J|A|C|S ARTICLES

Published on Web 11/03/2010

Characterization of Bleomycin Cleavage Sites in Strongly Bound Hairpin DNAs

Rachel A. Giroux and Sidney M. Hecht*

Center for BioEnergetics, Biodesign Institute and Department of Chemistry, Arizona State University, Tempe, Arizona 85287, United States

Received August 11, 2010; E-mail: sid.hecht@asu.edu

Abstract: The first complete, systematic study of DNA degradation by bleomycin under conditions analogous to those likely in a therapeutic setting has been carried out. Hairpin DNAs selected for their ability to bind strongly to BLM A_5 were used to determine the relationship between high-affinity DNA binding sites and the cleavage efficiency and selectivity of BLM A_5 and deglycoBLM A_5 on these DNAs. Of the 10 hairpin DNAs examined, 8 contained at least one 5′-GC-3′ or 5′-GT-3′ cleavage site, which have traditionally been associated with strong cleavage by Fe·BLM. In the hairpin DNAs, these included the strongest cleavage sites for BLM A_5 and were generally among those for deglycoBLM A_5. However, numerous additional cleavages were noted, many at sequences not usually associated with (deglyco)BLM-mediated cleavage. The remaining DNAs lacked the preferred (5′-GC-3′ or 5′-GT-3′) BLM cleavage sequences; however, strong cleavage was nonetheless observed at a number of unusual cleavage sites. The most prominent cleavage sequences were 5′-AT-3′, 5′-AA-3′, 5′-GA-3′, and 5′-TT-3′; treatment with Fe(II)·BLM A_5 or Fe(II)·deglycoBLM A_5 resulted in strong cleavage at these sequences. Additionally, in contrast with BLM A_5, which produced cleavage within the randomized and flanking invariant regions, deglycoBLM A_5 showed a preference for cleavage in the randomized region of the DNAs. Previous reports have established that deglycoBLM exhibits decreased DNA cleavage efficiency relative to BLM. This was also generally observed when comparing cleavage efficiencies for the strongly bound hairpin DNAs. However, some cleavage bands produced by Fe·deglycoBLM A_5 were stronger in intensity than those produced by BLM A_5 at concentrations optimal for both compounds. To investigate the chemistry of DNA degradation, selected hairpin DNAs were treated with *n*-butylamine following cleavage with Fe(II)·BLM A_5 or Fe(II)·deglycoBLM A_5 to explore the alkali labile pathway of DNA degradation by BLM. While all 10 DNAs showed evidence of alkali labile products, five DNA hairpins afforded some products formed solely via the alkali labile pathway.

Introduction

The bleomycins (BLMs) are a family of glycopeptides first isolated from *Streptomyces verticillus*.[1] They have shown success against various malignancies including testicular cancer and certain types of lymphomas.[2] BLM (Figure 1) has four functional domains: the metal binding domain, which also recognizes DNA[3,4] and activates oxygen,[5−8] the bithiazole and C-terminal substituents, which are involved in DNA binding,[9−14] the linker region, which is important for efficient DNA cleavage,[15−18] and the carbohydrate moiety, which is posited

(1) Umezawa, H.; Maeda, K.; Takeuchi, T.; Okami, Y. *J. Antibiot.* **1966**, *19*, 200–209.
(2) (a) Sikic, B. I.; Rozencweig, M.; Carter, S. K. *Bleomycin Chemotherapy*; Academic Press: New York, 1985. (b) Christian, J. A.; Huddart, R. A.; Norman, A.; Mason, M.; Fossa, S.; Aass, N.; Nicholl, E. J.; Dearnaley, D. P.; Horwich, A. *J. Clin. Oncol.* **2003**, *21*, 871–877. (c) Martinelli, G.; Cocorocchio, E.; Saletti, P. C.; Orecchia, R.; Bernier, J.; Tradati, N.; Santoro, P.; Robertson, C.; Peccatori, F. A.; Zucca, E.; Cavalli, F. *Leuk. Lymphoma* **2003**, *44*, 1919–1923. (d) Muir, T.; Kirsten, M.; Fourie, P.; Dippenaar, N.; Ionescu, G. O. *Pediatr. Surg. Int.* **2004**, *19*, 766–773. (e) Chevreau, C.; Mazerolles, C.; Soulie, M.; Gaspard, M. H.; Mourey, L.; Bujan, L.; Plante, P.; Rischmann, P.; Bachaud, J. M.; Malavaud, B. *Eur. Urol.* **2004**, *46*, 209–214. (f) Saitta, P.; Krishnamurthy, K.; Brown, L. H. *Dermatol. Surg.* **2008**, *34*, 1299–1313. (g) Linnert, M.; Gehl, J. *Anti-Cancer Drugs* **2009**, *20*, 157–164. (h) Steinbok, P.; Hukin, J. *Neurosurg. Focus* **2010**, *28*, E13.
(3) Carter, B. J.; Murty, V. S.; Reddy, K. S.; Wang, S. N.; Hecht, S. M. *J. Biol. Chem.* **1990**, *265*, 4193–4196.
(4) Guajardo, R. J.; Hudson, S. E.; Brown, S. J.; Mascharak, P. K. *J. Am. Chem. Soc.* **1993**, *115*, 7971–7977.
(5) Ishida, R.; Takahashi, T. *Biochem. Biophys. Res. Commun.* **1975**, *66*, 1432–1438.
(6) Sausville, E. A.; Peisach, J.; Horwitz, S. B. *Biochemistry* **1978**, *17*, 2740–2746.
(7) Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1981**, *256*, 11636–11644.
(8) Kilkuskie, R. E.; Suguna, H.; Yellin, B.; Murugesan, N.; Hecht, S. M. *J. Am. Chem. Soc.* **1985**, *107*, 260–261.
(9) Chien, M.; Grollman, A. P.; Horwitz, S. B. *Biochemistry* **1977**, *16*, 3641–3647.
(10) Berry, D. E.; Chang, L. H.; Hecht, S. M. *Biochemistry* **1985**, *24*, 3207–3214.
(11) Hamamichi, N.; Natrajan, A.; Hecht, S. M. *J. Am. Chem. Soc.* **1992**, *114*, 6278–6291.
(12) Kane, S. A.; Natrajan, A.; Hecht, S. M. *J. Biol. Chem.* **1994**, *269*, 10899–10904.
(13) Boger, D. L.; Colletti, S. L.; Honda, T.; Menezes, R. F. *J. Am. Chem. Soc.* **1994**, *116*, 5607–5618.
(14) Zuber, G.; Quada, J. C., Jr.; Hecht, S. M. *J. Am. Chem. Soc.* **1998**, *120*, 9368–9369.
(15) Boger, D. L.; Colletti, S. L.; Teramoto, S.; Ramsey, T. M.; Zhou, J. *Bioorg. Med. Chem.* **1995**, *3*, 1281–1295.
(16) Boger, D. L.; Ramsey, T. M.; Cai, H.; Hoehn, S. T.; Stubbe, J. *J. Am. Chem. Soc.* **1998**, *120*, 9139–9148.

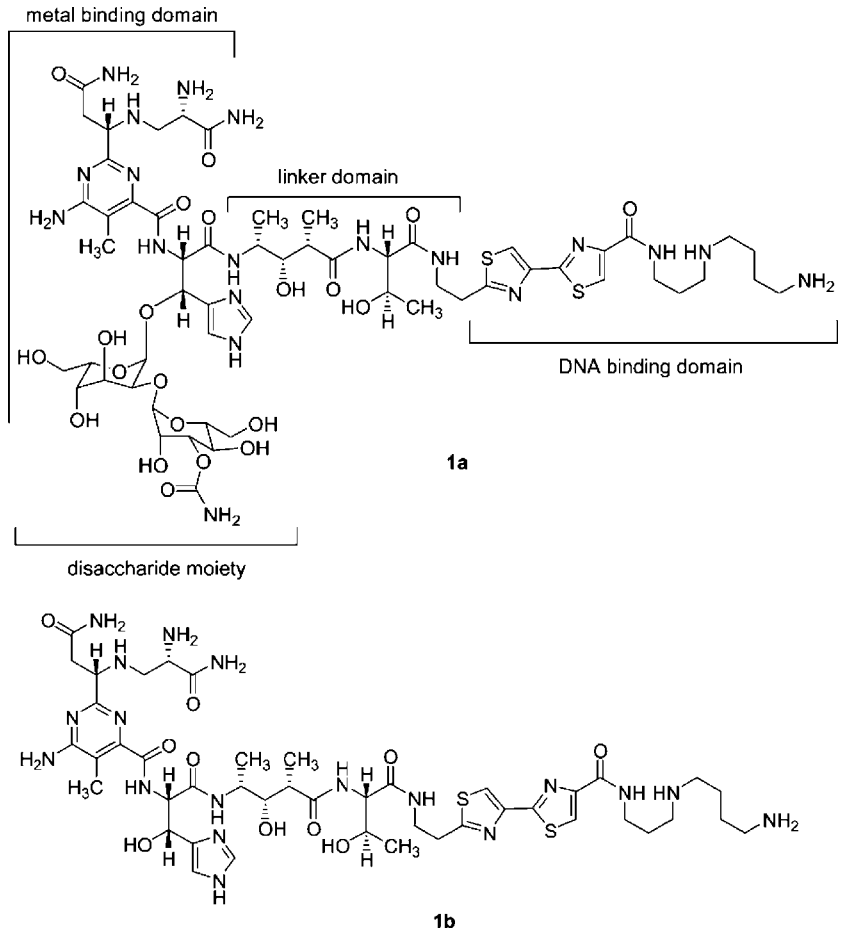

Figure 1. Structures of BLM A_5 (**1a**) and deglycoBLM A_5 (**1b**).

