Sequence Selective Cleavage of a DNA Octanucleotide by Chlorinated Bithiazoles and Bleomycins

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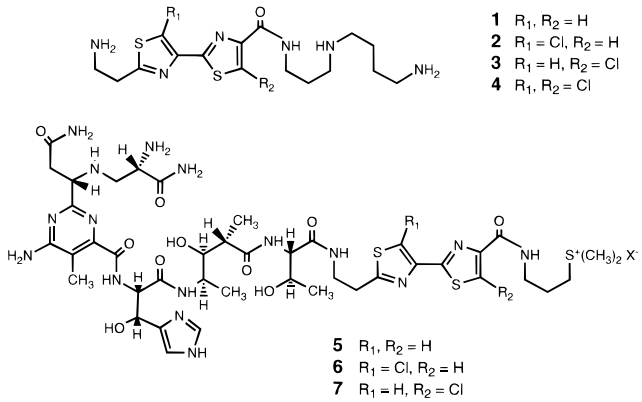
The bleomycins (BLM) are a family of clinically used antitumor antibiotics.[1] Their mechanism of action is believed to involve the degradation of DNA[2] and possibly of RNA.[3] BLM-mediated degradation requires a redox-active metal ion as well as oxygen and involves the intermediacy of a metal-bound oxo or peroxy species.[2,4] The selectivity of DNA and RNA cleavage must reflect initial site-selective substrate binding, followed by oxidative degradation at sites that are sterically accessible following binding. While the bithiazole moiety clearly contributes to the DNA and RNA affinity of the BLMs,[2,5] there is compelling evidence that the metal binding domain is responsible for the observed sequence selectivity of cleavage.[6]

Recently, attention has focused on the mode of DNA binding by metallobleomycins. Approaches have included DNA footprinting[7] and NMR spectroscopy, the latter of which has been combined with molecular dynamics calculations to provide models of Zn[8] and Co·BLMs[9] bound to DNA oligonucleotide substrates. The models are in agreement that the metal binding domain is oriented in the minor groove. In fact, the products of BLM-mediated DNA degradation must form by initial H atom abstraction from C4′H of deoxyribose, which resides in the minor groove of DNA.

Many studies have attempted to define the mode(s) of DNA binding by the bithiazole moiety of BLM, but these fail to lead uniquely to a single type of interaction. Good evidence exists for groove binding, intercalation and partial intercalation in specific cases;[5d,8−10] this may reflect actual variations in the mode of binding from one system to another.[11] The uncertainty concerning the nature of DNA binding by the bithiazole reflects the lack of any DNA structure modification mediated by this part of the BLM molecule.

Early efforts to equip the bithiazole with reactive prosthetic groups failed to define the nature of bithiazole−DNA interaction.[5e,12] However, chlorinated bithiazoles **2**−**4** were found to effect potent



light-mediated DNA cleavage, apparently via chlorine radicals (Cl·) produced by homolysis of C−Cl bonds.[13] Interestingly, these cleaved duplex DNA in a fashion that was nonrandom but not the same as that of BLM.[13b] Presently, we report the site-selective chemistry by which these bithiazoles modified the high efficiency BLM substrate d(CGCTAGCG)$_2$ and the nature of oxidative and light-mediated DNA oligonucleotide cleavage by analogues of BLM containing chlorinated thiazoles. Our findings strongly support minor groove binding by these BLM analogues and an intrinsic binding preference of the bithiazole that may contribute to the efficient site-selective cleavage of d(CGCTAGCG)$_2$ by BLM.

Irradiation of chlorobithiazoles **2**−**4** in the presence of the [5′-^{32}P]-end labeled octanucleotide resulted in DNA cleavage at a single site (Figure 1), identified as A_5 by comparison with sequencing reactions (not shown). Notably, cleavage by bithiazoles **3** and **4**, containing Cl attached to ring B, was much more efficient than cleavage by **2**. Treatment with 0.1 M piperidine and heating to 90 °C for 15 min prior to electrophoretic analysis gave an additional band corresponding to cleavage at T_4 in some of the lanes. This cleavage obtained specifically for bithiazoles **2** and **4**, i.e., for the ring A chlorinated derivatives. No DNA oligonucleotide cleavage was observed using non-chlorinated bithiazole **1**.[14]

