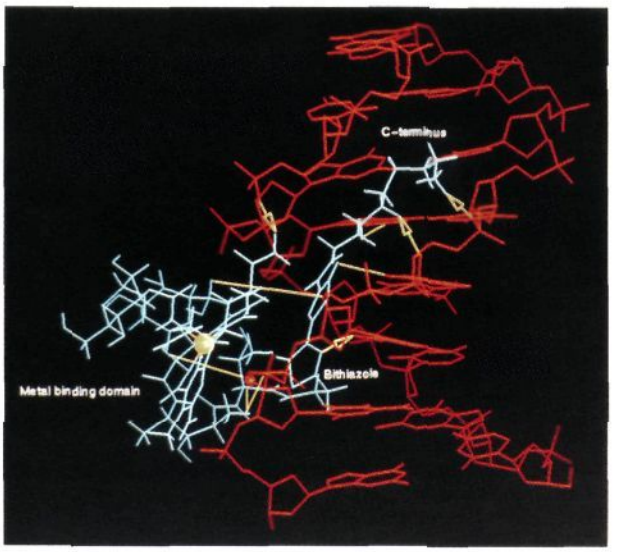

Figure 4. Model of the Zn(II)·BLM A_5–d(CGCTAGCG)$_2$ complex that satisfies the NOE restraints to within 0.2 Å. BLM (light blue) is positioned in the minor groove of the B-form octamer (red). The six intermolecular NOEs are shown as yellow lines; the apparent length difference is due to perspective only. H-bonding contacts are shown as yellow arrows pointing toward the hydrogen atoms. The metal ion (gold ball) is 3.3 Å from C_7 H4′ (red ball).

(21) Because the individual duplex strands cannot be distinguished, there is an ambiguity in the assignments. Molecular modeling yielded lower energy structures using the strand-specific assignments shown in Figure 3. Also, Bit 5′ appeared to show contacts to T_4 H6 and C_3 H6, which lie in the major groove. Although line broadening and spectral overlap make this assignment tentative, this may reflect a second binding mode for BLM.

(22) An intramolecular NOE cross peak between His 2 and Thr Me requires the close spatial proximity of these domains.

(23) This model was developed using the Insight II/Discover program and the Biosym CVFF force field. Heme parameters provided with Insight II were used to model BLM–metal ion interaction. The five BLM ligands identified previously[6e] were arranged around the metal in a square pyramid arrangement, with the Man carbamoyl as the axial ligand. Thirty starting structures were generated using the simulated annealing protocol (Nilges, M.; Clore, G. M.; Gronenborn, A. M. *FEBS Lett.* **1988**, *229*, 317); of these, 18 structures with the lowest energies were minimized initially using 100 steps of a steepest descents algorithm and further using 30 000 steps of conjugate gradient minimization (178 NOE restraints, 23 distance restraints) to a final root mean square derivative of <0.001 kcal/mol/Å^2.

(24) Retention of DNA symmetry upon BLM binding and the small number and intensity of BLM–DNA NOEs suggest that the structure in Figure 4 is not unique. The bithiazole ring-current induced shifts noted, for example, could be accommodated by an additional structure in which the bithiazole is (partially) intercalated. In this regard, preliminary analysis of a Zn·BLM A_2-d(CGCTAGCG)$_2$ complex indicates the presence of a kinked DNA structure that may favor a (partial) intercalation binding mode.[14c] The structure shown in Figure 4 represents the lowest energy structure consistent with both NMR-derived distance data and the known chemistry of BLM–DNA interaction.[1,8] Two other low-energy structures that satisfy the NOE restraints to within 0.2 Å were also found. Both structures are groove binding models but differ from the structure shown in Figure 4 in that the H-bond from the bithiazole ring nitrogen to the 2-amino group of G_6 is not formed and the metal ion lies ca. 5.0 Å from C_7 H4′.

(25) An average minor groove width of 5.7 Å is anticipated for a B-form duplex (Conner, B. N.; Takano, T.; Tanaka, S.; Itakura, K.; Dickerson, R. E. *Nature* **1982**, *295*, 294). In the model, minor groove widths range from 3.62 to 7.56 Å, with the metal binding domain occupying a minor groove width of 6.5–7.5 Å. Minor groove widths were measured as the shortest distances between sugar O4′ atoms and phosphate P atoms across the groove. The O4′–O4′ distances are decreased by 2.8 Å, or two oxygen van der Waals radii; those for P are decreased by 5.8 Å (Yuan, H.; Quintana, J.; Dickerson, R. E. *Biochemistry* **1992**, *31*, 8009).