to be important in metal binding, cell surface recognition, and DNA cleavage efficiency as well as RNA recognition and cleavage.[19−23]

The chemotherapeutic effects of BLM are believed to be the result of its ability to mediate sequence-selective cleavage of DNA, and possibly RNA, which requires a metal ion cofactor and O_2.[5,6,24−28] DNA degradation by Fe·BLM proceeds via two pathways, which afford two sets of products.[26,29−36] Both sets are derived from a DNA sugar C-4′ radical that forms by H atom abstraction.[37]

Unfortunately, almost all of studies of BLM-mediated DNA degradation have been carried out using a large molar excess of BLM. In contrast, BLM is employed clinically at atypically low dose (∼5 μmol), implying that those DNAs bound exceptionally strongly by BLM may be the only ones available for cleavage when BLM is used therapeutically. The nature of BLM binding and cleavage in the presence of excess DNA has not been studied. Relatively little is presently known about the factors that control the binding selectivity of BLM for DNA, or the correlation between such binding and DNA cleavage. The reported 5′-GC-3′ and 5′-GT-3′ sequence selectivity of BLM observed with excess Fe·BLM may be a consequence of the nature of the metal binding domain, preferential binding of the

(17) Boger, D. L.; Ramsey, T. M.; Cai, H.; Hoehn, S. T.; Stubbe, J. *J. Am. Chem. Soc.* **1998**, *120*, 9149–9158.
(18) Rishel, M. J.; Thomas, C. J.; Tao, Z.-F.; Vialas, C.; Leitheiser, C. J.; Hecht, S. M. *J. Am. Chem. Soc.* **2003**, *125*, 10194–10205.
(19) Boger, D. L.; Teramoto, S.; Honda, T.; Zhou, J. *J. Am. Chem. Soc.* **1995**, *117*, 7338–7343.
(20) Boger, D. L.; Teramoto, S.; Zhou, J. *J. Am. Chem. Soc.* **1995**, *117*, 7344–7356.
(21) Tounekti, O.; Kenani, A.; Foray, N.; Orlowski, S.; Mir, L. M. *Br. J. Cancer* **2001**, *84*, 1272–1279.
(22) Thomas, C. J.; Chizhov, A. O.; Leitheiser, C. J.; Rishel, M. J.; Konishi, K.; Tao, Z.-F.; Hecht, S. M. *J. Am. Chem. Soc.* **2002**, *124*, 12926–12927.
(23) Chapuis, J.-C.; Schmaltz, R. M.; Tsosie, K. S.; Belohlavek, M.; Hecht, S. M. *J. Am. Chem. Soc.* **2009**, *131*, 2438–2439.
(24) Sausville, E. A.; Peisach, J.; Horwitz, S. B. *Biochem. Biophys. Res. Commun.* **1976**, *73*, 814–822.
(25) Hecht, S. M. *Bioconjugate Chem.* **1994**, *5*, 513–526.
(26) Kane, S. A.; Hecht, S. M. *Prog. Nucleic Acid Res. Mol. Biol.* **1994**, *49*, 313–352.
(27) Hecht, S. M. *J. Nat. Prod.* **2000**, *63*, 158–168.
(28) Chen, J.; Stubbe, J. *Nat. Rev.* **2005**, *5*, 102–112.
(29) Giloni, L.; Takeshita, M.; Johnson, F.; Iden, C.; Grollman, A. P. *J. Biol. Chem.* **1981**, *256*, 8608–8615.
(30) Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1982**, *257*, 8612–8614.
(31) Sugiyama, H.; Xu, C.; Murugesan, N.; Hecht, S. M. *J. Am. Chem. Soc.* **1985**, *107*, 4104–4105.
(32) Sugiyama, H.; Kilkuskie, R. E.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1985**, *107*, 7765–7767.
(33) Rabow, L.; Stubbe, J.; Kozarich, J. W.; Gerlt, J. A. *J. Am. Chem. Soc.* **1986**, *108*, 7130–7131.
(34) Sugiyama, H.; Xu, C.; Murugesan, N.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *Biochemistry* **1988**, *27*, 58–67.
(35) Rabow, L. E.; Stubbe, J.; Kozarich, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 3196–3203.
(36) Rabow, L. E.; Stubbe, J.; Kozarich, J. W.; McGall, G. H. *J. Am. Chem. Soc.* **1990**, *112*, 3203–3208.
(37) (a) Wu, J. C.; Kozarich, J. W.; Stubbe, J. *Biochemistry* **1985**, *24*, 7562–7568. (b) Kozarich, J. W.; Worth, L., Jr.; Frank, B. L.; Christner, D. F.; Vanderwall, D. E.; Stubbe, J. *Science* **1989**, *245*, 1396–1399.

drug to specific DNA sequences, or other factors not yet identified.[3,14,38−40]

We recently described a novel strategy to identify DNA motifs that bind strongly to BLM A_5.[41] This strategy led to the identification of 10 hairpin DNAs that bind strongly to BLM A_5. The structural properties of these hairpin DNAs were characterized at the level of sequence analysis, and four of 10 DNAs were treated with Fe(II)·BLM A_5 to permit the sequence selectivity of cleavage to be determined so that a direct comparison of DNA binding and cleavage by BLM A_5 could be made.[42]

Key findings of that initial study included the existence of many binding sites not represented in the cleavage patterns when DNA was treated with an excess of Fe·BLM, and cleavage at unique sites in addition to canonical 5′-GC-3′ and 5′-GT-3′ sites. Also noted preliminarily was the presence of alkali labile lesions at a few sites not subject to frank strand scission. The 10 hairpin DNAs identified previously have now been used as substrates for degradation both by Fe·BLM A_5 and by Fe·deglycoBLM A_5. Both pathways of BLM-mediated DNA cleavage were studied. These strongly bound hairpin DNAs exhibit unusual cleavage patterns and additional lesions produced solely through the alkali labile pathway. The cleavage patterns include numerous sites in AT-rich regions, while the alkali labile lesions predominantly involve oxidative destruction of G residues. Further, there were sites apparent at which the effects of deglycoBLM A_5 were much more pronounced than at any site reported previously for this BLM analogue.

Results

Sequence-Selective Degradation of 10 Hairpin DNAs by Fe(II)·BLM A_5 and Fe(II)·deglycoBLM A_5: Frank Strand Scission Pathway. As described previously, a 64-nucleotide DNA hairpin library was prepared to define a set of DNAs with high avidity for BLM and permit analysis of the relationship between high affinity DNA binding sites and site selective DNA degradation.[42] This was done by first synthesizing a 41-nucleotide DNA substrate, which contained an 8-nucleotide randomized sequence in positions 11−18 and which contained a self-complementary region within nucleotides 24−41 that would be expected to form a (partial) DNA hairpin. The remaining 23 bases were added to the DNA substrate via the action of DNA polymerase (Klenow fragment, which also assured the complementarity of the newly created base pairs). To select for sequences that bound strongly to BLM A_5, the mixture of hairpins was incubated with resin-bound BLM A_5 and was then washed with buffer to remove unbound DNAs.[41,42] This procedure permitted the selection of 10 hairpin DNAs whose sequences were defined and which were subsequently chemically synthesized for biochemical characterization.[41,42] The ability of BLM A_5 and deglycoBLM A_5 to cleave members of this library has currently been examined to characterize the nature of that cleavage in detail and to determine whether the presence of the BLM sugar moiety had an effect on cleavage efficiency and site selectivity. All of the hairpin DNAs examined (Table 1) proved to be good substrates for Fe(II)·BLM A_5-mediated degradation. With the exceptions of hairpin DNAs **2**, **7**, and **10**, these hairpin DNAs were also cleaved efficiently by Fe(II)·deglycoBLM A_5. In all previous studies, Fe(II)·deglycoBLM exhibited lower cleavage efficiency than that observed for Fe(II)·BLM, even when used at somewhat higher concentrations than Fe(II)·BLM.[20,38,43] That was not uniformly true in the present study.