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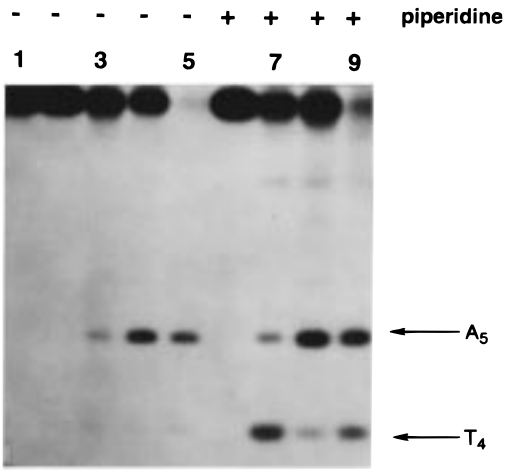

Figure 1. Light-mediated DNA cleavage by chlorobithiazoles **2**−**4**. Reaction mixtures having 10 μM [5′-^{32}P]-end labeled d(CGCTAGCG)$_2$ and 10 μM **2**−**4** were irradiated (Rayonet photoreactor, 2537 Å lamp, Pyrex filter) in 10 mM Na cacodylate, pH 7.0, for 15 min and then analyzed by 20% denaturing PAGE. Lanes 1 and 2, DNA alone; lanes 3−5, bithiazoles **2**−**4**, respectively; lane 6, DNA + piperidine treatment; lanes 7−9, bithiazoles **2**−**4**, respectively, + piperidine treatment. The samples in lanes 2−9 were irradiated. The paucity of intact DNA in lanes 5 and 9 reflects the extraordinary potency of bithiazole **4**.

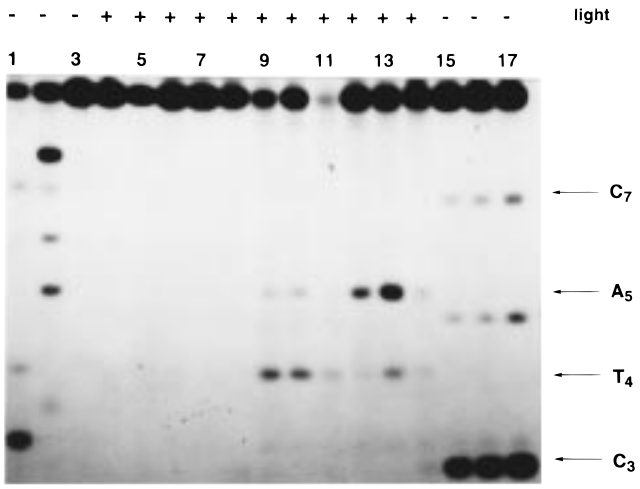

Figure 2. Cleavage of DNA by deglycoBLMs **5**−**7** in the presence of Fe^{2+} or light. Reaction mixtures containing 10 μM [5′-^{32}P]-end labeled d(CGCTAGCG)$_2$ and 50 μM BLMs **5**−**7** were irradiated in 10 mM Na cacodylate, pH 7.0, for 15 min, heated at 90 °C for 20 min, and then analyzed by 20% denaturing PAGE. Lane 1, Maxam−Gilbert C + T lane; lane 2, G + A lane; lanes 3 and 4, DNA alone; lane 5, 50 μM Fe^{3+}; lane 6, deglycoBLM **5**; lane 7, deglycoBLM **5** + equimolar Cu^{2+}; lane 8, deglycoBLM **5** + equimolar Fe^{3+}; lane 9, deglycoBLM **6**; lane 10, deglycoBLM **6** + equimolar Cu^{2+}; lane 11, deglycoBLM **6** + equimolar Fe^{3+}; lane 12, deglycoBLM **7**; lane 13, deglycoBLM **7** + equimolar Cu^{2+}; lane 14, deglycoBLM **7** + equimolar Fe^{3+}; lane 15, deglycoBLM **5** + equimolar Fe^{2+}; lane 16, deglycoBLM **6** + equimolar Fe^{2+}; lane 17, deglycoBLM **7** + equimolar Fe^{2+}.

