Interaction of Bleomycin with a Methylated DNA Oligonucleotide

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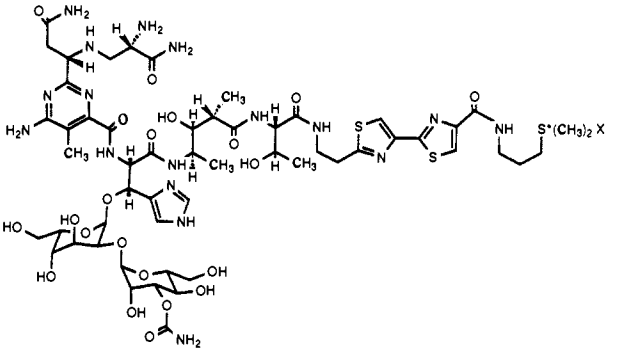
Abstract: The extent of DNA cleavage by Fe·bleomycin A_2 (Fe·BLM A_2) can be diminished substantially in proximity to (5-)methylated cytidine residues. This phenomenon has been attributed to limitations in the ability of certain bleomycin congeners to bind optimally to structurally altered DNA domains, such as those at or adjacent to a site of cytidine methylation, thus reducing reactivity toward bleomycin within those domains. However, diminution of strand scission has been limited to a subset of cleavage sites, generally has not been found to involve the methylated cytidine per se, and has been shown to function only with two BLM congeners. In order to examine the interaction of Fe·BLM A_2 with DNA at an isolated site of cytidine methylation at high resolution, and thereby determine whether any change can occur at the actual site of methylation, we have employed the self-complementary DNA oligonucleotide d(CGCTTTAAAG^{Me}CG) as a substrate. Fe·BLM A_2 was found to degrade this substrate as effectively as the respective nonmethylated dodecanucleotide, presumably reflecting comparable binding efficiencies for the two oligonucleotides. Two sets of chemical products were obtained when the methylated oligonucleotide was employed as a substrate; these paralleled the products observed when Fe·BLM A_2 was used for degradation of the nonmethylated dodecanucleotide d(CGCTTTAAAGCG). However, for the methylated dodecanucleotide, the products included a significantly larger proportion of alkali-labile lesions, as compared with actual strand scission. This finding establishes unequivocally that DNA methylation can affect the chemistry of DNA degradation by BLM even where no diminution of overall degradation occurs. Since both sets of products are believed to derive from a common C-4′ deoxyribose radical intermediate, these results suggest that the facility of some subsequent process is altered in the presence of a methylated cytidine moiety.

The bleomycins (BLMs) are a family of antitumor antibiotics that are thought to elicit their chemotherapeutic effects via degradation of chromosomal DNA.[1] Studies carried out in cell-free systems using isolated DNAs have indicated that DNA degradation involves metallobleomycins that are activated in the presence of dioxygen[2] and subsequently bind to and degrade substrate DNAs.[3] Bleomycin-mediated DNA degradation is sequence-selective, occurring predominantly at a subset of available 5′-GT-3′ and 5′-GC-3′ sequences.[4] Two types of products are formed when Fe·BLM degrades DNA. One of these results in DNA strand scission and the release of base propenals; the other does not involve DNA strand scission per se, but rather the formation of alkali-labile lesions with concomitant formation of free bases.[5]

An ongoing pursuit of this laboratory involves definition of the interaction of BLM with structurally altered DNAs,[6] especially where those alterations may bear relevance to the selectivity of bleomycin as a therapeutic agent. One such alteration involves the methylation of cytidine.[6c,d] DNA methylation is a means by which eukaryotic systems regulate, and in some instances inactivate, the expression of individual genes.[7] Aberrant gene expression, as observed in cancer cells, has also been correlated with altered[8] or lowered genomic methylation.[9] The finding that the extent of DNA methylation in cancer cells is often less than in their normal counterparts prompted us to study the effects of DNA methylation on the nature of DNA degradation by bleomycin.