Table 1. Nucleotide Sequences of 10 Hairpin DNAs

5′TTTAATTAAT--------AAAAAATTTTAA$^{C}_{C}$ 3′AAATTAATTA--------TTTTTTAAAATT$_{C}^{C}$	
5′AGATCATG 3′TCTAGTAC **DNA 1**	5′CTACTAAA 3′GATGATTT **DNA 6**
5′CGTGACGC 3′GCACTGCG **DNA 2**	5′TACGCGCA 3′ATGCGCGT **DNA 7**
5′TAAGTGGG 3′ATTCACCC **DNA 3**	5′GGGTACCT 3′CCCATGGA **DNA 8**
5′GAGAGGAT 3′CTCTCCTA **DNA 4**	5′CGTTGTTA 3′GCAACAAT **DNA 9**
5′ACAGAATA 3′TGTCTTAT **DNA 5**	5′CGCCATTG 3′GCGGTAAC **DNA 10**

For hairpin DNA **1**, nine sites of cleavage were noted both for Fe(II)·BLM A_5 and for Fe(II)·deglycoBLM A_5 (Figures 2 and 3), albeit not with the same relative intensities. Only two of these sites (5′-GA_{13}-3′ and 5′-GA_{19}-3′) involved a sequence that appears with reasonable frequency when arbitrarily selected DNA sequences are cleaved with an excess of Fe(II)·BLM,[44] and cleavage at these sites was not particularly strong relative to the other sites, especially for Fe(II)·deglycoBLM. Interestingly, Fe(II)·BLM A_5 and Fe(II)·deglycoBLM A_5 both cleaved the hairpin DNA at five sites outside of the 8-nt randomized region.

Hairpin DNA **2** was cleaved relatively weakly by both Fe(II)·BLM A_5 and Fe(II)·deglycoBLM A_5 (Figure 3; Supporting Information, Figure S1). The strongest cleavage sites produced both by BLM A_5 and by deglycoBLM A_5 were observed in the randomized region of the hairpin DNA (Figure 3). In this case, the strongest cleavage sites were observed at 5′-GT_{13}-3′ and 5′-GC_{18}-3′, while all other bands observed were quite weak.

(38) 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.
(39) (a) Wu, W.; Vanderwall, D. E.; Teramoto, S.; Lui, S. M.; Hoehn, S.; Tang, X.-J.; Turner, C. J.; Boger, D. L.; Kozarich, J. W.; Stubbe, J. *J. Am. Chem. Soc.* **1998**, *120*, 2239–2250. (b) Ma, Q.; Xu, Z.; Schroeder, B. R.; Sun, W.; Wei, F.; Hashimoto, S.; Konishi, K.; Leitheiser, C. J.; Hecht, S. M. *J. Am. Chem. Soc.* **2007**, *129*, 12439–12452.
(40) (a) McLean, M. J.; Waring, M. J. *J. Mol. Recognit.* **1989**, *1*, 184–192. (b) Keck, M. V.; Manderville, R. A.; Hecht, S. M. *J. Am. Chem. Soc.* **2001**, *123*, 8690–8700. (c) Chen, J.; Ghorai, M. K.; Kenney, G.; Stubbe, J. *Nucleic Acids Res.* **2008**, *36*, 3781–3790.
(41) Akiyama, Y.; Ma, Q.; Edgar, E.; Laikhter, A.; Hecht, S. M. *J. Am. Chem. Soc.* **2008**, *130*, 9650–9651.
(42) Ma, Q.; Akiyama, Y.; Xu, Z.; Konishi, K.; Hecht, S. M. *J. Am. Chem. Soc.* **2009**, *131*, 2013–2022.
(43) Sugiyama, H.; Ehrenfeld, G. M.; Shipley, J. B.; Kilkuskie, R. E.; Chang, L.-H.; Hecht, S. M. *J. Nat. Prod.* **1985**, *48*, 869–877.
(44) (a) Takeshita, M.; Kappen, L. S.; Grollman, A. P.; Eisenberg, M.; Goldberg, I. H. *Biochemistry* **1981**, *20*, 7599–7606. (b) Mirabelli, C. K.; Ting, A.; Huang, C. H.; Mong, S.; Crooke, S. T. *Cancer Res.* **1982**, *42*, 2779–2785. (c) Kross, J.; Henner, W. D.; Hecht, S. M.; Haseltine, W. A. *Biochemistry* **1982**, *21*, 4310–4318. (d) Shipley, J. B.; Hecht, S. M. *Chem. Res. Toxicol.* **1988**, *1*, 25–27.

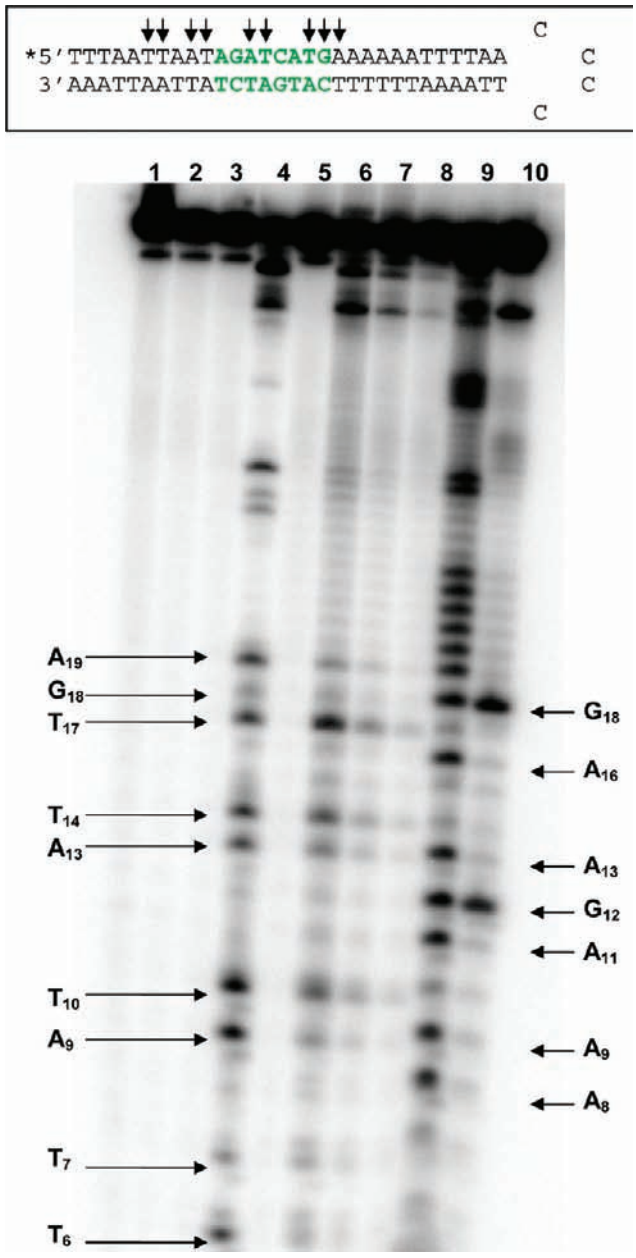

Figure 2. Sequence-selective cleavage of [5′γ-^{32}P] end-labeled DNA **1** by BLM A_5 and deglycoBLM A_5. Lane 1, radiolabeled **1** alone; lane 2, 5 μM Fe^{2+}; lane 3, 5 μM BLM A_5; lane 4, 5 μM Fe(II)·BLM A_5; lane 5, 20 μM deglycoBLM A_5; lane 6, 20 μM Fe(II)·deglycoBLM A_5; lane 7, 15 μM Fe(II)·deglycoBLM A_5; lane 8, 10 μM Fe(II)·deglycoBLM A_5; lane 9, G + A lane; lane 10, G lane.

Treatment of hairpin DNA **3** with Fe(II)·BLM A_5 produced 10 cleavage bands, with the strongest cleavage observed at 5′-GT_{15}-3′ (Supporting Information, Figure S2; summarized in Figure 3). The pattern was quite similar for Fe(II)·deglycoBLM A_5, albeit rather weak at a few positions. A total of 10 sites were observed when hairpin DNA **4** was treated with Fe(II)·BLM A_5, while only six sites were apparent following treatment of the same hairpin DNA with Fe(II)·deglycoBLM A_5 (Figure 3), and all of these were much less pronounced. The strongest cleavage produced by both compounds was at 5′-GA_{12}-3′ (Supporting Information, Figure S3). However, the cleavage efficiency at 5′-AT_{18}-3′ by Fe(II)·BLM A_5 was comparable to that of cleavage at 5′-GA_{17}-3′. While the cleavage at 5′-GT_{12}-3′ by Fe(II)·BLM A_5 was noticeably more efficient than that at 5′-GA_{14}-3′, Fe(II)·deglycoBLM A_5 produced (weaker) bands of comparable efficiency at both of these 5′-GA-3′ sites.

Hairpin DNA **5** was a very efficient substrate for BLM, affording 10 strong cleavage bands and four weak cleavage bands following treatment with Fe(II)·BLM A_5. In contrast, Fe(II)·deglycoBLM A_5 produced only two strong cleavage bands with the remaining 10 bands showing only weak intensity (Figure 3). Interestingly, T_{17}, which was one of the strongest cleavage sites for BLM A_5, was one of the weaker sites for deglycoBLM A_5 (Supporting Information, Figure S4).