The chemistry of DNA cleavage at positions A_5 and T_4 was investigated via product analysis by HPLC. Treatment of 100 μM d(CGCTAGCG)$_2$ with 100 μM **2** resulted in the release of 14 μM thymine; heating with piperidine did not significantly alter thymine release but did afford 6 μM 5-methylenefuranone (5-MF).[15] These observations are consistent with chlorine radical-mediated abstraction of C1′H from T_4, producing an alkali-labile lesion that affords strand scission only upon subsequent heating with piperidine (Scheme 1, Supporting Information).[15] In contrast, treatment of d(CGCTAGCG)$_2$ with **3** afforded only 2 μM free adenine despite the considerable cleavage evident at this position (Figure 1). Heating produced 12 μM adenine and 5 μM furfural.[16] These findings are fully consistent with abstraction of C5′H from adenosine$_5$, affording DNA cleavage with the formation of a tetranucleotide having a 5′-terminal aldehyde. That bithiazole **4** mediated both types of chemistry argues that bithiazoles **2**−**4** all bound to the DNA octanucleotide substrate in the same fashion.

To assess the relevance of these findings to DNA binding by bleomycin, **2** and **3** were used in the synthesis of two analogues of (deglyco)BLM A_2 (**6** and **7**, respectively).[17] When BLMs **6** and **7** were admixed with equimolar Fe^{2+}, the patterns of d(CGCTAGCG)$_2$ cleavage were the same as those observed for (non-chlorinated) deglyco BLM A_2 (**5**) itself (Figure 2). The major site of cleavage was at C_3, as noted previously,[6a] with lesser amounts at C_7. Thus the Cl atoms did not alter the (oxidative) chemistry. Also studied was d(CTCTAGCG)$_2$ cleavage by **5**−**7** upon irradiation, using the same conditions employed for cleavage by **2**−**4**. BLM analogue **6** produced cleavage predominantly at T_4, while **7** produced cleavage predominantly at A_5 (Figure 2). Similar effects were obtained for the Cu(II) and, to a lesser extent, for the Fe(III) complexes of **6** and **7**. As anticipated, the bands produced comigrated with the corresponding bands of the Maxam−Gilbert sequencing lanes, reflecting the presence of 3′-phosphate groups (cf. Scheme 1, Supporting Information);[18] irradiation in the presence of **5** afforded no DNA cleavage. Thus, the chlorinated bithiazoles present as constituents of BLMs **6** and **7** gave the same DNA products as the free bithiazoles themselves (cf. Figures 1 and 2), suggesting strongly that their mode of DNA binding was unaltered by inclusion within the BLM structure.

Molecular modeling studies were carried out to determine what mode of DNA binding common to bithiazoles **2**−**4** and BLMs **6** and **7** could afford the products actually observed. Assuming that H atom abstraction was mediated by Cl·,[13,19] and would be maximally efficient and specific if the Cl atoms were positioned in proximity to the H atoms abstracted, one binding mode was clearly preferred (Figure 1, Supporting Information). In this mode, the bithiazole H's (or Cl's) are "cisoid" and pointed toward the floor of the minor groove. This orientation is quite similar to that of the DNA binding mode determined for Zn(II)·BLM A_2 and Zn(II)·BLM A_5 by ^{1}H NMR spectroscopy/molecular dynamics calculations.[8]

These findings provide additional strong support for a minor groove binding mode by deglycoBLM since the chemistry observed involves H atoms accessible within the minor groove. They also suggest that the preferred binding of the bithiazole moiety within the AT domain of d(CGCTAGCG)$_2$ may reinforce the preferred binding of the metal binding domain at the GC sequence,[2,8,9] contributing to the high efficiency and selectivity of cleavage of this substrate by (deglyco)BLM.[6a] Further, the data in Figure 2 establish the orientation of the (chloro)bithiazole moiety in deglycoBLM as being 3′ to the observed site of oxidative cleavage, in full agreement with the structure proposed for the Zn(II)·deglycoBLM-d(CGCTAGCG)$_2$ complex.[8b]

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Supporting Information Available: Figure and scheme showing proposed DNA interaction by **2**−**4** (4 pages, print/PDF). See any current masthead for ordering information and Web access instructions.

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(14) Analogous results were obtained for chlorinated bithiazoles structurally related to BLM A_2, reflecting the similarities in DNA binding by BLM A_2 and A_5.[8a]

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(16) Furfural was detected as its *O*-benzyloxime derivative. Boturyn, D.; Hecht, S. M., unpublished results.

(17) Prepared starting from the chlorinated bithiazoles[13] in analogy with the synthesis of other deglycoBLMs (see, e.g., Hamamichi, N.; Natrajan, A.; Hecht, S. M. *J. Am. Chem. Soc.* **1992**, *114*, 6278).

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