We have reported previously[6c] that BLM-mediated DNA strand scission was diminished significantly in proximity to cytidine residues that had been methylated on the 5-position of the cytosine moiety by restriction methylases. More recently, the effects of modifying the C-terminal substituent of BLM were investigated; of the four BLM congeners studied, only BLM A_2 and one other congener demonstrated diminished degradation of DNAs in regions containing 5-methylcytidine,[6d] although all four exhibited diminished cleavage in proximity to N^6-methylated adenosines. An analysis of the structural factors in the methylated DNAs that led to diminished degradation by BLM suggested that conformational alteration of DNA structure in the regions containing



bleomycin A_2

5-methylcytidine had probably produced DNA substrates to which Fe·BLM A_2 bound with lowered affinity or in some alternative

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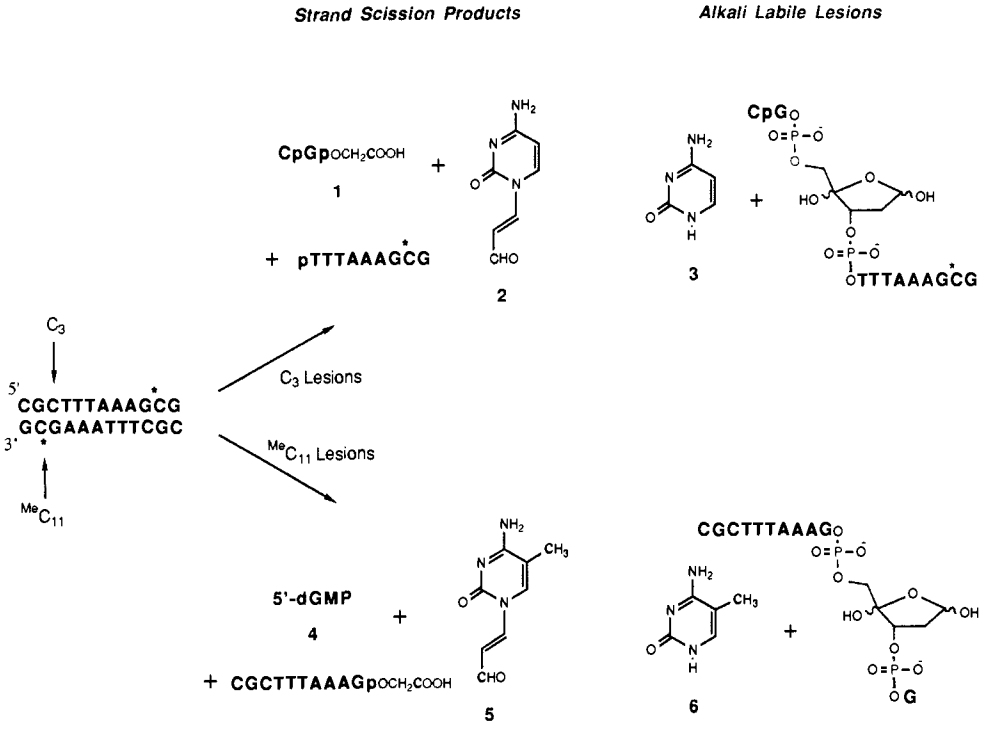
Scheme I. Products Resulting from the Degradation of d(CGCTTTAAAG^{Me}CG) by Fe(II)·BLM A_2 (*C Denotes 5-Methylcytidine)