Hairpin DNA **6** lacked the 5′-GT-3′ and 5′-GC-3′ sequences (within nucleotides 11−18) commonly cleaved by Fe(II)·BLM. Nonetheless, four strong cleavage bands were observed following treatment with Fe(II)·BLM A_5 in addition to four weaker bands. Three strong bands were noted following treatment with Fe(II)·deglycoBLM A_5 as well as four weaker bands (Supporting Information, Figure S5; summarized in Figure 3). DNA **7**, which contained two 5′-GC-3′ sequences within nucleotides 11−18 (Figure 3), was nonetheless a poor substrate for Fe(II)·deglycoBLM A_5 which produced only four cleavage bands, two of which were quite weak. All of the cleavage sites were within the randomized region of the hairpin DNA (Supporting Information, Figure S6). In comparison, Fe(II)·BLM A_5 cleaved hairpin DNA **7** at a total of 11 sites (Figure 3). Further, while Fe(II)·BLM A_5 cleaved the hairpin DNA with much greater efficiency at 5′-GC_{17}-3′ than at 5′-GC_{15}-3′, Fe(II)·deglycoBLM A_5 cleaved the DNA with comparable (albeit lesser) efficiency at both sites.

Fe(II)·deglycoBLM A_5 produced a total of 12 cleavage sites when hairpin DNA **8** was used as a substrate. However, only one site (5′-GT_{14}-3′) was cleaved strongly (Supporting Information, Figure S7). Fe(II)·BLM A_5 also cleaved hairpin DNA **8** at the same 12 sites, albeit with seven efficient sites (5′-AA_5-3′, 5′-AT_6-3′, 5′-TT_7-3′, 5′-AA_9-3′, 5′-AT_{10}-3′, 5′-GG_{12}-3′, and 5′-GT_{14}-3′) (Figure 3). Hairpin DNA **9**, which has two 5′-GT-3′ sites, was cleaved only in the randomized region of the hairpin DNA by Fe(II)·deglycoBLM A_5 (strongly at 5′-GT_{13}-3′ and 5′-GT_{15}-3′, and weakly at 5′-TT_{14}-3′). In contrast, Fe(II)·BLM A_5 produced a total of eight cleavage bands with five sites lying outside the randomized region (Supporting Information, Figure S8; summarized in Figure 3). Three of these five bands were strong. The three cleavage bands produced by Fe(II)·BLM A_5 within the randomized region were at the same sites as those produced by Fe(II)·deglycoBLM A_5, and the relative intensities of the three bands at these sites were similar for the two compounds (although the absolute amount of cleavage was much greater for Fe(II)·BLM A_5).

Hairpin DNA **10** was a poor substrate for Fe(II)·deglycoBLM A_5, which produced one strong cleavage band at 5′-GC_{13}-3′ and five weak cleavage bands. Fe(II)·BLM A_5 cleaved this substrate at 10 positions, with three strong bands at 5′-AT_6-3′, 5′-GC_{13}-3′, and 5′-GA_{19}-3′ (Supporting Information, Figure S9; summarized in Figure 3).

It is interesting that a number of 5′-AA-3′, 5′-AT-3′, and 5′-TA-3′ cleavage sites were produced by treatment with Fe(II)·BLM A_5 and Fe(II)·deglycoBLM A_5. However, these sites were less common for deglycoBLM A_5 in part because deglycoBLM showed a preference for the randomized regions of the DNA hairpins where these sequences were less abundant.

Sequence-Selective Degradation of 10 Hairpin DNAs by Fe(II)·BLM A_5 and Fe(II)·deglycoBLM A_5: Alkali Labile Pathway. To investigate the alkali labile pathway, all 10 hairpin DNAs were subjected to treatment with 0.2 M *n*-butylamine

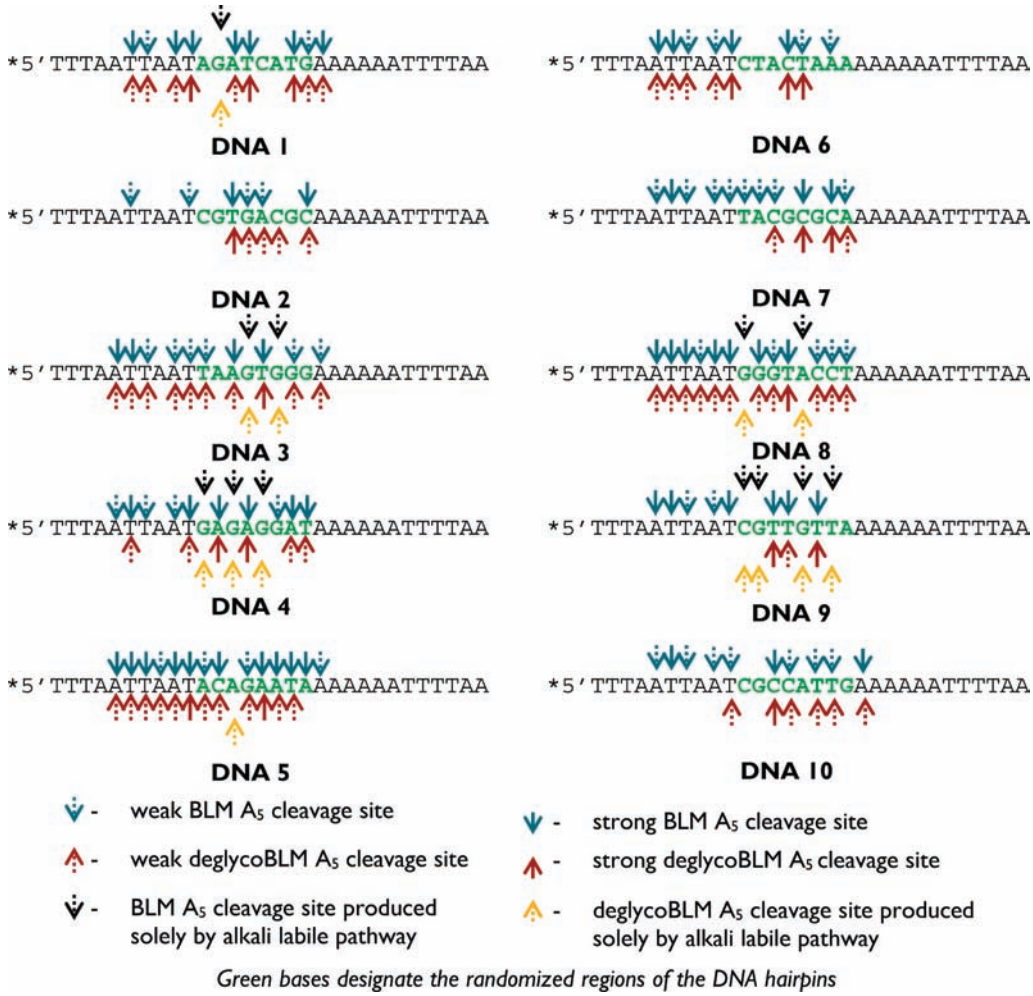

Figure 3. Summary of sequence selectivity of hairpin DNA degradation by Fe(II)·BLM A_5 and Fe(II)·deglycoBLM A_5. Green bases indicate regions of the DNA hairpins that were randomized in the original library.

subsequent to treatment with Fe·BLM A_5 or Fe·deglycoBLM A_5.[34,45] Enhancement of numerous cleavage bands was observed for all 10 hairpin DNAs, as expected. However, new cleavage bands not present after Fe·BLM A_5 or Fe·deglycoBLM A_5 treatment alone were also observed for several of the hairpin DNAs. New cleavage sites produced by unmasking alkali labile lesions were observed for hairpin DNAs **1**, **3**, **4**, **5**, **8**, and **9**.

Treatment of hairpin DNA **1** with *n*-butylamine resulted in one additional cleavage band at 5′-AG_{12}-3′, as observed in lanes 5 and 8 (Supporting Information, Figure S10). Treatment of hairpin DNA **3** revealed two alkali labile cleavage sites (at 5′-AG_{14}-3′ and 5′-TG_{16}-3′) (Figure 4). Both of these sites were clearly evident following Fe(II)·deglycoBLM A_5 treatment; however, they were weaker following Fe(II)·BLM A_5 treatment. Additionally, the alkali labile lesion band produced at T_{15} increased 2.5-fold in intensity following Fe(II)·deglycoBLM A_5 treatment; Fe(II)·BLM A_5 produced only a slight increase in cleavage intensity at T_{15}.

Three new cleavage bands (at 5′-TG_{11}-3′, 5′-AG_{13}-3′, and 5′-AG_{15}-3′) resulting from alkali labile lesions were evident when hairpin DNA **4** was treated with Fe(II)·BLM A_5 or Fe(II)·deglyco BLM A_5, and then with *n*-butylamine (Figure 5). Another interesting observation was found at the T_6 cleavage site where the two bands seen correspond to the frank strand scission product (lower band) and the alkali labile lesion (upper band), the latter of which is normally produced abundantly either at low oxygen tension or when BLM cleavage products are treated with *n*-butylamine.[31,33−35] In accordance with previous findings, prior to treatment with *n*-butylamine, the ratio of these products was 3:1 favoring frank strand scission. However, after treatment with *n*-butylamine the ratios were nearly 1:1.[34,45,46] This observation was common in most of the hairpin DNAs studied specifically at the T_6 position.

