Sequence Specificity of DNA Alkylation by the Antitumor Natural Product Leinamycin

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Reaction with thiol converts the antitumor natural product leinamycin to an episulfonium ion that alkylates the N^7-position of guanine residues in double-stranded DNA. The sequence specificity for DNA alkylation by this structurally novel compound has not previously been examined. It is reported here that leinamycin shows significant (>10-fold) preferences for alkylation at the 5′-G in 5′-GG and 5′-GT sequences. The sequence preferences for activated leinamycin are significantly different from that observed for the structurally simple episulfonium ion generated from 2-chloroethyl ethyl sulfide. DNA alkylation by activated leinamycin is inhibited by addition of salt (100 mM $NaClO_4$), although the degree of inhibition is somewhat less than that seen for 2-chloroethyl ethyl sulfide. This result suggests that electrostatic interactions between the activated leinamycin and the N^7-position of guanine residues facilitate efficient DNA alkylation. However, the observed sequence preferences for DNA alkylation by activated leinamycin do not correlate strongly with calculated sequence-dependent variations in the molecular electrostatic potential at the N^7-atom of guanine residues in duplex DNA. Thus, electrostatic interactions between activated leinamycin and DNA do not appear to be the primary determinant for sequence specificity. Rather, the results suggest that sequence-specific noncovalent interactions of leinamycin with the DNA double helix on the 3′-side of the alkylated guanine residue play a major role in determining the preferred alkylation sites. Consistent with the notion that noncovalent binding plays an important role in DNA alkylation by leinamycin, experiments with 2′-deoxyoligonucleotide substrates confirm that the natural product does not alkylate single-stranded DNA under conditions where duplex DNA is efficiently alkylated.

Introduction

When exposed to a thiol-rich environment such as that found inside cells, the antitumor natural product leinamycin (LM) (*1, 2*) is converted to a potent DNA-alkylating agent via a unique chemical mechanism (Scheme 1) (*3– 6*). The activated form of LM exists predominantly as the epoxide **3**, but product analysis indicates that DNA alkylation occurs exclusively via Markovnikov attack of the N^7-position of guanine residues on the episulfonium ion (**2**) to yield the adduct **4** (*5, 7*). Although the site at which activated LM bonds to DNA is well-established (N^7-G), the effects that flanking DNA base sequences exert on the alkylation reaction have not previously been examined. It is important to examine the sequence specificity for reactions of alkylating agents with DNA because sequence preferences undoubtedly play a role in the biological responses elicited by any given DNA-damaging agent (*8–10*). In addition, studies of sequence specificity can provide insight about the mechanisms of the DNA damage and sometimes reveal important information regarding noncovalent association of the small molecule with the double helix (*8–20*). For these reasons, we investigated the sequence specificity of DNA alkylation by the structurally unique natural product LM.

A number of factors can contribute to the sequence specificity observed for agents that alkylate DNA at the N^7-position of guanine residues. For example, sequence-dependent changes in the electrostatic potential at the N^7-position of guanine residues can affect the sequence preferences for the reaction of charged electrophiles such as diazonium ions and aziridinium ions with DNA (*21– 24*). In addition, sequence-dependent changes in the steric accessibility of N^7-nitrogen in guanine residues may be important in some cases (*24, 25*). Finally, there are many examples demonstrating that sequence-specific noncovalent interactions of small molecules with the double helix can lead to sequence-specific chemical modification of DNA (*11, 26–30*).

Here, we report on the sequence specificity of DNA alkylation by the episulfonium ion (**2**) generated in the reaction of the antitumor natural product LM with thiols. We find that activated LM (**2/3**; Scheme 1) displays distinct preferences for reaction at certain sequences in double-stranded DNA. Our results suggest that the efficiency of the reaction is facilitated by electrostatic interaction between the positively charged alkylating agent and the negative electrostatic potential at the N^7-position of guanine residues in DNA. However, the observed sequence specificity likely arises primarily through noncovalent interactions of the antibiotic with the double helix on the 3′-side of the alkylated base.

Experimental Procedures

Materials. Reagents were purchased from the following suppliers and were of the highest purity available: supercoiled