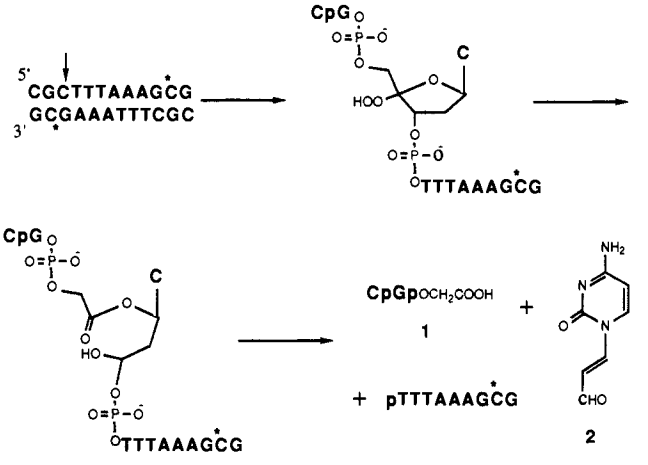


orientation that resulted in less DNA modification by BLM.

While the foregoing studies have contributed to an understanding of the way in which an important biochemical control mechanism may affect susceptibility to BLM-mediated DNA degradation, at a chemical level several issues require attention. For example, diminution of DNA strand scission is limited to a subset of cleavage sites and generally does not occur at the methylated cytidine per se. Only two BLM congeners have been found to exhibit diminished strand scission in response to cytidine methylation, and no detailed analysis of the chemistry of strand scission at a single, defined site has been reported.

In order to study the mechanism of BLM interaction with DNA at an *isolated* methylated cytidine at high resolution, we have employed the self-complementary oligonucleotide 5′-d-(CGCTTTAAAG^{Me}CG) as a substrate for Fe·BLM A_2. This oligonucleotide contains 5-methylcytidine at a position shown previously[10] to undergo highly efficient modification by Fe·BLM A_2 in the absence of methylation. The modified oligonucleotide thereby provided a powerful tool for dissection of the chemistry of methylated DNA–ligand interaction at high resolution, especially because the overall extent of degradation at the actual site of methylation was *unaltered*, as found most often for larger methylated DNA oligonucleotides.[6d] Presently, we report the first complete analysis of the products resulting from Fe·BLM-mediated degradation of DNA at a site of methylation. Key findings include the determinations that the chemical products derived from 5-methylcytidine were analogous to those obtained from cytidine,

Scheme II. Production of CpGp-OCH$_2$COOH (**1**) and Cytosine Propenal (**2**) from a Putative Reaction Intermediate



that the putative C-4′ deoxyribose radical intermediates in the methylated DNA oligonucleotide exhibited a sensitivity to O_2 tension, and that BLM congeners believed to bind to the non-methylated dodecanucleotide in an antiparallel fashion to produce damage on both strands of the duplex were able to exhibit the same behavior with the methylated substrate. While the extent of degradation of the substrate oligonucleotide was not altered upon methylation, reflecting an apparently unaltered affinity for Fe·BLM A_2, degradation of the methylated oligonucleotide produced a significantly greater proportion of alkali-labile lesions as compared with DNA strand scission. In addition to its chemical novelty and importance as the first example of analysis of the chemistry of BLM-mediated degradation of a modified nucleoside in DNA, the present finding may have important implications at the level of facility of repair of DNA lesions.

Results and Discussion

The methylated dodecanucleotide d(CGCTTTAAAG^{Me}CG) (numbered as 5′-$C_1G_2C_3T_4T_5T_6A_7A_8A_9G_{10}{}^{Me}C_{11}G_{12}$) was incubated with varying concentrations of Fe(II)·BLM A_2 under ambient conditions, resulting in the formation of degradation products amenable to HPLC analysis and quantitation through comparison to purified synthetic standards.[10,11] Products essential for