DNA hairpin **5** was the only oligonucleotide that produced an additional cleavage site (at 5′-CA_{13}-3′), albeit weak, solely following deglycoBLM A_5 (Figure 6). Strong enhancement of alkali labile bands following *n*-butylamine treatment was noted at 5′-AT_6-3′, 5′-AT_{10}-3′, and 5′-AC_{12}-3′. Treatment of DNA **8** with Fe(II)·BLM A_5 or Fe(II)·deglycoBLM A_5, and then *n*-butylamine, produced a cleavage band at 5′-TA_{15}-3′ that was not apparent in the absence of *n*-butylamine and a second faint band at 5′-TG_{11}-3′ (Figure 7). Also notable in this figure is the enhancement of the bands due to alkali labile lesions at T_6 and T_{10} following *n*-butylamine treatment. Additional bands were observed when DNA **9** was subjected to treatment with *n*-butylamine following treatment with Fe(II)·BLM A_5 or Fe(II)·deglyco BLM A_5 (Figure 8). Treatment with *n*-butylamine produced three additional cleavage bands for both

(45) Aso, M.; Kondo, M.; Suemune, H.; Hecht, S. M. *J. Am. Chem. Soc.* **1999**, *121*, 9023–9033.

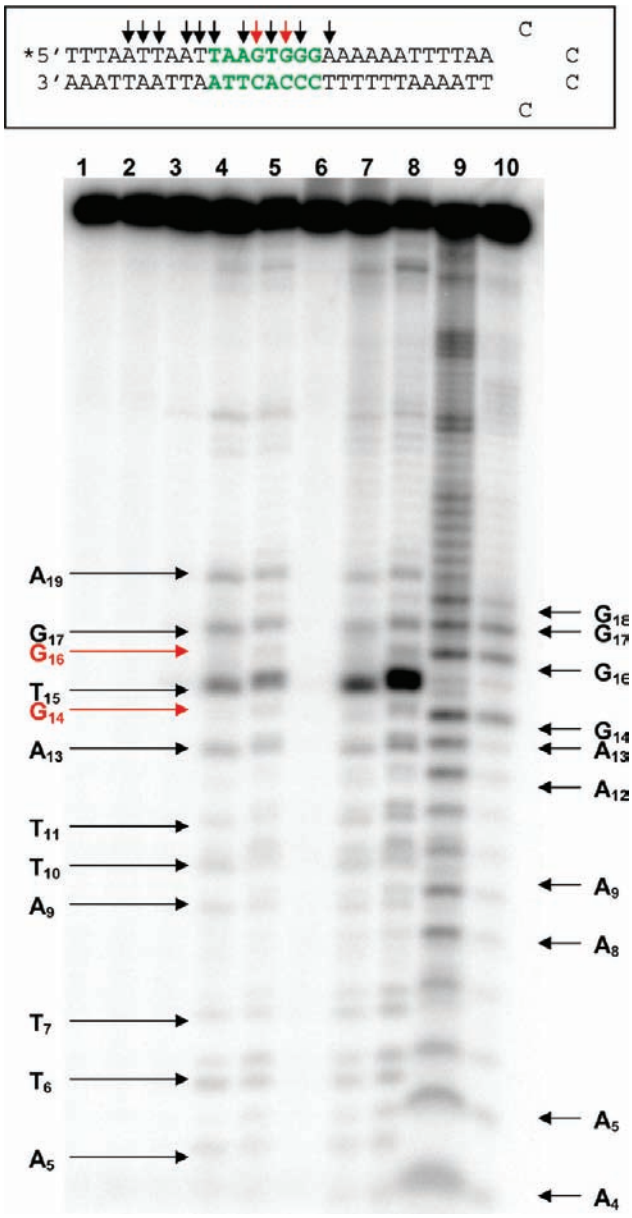

Figure 4. Sequence-selective cleavage of [5′γ-^{32}P] end-labeled DNA **3** by BLM A_5 and deglycoBLM A_5 followed by *n*-butylamine treatment. Lane 1, DNA alone; lane 2, 5 μM Fe^{2+}; lane 3, 5 μM BLM A_5; lane 4, 5 μM Fe(II)·BLM A_5; lane 5, 5 μM Fe(II)·BLM A_5, followed by treatment with 0.2 M *n*-butylamine; lane 6, 20 μM deglycoBLM A_5; lane 7, 20 μM Fe(II)·deglycoBLM A_5; lane 8, 20 μM Fe(II)·deglycoBLM A_5, followed by treatment with 0.2 M *n*-butylamine; lane 9, G + A lane; lane 10, G lane. (Alkali labile) cleavage sites not apparent in the absence of *n*-butylamine treatment are noted in red.

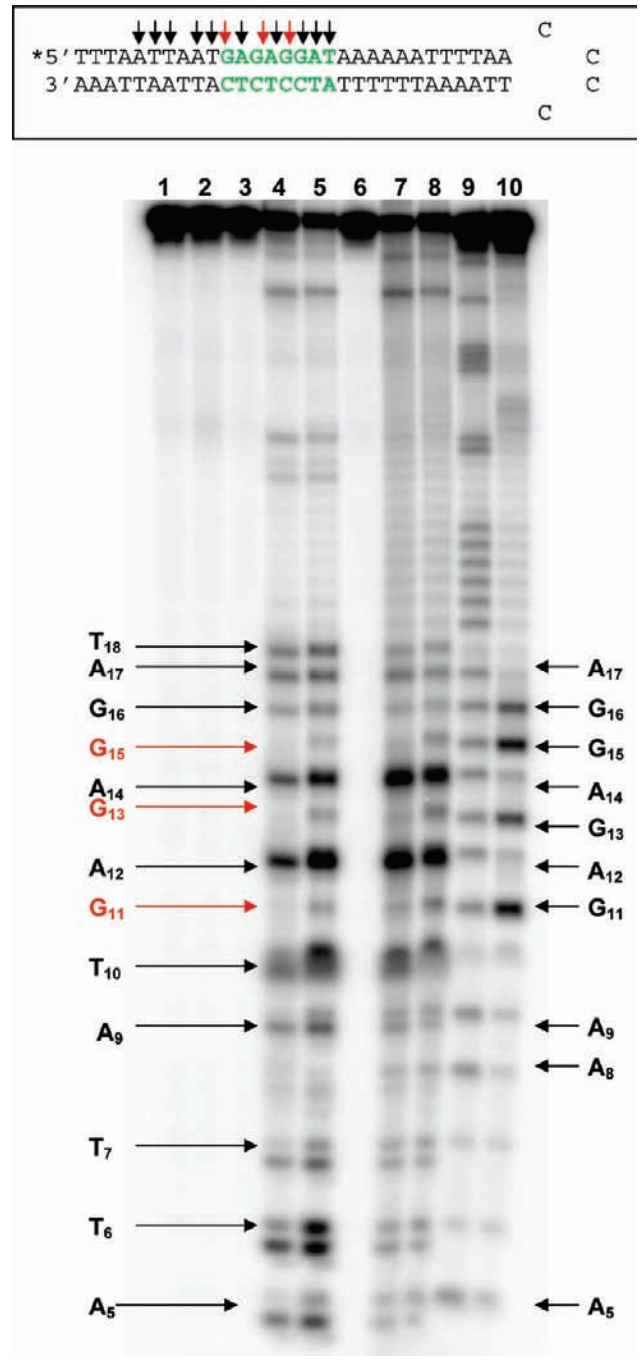

Figure 5. Sequence-selective cleavage of [5′γ-^{32}P] end-labeled DNA **4** by BLM A_5 and deglycoBLM A_5, followed by *n*-butylamine treatment. Lane 1, DNA alone; lane 2, 5 μM Fe^{2+}; lane 3, 5 μM BLM A_5; lane 4, 5 μM Fe(II)·BLM A_5; lane 5, 5 μM Fe(II)·BLM A_5, followed by treatment with 0.2 M *n*-butylamine; lane 6, 20 μM deglycoBLM A_5; lane 7, 20 μM Fe(II)·deglycoBLM A_5; lane 8, 20 μM Fe(II)·deglycoBLM A_5, followed by treatment with 0.2 M *n*-butylamine; lane 9, G + A lane; lane 10, G lane. New alkali labile cleavage sites are noted in red.

compounds at 5′-TT_{17}-3′, 5′-TG_{15}-3′, and 5′-TC_{11}-3′. Deglycoblm A_5 produced an alkali labile cleavage site at 5′-CG_{12}-3′, which was only weakly observable following BLM A_5 treatment. Again, the alkali labile bands for T_6 and T_{10} were strongly enhanced following treatment with *n*-butylamine.

Thus, a total of 13 sites were noted in hairpin DNAs **1**, **3**, **4**, **5**, **8**, and **9** after treatment with a BLM + *n*-butylamine, but not in the absence of *n*-butylamine. The exclusive formation of alkali labile lesions in the absence of frank strand scission has not been reported previously. It may also be noted that nine of these sites involved guanine nucleotides, which are rarely substrates for oxidative cleavage by Fe·BLM in randomly chosen DNAs.

Discussion

Several earlier studies have shown that hairpin DNAs can be cleaved quite efficiently by metalloBLMs.[40b,41,42,47] To probe the relationship between DNA binding and efficient BLM cleavage, a 64-nucleotide DNA hairpin library with an eight

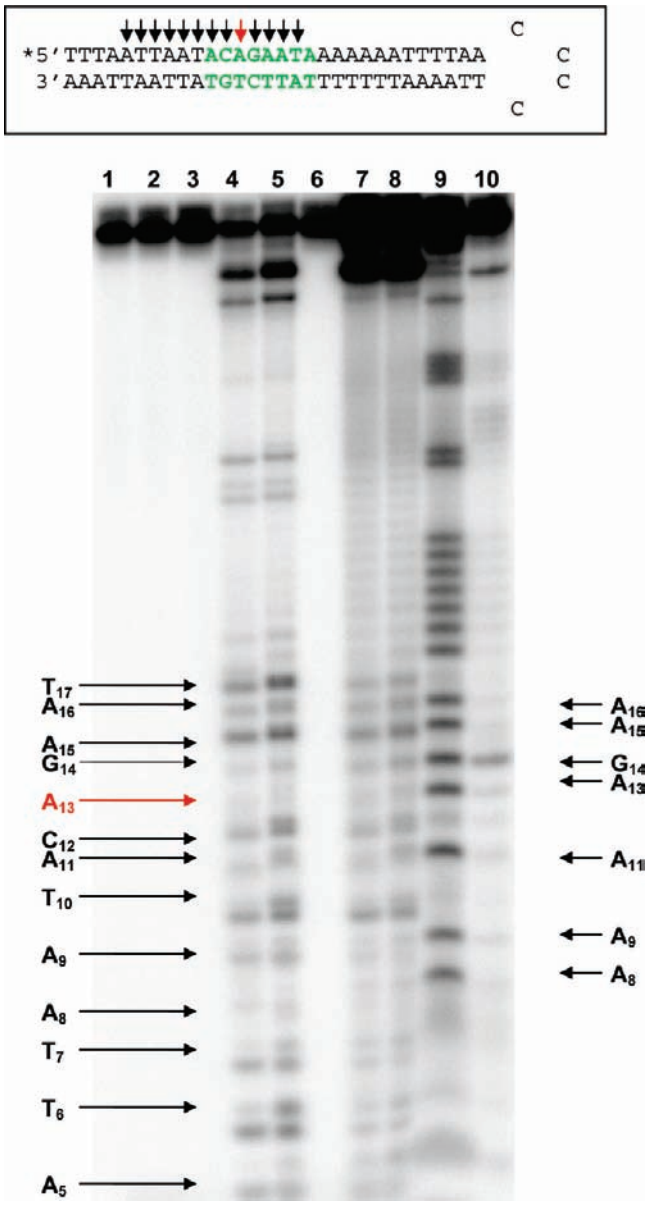

Figure 6. Sequence-selective cleavage of [5′γ-^{32}P] end-labeled hairpin DNA **5** by BLM A_5 and deglycoBLM A_5, followed by *n*-butylamine treatment. Lane 1, DNA alone; lane 2, 5 μM Fe^{2+}; lane 3, 5 μM BLM A_5; lane 4, 5 μM Fe(II)·BLM A_5; lane 5, 5 μM Fe(II)·BLM A_5, followed by treatment with 0.2 M *n*-butylamine; lane 6, 20 μM deglycoBLM A_5; lane 7, 20 μM Fe(II)·deglycoBLM A_5; lane 8, 20 μM Fe(II)·deglycoBLM A_5, followed by treatment with 0.2 M *n*-butylamine; lane 9, G + A lane; lane 10, G lane. A new alkali labile cleavage site is noted in red.

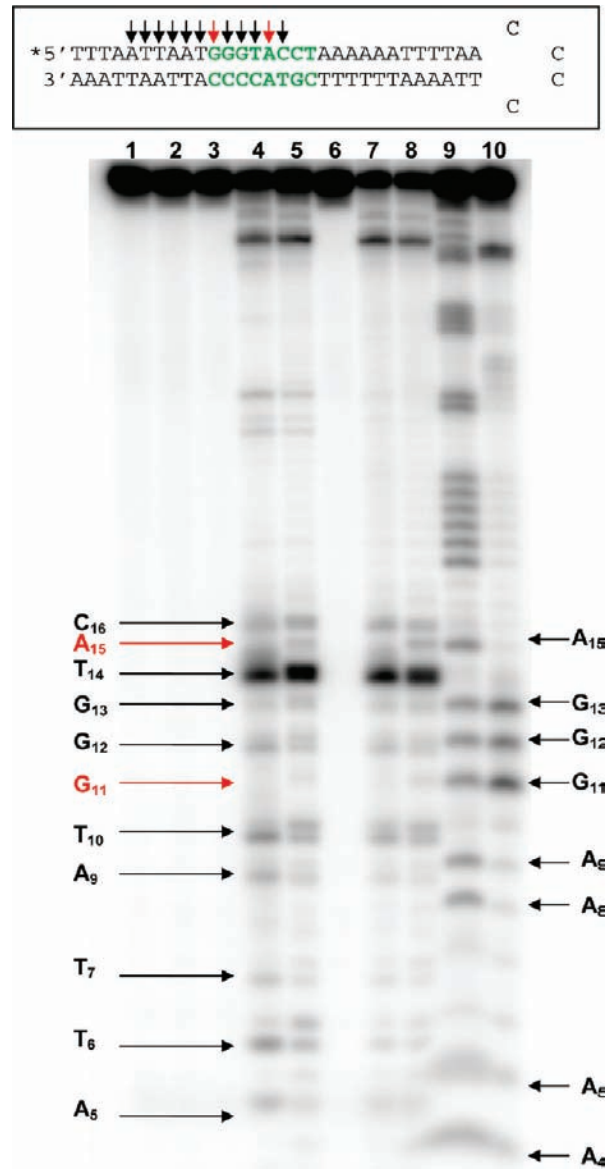

Figure 7. Sequence-selective cleavage of [5′γ-^{32}P] end-labeled DNA **8** by BLM A_5 and deglycoBLM A_5 followed by *n*-butylamine treatment. Lane 1, DNA alone; lane 2, 5 μM Fe^{2+}; lane 3, 5 μM BLM A_5; lane 4, 5 μM Fe(II)·BLM A_5; lane 5, 5 μM Fe(II)·BLM A_5, followed by treatment with 0.2 M *n*-butylamine; lane 6, 20 μM deglycoBLM A_5; lane 7, 20 μM Fe(II)·deglycoBLM A_5; lane 8, 20 μM Fe(II)·deglycoBLM A_5, followed by treatment with 0.2 M *n*-butylamine; lane 9, G + A lane; lane 10, G lane. New alkali labile cleavage sites are noted in red.

base pair randomized region was recently prepared. Of the 4^8 (65 536) different sequences potentially generated by this procedure, 10 hairpin DNAs, which had bound BLM A_5 quite strongly, were selected for further study.[41,42] In the early study, only four of the 10 hairpin DNAs were studied for their DNA cleavage characteristics. Presently all 10 were treated with Fe(II)·BLM A_5 and deglycoBLM A_5, in the absence and presence of *n*-butylamine. These 10 hairpin DNAs were used to analyze the structural elements in the hairpin DNAs, which contributed to efficient DNA binding and cleavage. Because the rate-limiting step in BLM-mediated degradation of DNA is the abstraction of the C-4′ H,[37b] and not the binding of BLM to DNA per se, it seemed plausible that DNAs selected for tight binding to BLM might exhibit diminished off-rates of Fe·BLM from the formed Fe·BLM−DNA complexes. If this proved to be true, it might enable other relatively slow chemical processes not normally observed. While there is presently little direct experimental evidence regarding the dynamics of Fe·BLM binding to DNA, the issue is clearly worthy of investigation.