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Table I. Degradation of d(CGCTTTAAAG^{Me}CG) or d(CGCTTTAAAGCG) by Fe(II)·BLM A_2

entry	oligonucleotide	Fe(II)·BLM A_2, μM	C_3 lesions: cytosine, μM	C_3 lesions: cytosine propenal, μM	C_{11} lesions: 5-methylcytosine, μM	C_{11} lesions: 5-methylcytosine propenal, μM	total lesions (C_3 + C_{11}), μM	strand selectivity (C_3/C_{11})	% DNA strand scission events[a]	% methylated/% nonmethylated
1	d(CGCTTTAAAG^{Me}CG)	50	1	2	11	11	25	12/88	52	
2	d(CGCTTTAAAG^{Me}CG)	100	1.5	3	20	21	46	11/89	52	
3	d(CGCTTTAAAG^{Me}CG)	200	3	6	37	34	80	11/89	50	
4	d(CGCTTTAAAG^{Me}CG)	400	6	7	46	36	95	14/86	45	
5	d(CGCTTTAAAGCG)	200	26	55			81	12/88[b]	68	
6	d(CGCTTTAAAG^{Me}CG) + d(CGCTTTAAAGCG)[c]	200	14	27	21	23	85	12/88[b]		52/48

[a] (Base propenal release/total lesions) × 100. [b] Determined as described previously.[10,11] [c] Each oligonucleotide was present at 83 μM concentration (1 mM DNA nucleotide).

quantification of the position and chemical nature of lesions formed from d(CGCTTTAAAG^{Me}CG) [and the structurally analogous oligonucleotide d(CGCTTTAAAGCG)] included (see Scheme I) strand scission products derived from destruction of cytidine$_3$/(2′-deoxycytidylyl(3′→5′) [2′-deoxyguanosine-3′-(phosphoro-2″-*O*-glycolate)] (**1**) and *trans*-3-(cytosin-1′-yl)propenal (**2**), and from the destruction of 5-methylcytidine$_{11}$/2′-deoxyguanosine 5′-phosphate (5′-dGMP; **4**) and *trans*-3-(5′-methylcytosin-1′-yl)propenal (**5**), as well as cytosine (**3**) and 5-methylcytosine (**6**) released during formation of alkali-labile lesions at these same two positions.

Mechanistically, Fe(II)·BLM-mediated DNA strand scission is thought to involve the intermediacy of a C-4′ hydroperoxide derivative of deoxyribose,[5a,11a,12] which is believed to undergo cleavage of the C-3′–C-4′ bond via a Criegee-type rearrangement. For an intermediate of this type formed at cytidine$_3$, collapse of the intermediate (Scheme II) would then afford dinucleotide **1** and cytosine propenal (**2**) in a 1:1 ratio. The analogous lesion at 5-methylcytidine$_{11}$ would produce 5′-dGMP (**4**) and 5-methylcytosine propenal (**5**) in a 1:1 ratio. The alkali-labile lesions are believed to arise by C-4′ hydroxylation of deoxyribose,[5b,c,h] followed by loss of cytosine (from cytidine$_3$) or 5-methylcytosine (from 5-methylcytidine$_{11}$). Since each of these lesions results in the formation of one free base or base propenal, the total number of lesions (alkali-labile + strand scission) may be quantified by summation of all bases and base propenals. Further, the identification of the free bases and base propenals, as well as the structural characterization of the nature of the oligonucleotide termini at the site of cleavage (Scheme II), indicated that Fe·BLM-mediated degradation of a DNA oligonucleotide at a site of methylation proceeded by the same chemical mechanism observed in the absence of methylation.

The actual quantitation of the products formed upon admixture of Fe(II)·BLM A_2 and d(CGCTTTAAAG^{Me}CG) is summarized in Table I. As shown, over an 8-fold concentration range for Fe(II)·BLM A_2, the majority of lesions occurred at 5-methylcytidine$_{11}$ (86–89%), rather than cytidine$_3$ (11–14%), as has also been observed for the corresponding nonmethylated oligonucleotide (Table I, entry 5).[11b] Also reflected in Table I are the comparable efficiencies of degradation of the methylated and nonmethylated oligonucleotide substrates (80 vs 81 μM total products, cf. entries 3 and 5). It has been shown previously that the extent of product formation from such oligonucleotide substrates and, especially, the ratio of degradation mediated at cytidine$_3$ and cytidine$_{11}$ are sensitive probes of the structural nature of BLM–DNA interaction.[11b] That these parameters were not altered by the presence of a methyl group on cytidine suggests strongly that introduction of a methyl group into the major groove of DNA did not alter the recognition or binding properties of Fe(II)·BLM A_2 for this particular substrate.

Additional evidence indicating that the presence of the 5-methyl group of cytidine$_{11}$ did not alter the ability of Fe(II)·BLM A_2 to interact with this oligonucleotide was obtained from a competition experiment in which Fe(II)·BLM A_2 was incubated in the presence of an excess of substrate, consisting of equimolar amounts of duplex d(CGCTTTAAAG^{Me}CG) and d(CGCTTTAAAGCG); these were mixed as preformed *homo*duplexes and maintained at 0 °C to limit *hetero*duplex formation.[13] The results of this experiment (Table I, entry 6) demonstrated that, under the conditions employed (which utilized limiting amounts of activated Fe·BLM), each dodecanucleotide was degraded to essentially the same extent, and that the strand selectivity of degradation [i.e., at cytidine$_3$ vs cytidine$_{11}$ (or 5-methylcytidine$_{11}$)] was maintained for each. Under conditions of limiting BLM, if the *non*methylated oligonucleotide cleavage site were a better substrate, one would have expected a greater extent of cleavage at this site. The fact that the extent of cleavage was not affected by cytidine methylation again suggested that the presence of the methyl group on cytidine had no significant effect on the nature of DNA oligonucleotide binding by Fe(II)·BLM A_2. These results are consistent with the belief that Fe(II)·BLM binds to and interacts with DNA predominantly from the minor groove and is, therefore, not perturbed by agents that bind in the major groove.[6d,14]

Table II. Effect of Excess O_2 on Product Formation from d(CGCTTTAAAG^{Me}CG) + d(CGCTTTAAAGCG) in the Presence of Fe(II)·BLM A_2

condition	cytosine,[a] μM	cytosine propenal,[a] μM	5-methylcytosine, μM	5-methylcytosine propenal, μM
ambient O_2	13	24	20	27
excess O_2	7	32	12	45
	Ratio Excess O_2/Ambient O_2			
	0.5	1.3	0.6	1.7

[a] These products are presumably derived almost exclusively from the nonmethylated oligonucleotide (cf. Table I).

The most pronounced effect of cytidine methylation on Fe(II)·BLM A_2 mediated degradation was the dramatic difference in the ratio of alkali-labile lesions formed vs strand scission events. The data shown in Table I indicate that, over a range of Fe(II)·BLM A_2 concentrations (50–200 μM) where dioxygen was not limiting, the observed ratio of alkali-labile lesions to DNA strand scission at 5-methylcytidine$_{11}$ was essentially 1:1.[15] This was in direct contrast to the results obtained with the nonmethylated dodecanucleotide which, under identical conditions, produced alkali-labile lesions and strand breaks in a 1:2 ratio (Table I, entry 5 and refs 10 and 11b). Remarkably, even for

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(13) That strand exchange does not occur under these conditions may be judged from the observation that d(CGCTTTAAAGCG), having an average of two BLM-induced lesions/duplex (localized at cytidine$_3$ and cytidine$_{11}$), underwent further BLM-induced damage with facility only if heated above the melting temperature and allowed to reanneal (H. Sugiyama and S. M. Hecht, unpublished data). This also argues for the existence of the oligonucleotides as duplexes under the reaction conditions employed.

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(15) A superficially similar, although less pronounced, effect has been reported previously for the alternating copolymer poly(dG-^{Me}dC)·poly(dG-^{Me}dC).[6d] Clearly, the complex nature of the substrate involved precludes any chemical analysis of the type reported here.