DNA cleavage by the BLMs has been studied extensively and shown to be sequence selective, occurring primarily at 5′-GT-3′ and 5′-GC-3′ sequences.[46−49] Of the 10 hairpin DNAs examined, DNAs **2**, **3**, and **7**−**10** contained 5′-GC-3′ or 5′-GT-3′ sequences within nucleotides 11−18 with hairpin DNA **2** containing both of these sequences. In all of the hairpin DNAs, these sites proved to include the strongest cleavage bands for Fe(II)·BLM A_5 and generally included the strongest for Fe(II)·deglycoBLM A_5 as well (Figure 3). Treatment of DNA **2** produced two strong cleavage bands using Fe(II)·BLM A_5 at

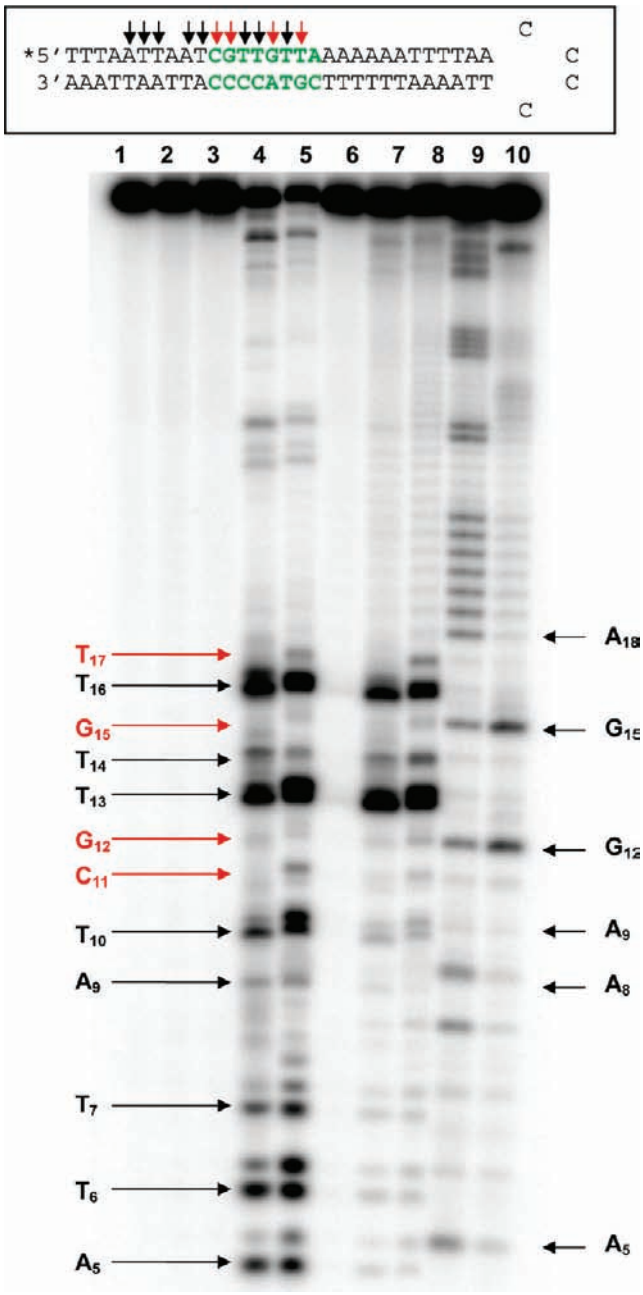

Figure 8. Sequence-selective cleavage of [5′γ-^{32}P] end-labeled DNA **9** by BLM A_5 and deglycoBLM A_5 followed by *n*-butylamine treatment. Lane 1, DNA alone; lane 2, 5 μM Fe^{2+}; lane 3, 5 μM BLM A_5; lane 4, 5 μM Fe(II)·BLM A_5; lane 5, 5 μM Fe(II)·BLM A_5, followed by treatment with 0.2 M *n*-butylamine; lane 6, 20 μM deglycoBLM A_5; lane 7, 20 μM Fe(II)·deglycoBLM A_5; lane 8, 20 μM Fe(II)·deglycoBLM A_5, followed by treatment with 0.2 M *n*-butylamine; lane 9, G + A lane; lane 10, G lane. New alkali labile cleavage sites are noted in red.

5′-GT_{13}-3′ and 5′-GC_{18}-3′; however, only 5′-GT_{13}-3′ proved to be a strong cleavage site for Fe(II)·deglycoBLM A_5. The 5′-GT_{15}-3′ sequence in hairpin DNA **3** was the only strong cleavage site in the randomized region for Fe(II)·deglycoBLM A_5, while Fe(II)·BLM A_5 showed an additional strong cleavage band at the 5′-AA_{13}-3′ sequence. The two 5′-GC-3′ cleavage sites in hairpin DNA **7** were the only strong cleavage sites both for Fe(II)·BLM A_5 and for Fe(II)·deglycoBLM A_5. Hairpin DNA **8** had a 5′-GT_{14}-3′ sequence, which was the only strong cleavage site in the randomized region for both Fe·BLMs examined. Hairpin DNA **9** contained two 5′-GT-3′ sequences, which were the sole strong cleavage sites for Fe(II)·deglycoBLM A_5. Additionally, Fe(II)·BLM A_5 also cleaved a third site in the randomized region of hairpin DNA **9**, 5′-TT_{14}-3′, which immediately followed a 5′-GT-3′ sequence. Hairpin DNA **10** had only one strong cleavage band in the randomized region, at 5′-GC_{13}-3′, for both Fe·BLMs studied.

The remaining hairpin DNAs lacked the cleavage sites preferred by BLM in randomly chosen DNAs; nonetheless, strong cleavage bands were observed for Fe·BLMs and involved a number of unusual cleavage sites (Figure 3). Hairpin DNA **1** produced strong bands at two 5′-AT-3′ sites located in the randomized region for both Fe(II)·BLM A_5 and Fe(II)·deglycoBLM A_5 and an additional strong band at the 5′-GA_{19}-3′ sequence for Fe(II)·BLM A_5. In the randomized region of hairpin DNA **4**, the two 5′-GA-3′ sequences were cleaved strongly by Fe(II)·deglycoBLM A_5. In addition to these two sites, Fe(II)·BLM A_5 also produced a strong band at a third 5′-GA-3′ sequence and at the 5′-AT_{18}-3′ sequence. Hairpin DNA **5** also contained a 5′-GA-3′ sequence in the randomized region, which was the sole strong site for cleavage by Fe(II)·deglycoBLM A_5. Fe(II)·BLM A_5 also mediated strong cleavage at 5′-AC-3′, 5′-AA-3′, 5′-AT-3′, and 5′-TA-3′ sequences. The 5′-AC-3′ sequence afforded reasonably strong bands following treatment with Fe(II)·BLM A_5 and Fe(II)·deglycoBLM A_5.

Both Fe·BLMs also produced a number of weaker cleavage bands in the randomized region of the hairpin DNAs, most of these occurring at unusual DNA sequences. Interestingly, cleavage was also observed in the nonrandomized region of the hairpin DNAs. Fe(II)·BLM A_5 showed a strong preference for cleavage at 5′-AT_6-3′, where it produced a strong cleavage band in all of the hairpin DNAs except hairpin DNA **2**. In general, Fe(II)·deglycoBLM A_5 failed to produce strong cleavage in the nonrandomized regions of the hairpin DNAs; however, numerous weak cleavage bands were observed.

The two most abundant cleavage sites produced by Fe(II)·BLM A_5 and Fe(II)·deglycoBLM A_5, observed in all hairpin DNAs tested, were 5′-AT-3′ and 5′-AA-3′ followed closely by 5′-TT-3′. Because these sites were not cleaved as efficiently as the 5′-GT-3′ and 5′-GA-3′ sites, this observation may suggest that these sequences are important recognition motifs for BLM binding. This concept is consonant with previous findings that bithiazoles and metalloBLMs have the ability to bind to AT-rich regions of DNA.[14,40a,48,49]

Previous studies have shown that the absence of the BLM sugar moiety produces only relatively subtle effects on DNA cleavage patterns;[38,43] however, it does result in a decreased cleavage efficiency and a decreased efficiency of double-strand DNA breaks.[19,39] This was also generally apparent when comparing the hairpin DNA cleavage produced by Fe(II)·BLM A_5 and Fe(II)·deglycoBLM A_5 although there were a few significant exceptions. Some cleavage bands produced by Fe(II)·deglycoBLM A_5 showed stronger intensity than Fe(II)·BLM A_5 at concentrations of compound optimal for each BLM. For

(46) Burger, R. M. *Chem. Rev.* **1998**, *98*, 1153–1169.
(47) (a) Absalon, M. J.; Kozarich, J. W.; Stubbe, J. *Biochemistry* **1995**, *34*, 2065–2075. (b) Absalon, M. J.; Wu, W.; Kozarich, J. W.; Stubbe, J. *Biochemistry* **1995**, *34*, 2076–2086. (c) Hashimoto, S.; Wang, B.; Hecht, S. M. *J. Am. Chem. Soc.* **2001**, *123*, 7437–7438. (d) Akiyama, Y.; Ma, Q.; Edgar, E.; Laikhter, A.; Hecht, S. M. *Org. Lett.* **2008**, *10*, 2127–2130.
(48) Quada, J. C.; Zuber, G. F.; Hecht, S. M. *Pure Appl. Chem.* **1998**, *70*, 307–311.
(49) Li, W.; Zhao, C.; Xia, C.; Antholine, W. E.; Petering, D. H. *Biochemistry* **2001**, *40*, 7559–7568.