Table III. Degradation of d(CGCTTTAAAG^{Me}CG) and d(CGCTTTAAAGCG) by Fe(II)·deglyco-BLM A_2

oligonucleotide	Fe(II)·deglyco-BLM A_2, μM	C_3 lesions: cytosine, μM	C_3 lesions: cytosine propenal, μM	C_{11} lesions: 5-methylcytosine, μM	C_{11} lesions: 5-methylcytosine propenal, μM	total lesions (C_3 + C_{11}), μM	strand selectivity (C_3/C_{11})
d(CGCTTTAAAG^{Me}CG)	200	7	20	4	3	34	80/20
d(CGCTTTAAAGCG)	200	11	26			37	80/20[a]

[a] Determined as described previously.[10,11]

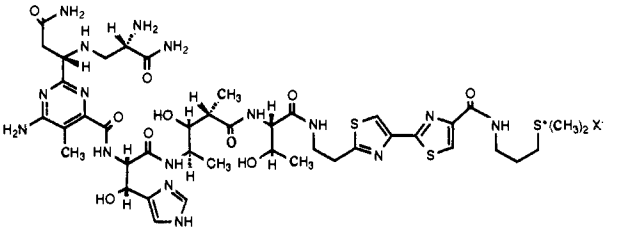
the methylated dodecanucleotide, the ratio of products formed at cytidine$_3$ appeared to be unaffected by methylation of cytidine$_{11}$ (Table I, entries 1–3), at least for lower Fe(II)·BLM A_2 concentrations where the amount of dissolved dioxygen was not limiting.

To assure that the mechanistic roles of dioxygen were the same for the methylated and nonmethylated dodecanucleotides, the effect of added dioxygen on Fe(II)·BLM A_2 mediated degradation of these two substrates was studied. This experiment employed the methylated and nonmethylated substrates in a fashion similar to that utilized in the competition experiment described above. As indicated in Table II, purging the reaction mixture with dioxygen caused similar increases in the production of cytosine propenal (**2**) and 5-methylcytosine propenal (**5**), and similar decreases in the production of the two free bases. These results, which paralleled those observed previously in other systems,[5b,d] again suggested that the same mechanism of DNA degradation by Fe(II)·BLM A_2 was operative for both methylated and nonmethylated oligonucleotides. Further, they indicated that the tendency toward increased alkali-labile lesion production in the case of the methylated substrate could be reversed through an increase in dioxygen concentration.

The above observations and product analysis indicated that DNA methylation of the oligonucleotide substrate did not change the chemical mechanism by which Fe(II)·BLM A_2 degraded the substrate, or the ability of Fe(II)·BLM A_2 to bind and degrade the dodecanucleotide. These findings imply the absence of any oligonucleotide-based conformational change or steric constraint grossly unfavorable to Fe(II)·BLM A_2 interaction. However, the alteration in production of strand scission vs alkali-labile lesions upon methylation does argue that the partitioning between the two observed lesions (Scheme I) was altered by methylation. It has been demonstrated previously[5d] for a poly(dA-dU) substrate tritium labeled specifically at C-4′ of the uridine deoxyribose moiety that there was an isotope effect associated with the production of both types of lesions outlined in Scheme I and that these were similar in magnitude, suggesting that (i) both sets of products derived from a common C-4′ deoxyribose radical and (ii) the formation of the radical was rate-limiting. The similarity of the products formed in the present case for the methylated and nonmethylated substrates, as well as the observed oxygen sensitivity of product formation, suggests that the same overall degradation scheme may be operative here.

Accordingly, the data reflecting a change in the ratio of strand scission and alkali-labile lesions for the methylated oligonucleotide are probably best interpreted as either a diminution in the rate of oxygenation of the initially formed C-4′ deoxyribose radical or a facilitation of the formation of the putative C-4′ hydroxyribose intermediate leading to the alkali-labile lesion.[5f–h] Clearly, even a relatively small conformational change in the DNA oligonucleotide, or the development of some limited steric constraint, resulting from cytidine methylation could be sufficient to alter the rate of diffusion of dioxygen required to form the C-4′ hydroperoxide intermediate (Scheme II). Likewise, a small alteration in the positioning of the Fe·BLM-derived metal–oxo complex[2f] in relation to the C-4′ deoxyribose radical could significantly enhance the rate of formation of the putative hydroxy intermediate leading to the alkali-labile lesion.

The apparent differences in the ratio of products resulting from Fe·BLM-mediated degradation at C_3 and C_{11} in the methylated oligonucleotide (Table I, vide supra) prompted us to study this phenomenon using a BLM congener known[11b] to mediate degradation preferentially at cytidine$_3$ of d(CGCTTTAAAGCG). Accordingly, Fe(II)·deglyco-BLM A_2 was employed for the



deglycobleomycin A_2

degradation of the methylated and nonmethylated dodecanucleotides under identical conditions. As summarized in Table III, the presence of the methyl group on cytidine$_{11}$ had no appreciable effect on the total extent of product formation or the selectivity of cleavage at cytidine$_3$ vs cytidine$_{11}$. As was observed for Fe(II)·BLM A_2, degradation of both oligonucleotides at C_3 gave predominantly cytosine propenal rather than free cytosine, reflecting a preference for DNA strand scission as the primary mode of degradation. However, in common with the results obtained for Fe(II)·BLM A_2 (Table I), Fe(II)·deglyco-BLM A_2 produced a disproportionately greater amount of free base for those lesions mediated at the site of methylation. In addition to its implications for the mode of Fe(II)·BLM interaction with a structurally altered DNA substrate, it may be noted that the observed switch in the ratio of base propenal to free base between C_3 and C_{11} reinforces an earlier suggestion[2f,11b] that DNA cleavage at these positions results from two distinct (antiparallel) orientations of Fe(II)·BLM, one of which is responsible for cleavage at C_3, and the other at C_{11}.

It is clear that the structural effects resulting from DNA methylation can potentially be transmitted some distance from the actual site of methylation via telestability[16] since previous studies have documented the diminution of DNA strand scission at sites far removed from a site of DNA methylation.[6c,d] Although it is not known what effect an isolated 5-methylcytidine moiety has on DNA conformation, it has been suggested[17] that the result is some non-B form structure that can be recognized, for example, by DNA binding proteins. It seems possible that such conformational changes may also be recognized by BLM, as was documented following DNA platination.[6a,b] Alternatively, as suggested by a recent report,[18] the sequence of DNA per se may alter the binding mode of a DNA interactive agent; the binding of BLM could very well be affected locally in a similar manner, especially following DNA methylation.

The results reported here constitute strong evidence that the conformational changes in DNA induced at a single site of methylation in some instances can be highly localized and need not affect the recognition or modification by Fe·BLM of the DNA strand complementary to the site of methylation. In this sense, the present findings are reminiscent of the recent report by Kozarich et al.[19] which demonstrated that the isotope effect associated

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with abstraction of C-4′ H of deoxyribose by Fe·BLM varied at individual positions within the same region of a DNA duplex, undoubtedly reflecting local variations in the facility of the rate-limiting step in BLM-mediated DNA degradation. While the present results do not suggest any effect of DNA methylation on the facility of formation of the putative C-4′ deoxyribose radical intermediate, they do reflect the same ability of BLM to alter parameters of its chemical behavior in a highly localized fashion in response to variations of substrate structure. Further, in contrast to the alteration in isotope effect at individual DNA nucleotide positions,[19] which would not be predicted to have any effect on product formation, DNA methylation clearly altered the ratio of chemical products formed at the methylated cytidine moiety in the substrate oligonucleotide.

It may be noted that the ability of BLM to mediate its therapeutic effects via DNA degradation is unquestionably affected by normal repair processes that occur in mammalian cells. While it is known that BLM-mediated DNA damage is subject to repair,[20] the facility of repair of strand breaks relative to that of alkali-labile lesions has not been studied. It would be interesting to determine whether, in addition to being somewhat less susceptible to BLM-mediated DNA damage,[6c,d] methylated DNA also affords a larger proportion of products amenable to repair.