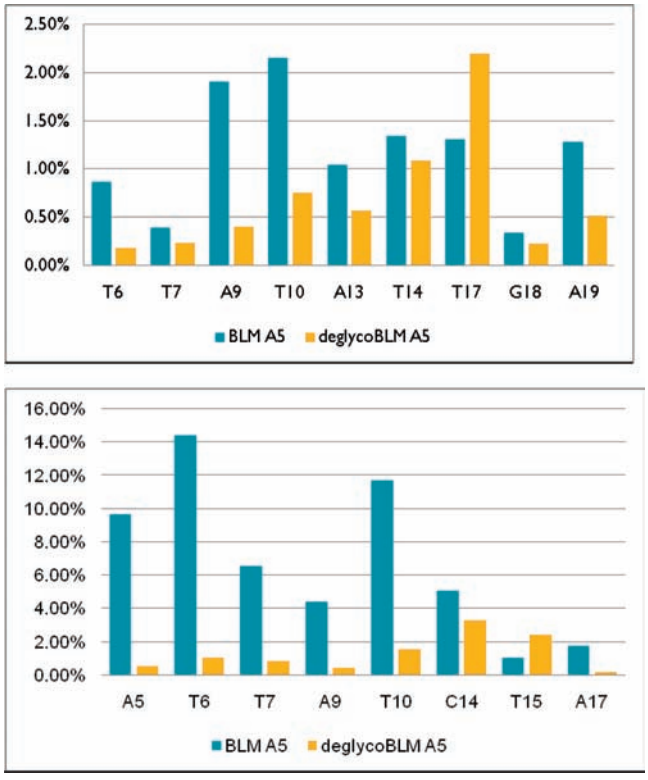

Figure 9. Relative percent cleavage for Fe(II)·BLM A_5 and Fe(II)·deglycoBLM A_5 at designated bases. (top) hairpin DNA **1**; (bottom) hairpin DNA **6**.

example, when comparing relative cleavage using densitometric analyses, it was found that cleavage of hairpin DNA **1** by Fe(II)·deglycoBLM A_5 at site T_{17} was 1.7 times greater than cleavage by Fe(II)·BLM A_5 (Figure 9A). Cleavage at 5′-AT_{14}-3′ was also enhanced for Fe(II)·deglycoBLM A_5 relative to the other cleavage sites. Comparison of Fe(II)·BLM A_5 and Fe(II)·deglycoBLM A_5 cleavage at 5′-CT_{15}-3′ of hairpin DNA **6** showed that the cleavage produced by Fe(II)·deglycoBLM A_5 at 5′-CT_{15}-3′ was 2.3 times greater than that produced by Fe(II)·BLM A_5 (Figure 9B), although neither cleaved the substrate strongly at that position. Cleavage at 5′-AC_{14}-3′ by deglycoBLM A_5 was also enhanced. It is interesting to note that hairpin DNA **2** was the only hairpin DNA for which both compounds produced strong cleavage only within the eight-nucleotide randomized region of the hairpin DNA. Because Fe(II)·deglycoBLM A_5 consistently showed a cleavage preference for this region of the hairpin DNAs, deglycoBLM A_5 may exhibit a greater binding selectivity for these regions of the chosen hairpin DNAs.

BLM-mediated cleavage of DNA can occur via two pathways. At ambient oxygen levels, DNA degradation occurs predominantly via the frank strand scission pathway, resulting in oligonucleotides having 5′-phosphate and 3′-phosphoroglycolate termini.[29,32] At low oxygen tension, degradation increasingly favors the release of the nucleobase at the site of the BLM-induced lesion with concomitant formation of an alkali labile lesion.[27,30,50,51] The alkali labile lesion does not lead directly to DNA strand scission, but the DNA containing the lesion can be cleaved by any of a number of different reagents including alkali,[30,32,33,50] alkylamines,[34,45] and hydrazine.[52]

To investigate the chemistry of hairpin DNA degradation, the selected hairpin DNAs were treated with *n*-butylamine following treatment with Fe(II)·BLM A_5 and Fe(II)·deglycoBLM A_5. All 10 hairpin DNAs showed evidence of alkali labile products. More interestingly, six of the hairpin DNAs (**1**, **3**, **4**, **5**, **8**, and **9**) produced additional cleavage products solely via the alkali labile pathway. Unlike the frank strand scission products from the hairpin DNAs, which most commonly involved 5′-AT-3′ and 5′-AA-3′ sequences, the exclusive alkali labile lesions were most common at 5′-TG-3′ and 5′-AG-3′ sequences.

Some polyacrylamide gel analyses showed very good resolution of BLM A_5 cleavage products near the 5′-end of the hairpin DNA, where it was possible to discern two bands for a single nucleotide. The upper band corresponds to oligonucleotide 3′-phosphate, while the lower band corresponds to the frank strand scission product. Prior to treatment with *n*-butylamine, the upper band was either absent or very weak, often in a 3:1 or greater ratio with the frank strand scission product being predominant. After treatment with *n*-butylamine, the upper band and lower band were present in approximately a 1:1 ratio, indicating the presence of the alkali labile lesion in quite significant quantities.[34,45] It proved difficult to perform these comparisons for Fe(II)·deglycoBLM A_5 because this BLM preferentially cleaved the hairpin DNAs in the randomized region and produced only very weak cleavage near the 5′-ends of the hairpin DNAs.

The use of hairpin DNAs having the ability to bind BLM A_5 strongly has provided insights into the relationship between DNA binding and cleavage by BLM. The patterns and intensities of cleavage bands observed in BLM- and deglycoBLM-mediated DNA degradation were not entirely consistent with the preferred 5′-GC-3′ and 5′-GT-3′ DNA cleavage patterns previously reported over a period of many years for Fe·BLM using randomly chosen DNAs as substrates. Of interest was the observation that deglycoBLM A_5 showed a preference for cleavage in the randomized region of the DNA hairpins, producing either very weak cleavage bands or failing altogether to cleave nucleotides near the 5′-end of the hairpin DNAs. Treatment with an Fe·BLM + *n*-butylamine afforded new cleavage bands in five of the hairpins examined. These studies show that a difference in both cleavage efficiency and cleavage selectivity is apparent for BLM A_5 and deglycoBLM A_5 in the case of some strongly bound DNAs.

Conclusions

Unlike studies of BLM-mediated DNA cleavage carried out with excess Fe(II)·BLM and arbitrarily chosen DNA substrates, the present study utilized hairpin DNAs selected for their ability to bind BLM strongly, more closely mimicking the situation likely to occur when the drug is employed as a therapeutic agent. Important observations included the presence of many sites of DNA cleavage not normally observed when using arbitrarily chosen DNA and the appearance of alkali labile lesions at sites that had not undergone frank strand scission. The BLM analogue deglycoBLM also showed somewhat different behavior from BLM using the hairpin DNA substrates, both with regard to the positions and relative intensities of cleavage. These results

(50) Wu, J. C.; Kozarich, J. W.; Stubbe, J. *J. Biol. Chem.* **1983**, *258*, 4694–4697.
(51) Uesugi, S.; Shida, T.; Ikehara, M.; Kobayashi, Y.; Kyogoku, Y. *Nucleic Acids Res.* **1984**, *12*, 1581–1592.
(52) Holmes, C. E.; Duff, R. J.; van der Marel, G. A.; van Boom, J.; Hecht, S. M. *Bioorg. Med. Chem.* **1997**, *5*, 1235–1248.

reinforce the initial study of strongly bound hairpin DNAs in establishing that BLM undergoes interactions with DNA rather differently from those described in the literature over a period of decades.

Experimental Procedures

Materials. Adenosine 5′-[γ-^{32}P]triphosphate was purchased from PerkinElmer Life and Analytical Sciences. T4 polynucleotide kinase was purchased from Fermentas Life Sciences. *n*-Butylamine and $Fe(SO_4)_2(NH_4)_2 \cdot 6H_2O$ were purchased from Sigma-Aldrich; the latter was used to prepare fresh aqueous solutions for admixture to BLM A_5 immediately prior to use. The hairpin DNAs used in this study were obtained as previously described.[41,42]

5′-^{32}P Radiolabeling of Hairpin DNA and Cleavage of the End-Labeled Hairpin DNA by Fe(II)·BLM A_5 and Fe(II)·deglycoBLM A_5. Briefly, the 64-nt hairpin DNA was [5′-^{32}P]-end-labeled with 5′-[γ-^{32}P]ATP and T4 polynucleotide kinase and purified by 16% polyacrylamide gel electrophoresis. The hairpin DNA cleavage reaction containing the 5′-^{32}P-labeled hairpin DNA (2×10^5 cpm, 0.5−5 μM) was carried out in 5 μL (total volume) of 10 mM sodium cacodylate, pH 8.0, containing different concentrations of Fe(II)·deglycoBLM A_5 and Fe(II)·BLM A_5 as noted in the figures. Each reaction was initiated by the simultaneous addition of BLM A_5 or deglycoBLM A_5 and equimolar $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (freshly prepared solution) to the buffered solution containing the hairpin DNA. Each reaction mixture was incubated at 37 °C for 30 min, and then the reaction mixture was dissolved in a gel loading buffer (98% formamide, 2 mM EDTA, 0.025% xylene FF, and 0.025% bromophenol blue). For the experiments examining the alkali labile reaction products, the reaction mixtures were then treated with 0.2 M *n*-butylamine at 90 °C for 12 min. The resulting solution was heated at 90 °C for 10 min and then analyzed by 16% polyacrylamide gel (31 cm × 38.5 cm × 0.4 cm) electrophoresis at 50 W for 2.5 h. The gel was analyzed using a phosphorimager.

Acknowledgment. This work was supported by NIH Research Grant CA140471, awarded by the National Cancer Institute.

Supporting Information Available: Polyacrylamide gel electrophoretic analyses of BLM-mediated cleavage of several of the hairpin DNAs studied. This material is available free of charge via the Internet at http://pubs.acs.org.

JA107228C