Experimental Section

Materials. Blenoxane was obtained from Bristol Laboratories through the courtesy of Dr. William Bradner and was fractionated chromatographically to provide bleomycin A_2.[21] Deglycobleomycin A_2 was obtained by partial hydrolysis of bleomycin A_2.[22] The oligonucleotides used as substrates for BLM were prepared as described previously.[10]

General Methods. (A) Oligonucleotide Degradation Mediated by Fe(II)·BLM A_2. Reaction mixtures (50 μL total volume) were prepared containing 1 mM (nucleotide concentration) of the oligonucleotide to be studied, 50, 100, 200, or 400 μM BLM A_2, and an equimolar amount of $Fe^{II}(NH_4)_2(SO_4)_2$ in 50 mM sodium cacodylate buffer, pH 7.0. Reactions were initiated by the addition of Fe(II), incubated at 0 °C for 15 min, and analyzed by HPLC. Reactions that included both non-methylated and methylated substrates were carried out as outlined above in the presence of 1.0 mM (nucleotide concentration) of each duplex substrate maintained at 0 °C and 200 μM Fe(II)·BLM. Reactions that employed deglyco-BLM A_2 (200 μM) were carried out as described above.

Dioxygen-purged oligonucleotide degradation reactions were carried out as described above with the addition of a 10-min purge of dioxygen gas before and during the addition of Fe(II), which initiated the reaction.

(B) HPLC Quantification of Oligonucleotide Cleavage Products. Oligonucleotide cleavage products were analyzed and quantitated on a Rainin Microsorb Short-One C-18, 3-μm column equipped with a Brownlee Laboratories HPLC analytical cartridge C-18 precolumn. The column was washed with 0.1 mM NH_4OAc, pH 6.8, at a flow rate of 1.6 mL/min. Products were detected by UV absorbance (A_{254}) with a Varian multiwavelength detector. Quantitation was carried out through the comparison of peak areas of reaction products to those of carefully purified synthetic standards. Product retention times (in minutes) were as follows: cytosine, 1.7; 5-methylcytosine, 3.9; 5′-dGMP, 4.4; $dCpGpCH_2COOH$, 7.8.

Reaction mixtures were analyzed additionally by gradient HPLC to effect the quantitation of base propenals formed. Analysis was carried out on an Alltech C-8 column, 5 μm, which was washed with a 100% H_2O to 25% acetonitrile linear gradient over a 25-min time period at a flow rate of 1.0 mL/min. Products were detected by UV absorbance (A_{300}); peaks were recorded and quantitated as described above. Retention times (in minutes) were as follows: cytosine propenal, 19.0; 5-methylcytosine propenal, 23.0. Also formed, albeit in very low yields, were the base propenals derived from deoxyadenosine and thymidine.[10]

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The Total Synthesis of (+)-Ionomycin

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Abstract: The total synthesis of (+)-ionomycin, a dibasic acid ionophore, is described by using a strategy that capitalizes on the chiron approach and on asymmetric processes. The C_1–C_{22} portion of ionomycin was constructed from smaller segments obtained in optically pure form by systematic functionalization and manipulation of chirons derived originally from L-glutamic acid. A key reaction in the synthesis of the deoxypropionate-containing segments relies on a novel sulfur-assisted organocuprate displacement of a secondary tosylate with complete inversion of configuration. The tetrahydrofuran segment of ionomycin was constructed from an optically pure epoxide obtained via a Sharpless asymmetric epoxidation and a sulfone derived from geraniol.

Since their discovery, the polyether class of antibiotics has commanded much interest on several scientific frontiers.[1,2] Their most fascinating biological function is the ability to chelate various inorganic cations and to transport them across lipid membranes, hence the term ionophore.[3] In view of their challenging structures and the presence of different stereochemical arrays of functional groups, this class of natural products has also been the subject of elegant synthetic[4] and biosynthetic studies.[5]

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