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Scheme 1. Mechanism of Thiol-Triggered DNA Alkylation by LM
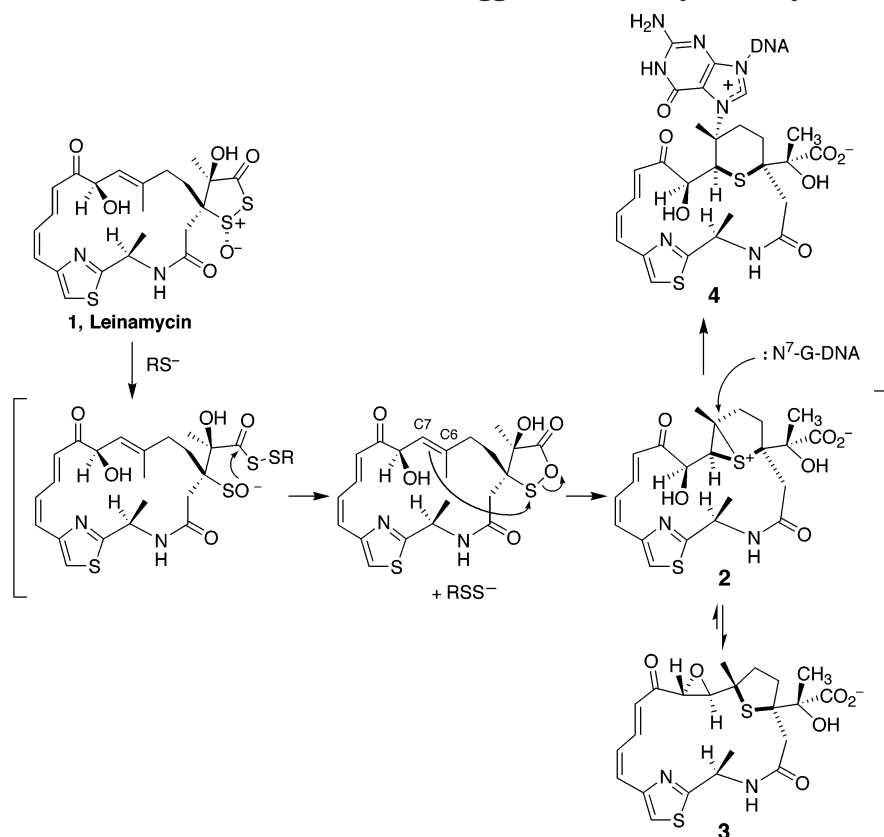
pBR322 DNA, ammonium persulfate, and *N,N*-bisacrylamide were purchased from Boehringer Mannheim; EcoR I, BamH I, T4 polynucleotide kinase, and calf intestinal phosphatase were purchased from New England Biolabs; [γ-^{32}P]dATP was purchased from New England Nuclear-Dupont; *N*-(2-hydroxyethyl)-piperazine-*N'*-ethanesulfonic acid (HEPES), dimethyl sulfate (DMS), G-50 Sephadex, and phenol were purchased from Sigma Chemical Company; ethylenediaminetetraacetic acid was purchased from Fluka Chemical Company; xylene cyanol, bromophenol blue, formamide, acrylamide, *N,N,N',N'*-tetraethylethylenediamine, and urea were purchased from United States Biochemical; sodium perchlorate, glutathione, β-mercaptoethanol, 2-chloroethyl ethyl sulfide (CEES), potassium phosphate, and sodium phosphate were purchased from Aldrich; ethanol was purchased from McCormick Distilling Co.; HPLC grade solvents (acetonitrile, chloroform, and methanol) were purchased from Fisher. LM was a gift from Dr. Yutaka Kanda and co-workers at Kyowa Hakko Kogyo, Ltd. Carrier DNA for these experiments was prepared by sonicating a 1.7 mM bp solution of herring sperm DNA (catalog no. 223646, Boehringer Mannheim) for 2 h (in a Fisher Scientific FS-110 sonicating bath).

Alkylation of a 377 Base Pair 5'-^{32}P-Labeled Restriction Fragment with Activated LM or CEES. The plasmid pBR322 was digested with EcoR I, followed by treatment with calf intestinal phosphatase. The linearized plasmid was 5'-labeled using [γ-^{32}P]ATP and T4 polynucleotide kinase (*31*) and then digested with BamH I. The resulting 377 base pair DNA fragment was purified on a 5% nondenaturing polyacrylamide gel. In a typical alkylation reaction, 40 000 cpm of labeled DNA, herring sperm DNA (250 μM base pair), LM (2.5 or 5 μM), and 10 equiv of glutathione were incubated at 37 °C for 2 h in HEPES buffer (10 mM, pH 7.0) containing NaClO$_4$ (50 mM). The reaction (20 μL final volume) was quenched by adding 180 μL of a solution prepared by mixing 1.6 mL of 3 M NaOAc (pH 5) and 48 mL of absolute ethanol. The DNA was precipitated by cooling this mixture on dry ice (15 min) and centrifuging for 20 min (12 000 rpm in an Eppendorf model 5415C). The supernatant was removed, and the pellet was washed with 70%

ethanol−water. The DNA pellet was redissolved in aqueous piperidine (100 μL of a 0.2 M solution) and incubated at 90 °C for 25 min (Maxam−Gilbert workup) (*32*). The solution was frozen on dry ice, lyophilized for 1.5 h in a SpeedVac Concentrator at 37 °C, redissolved in 100 μL of water, and evaporated again. The dried DNA fragments were dissolved in formamide loading buffer and denatured at 90 °C for 4 min, and an equal number of counts was loaded in each lane of an 11% denaturing polyacrylamide gel (19:1 cross-linking, containing 5 M urea). The gel was electrophoresed at 1500 V for 5 min, 800 V for 30 min, and 1200 V for 2 h (until the bromophenol blue dye migrated ∼36 cm from the top of the gel plates). The resolved DNA fragments were quantitatively analyzed by phosphorimager (Molecular ImagerFX, Imaging Screen-K, catalog no. 170-7841, Bio-Rad, using Quantity One Version 4, Bio-Rad). Data presented in the paper were gathered from reactions in which the final yield of alkylated DNA did not exceed 80%. Alkylation reactions with the sulfur mustard **5** were conducted and analyzed in a manner identical to that described above except using a 50 μM concentration of CEES in place of LM and glutathione.

Alkylation of a 978 Base Pair, 5'-^{32}P-Labeled Restriction Fragment by Activated LM. The plasmid pBR322 was digested with BamH I, followed by treatment with calf intestinal phosphatase. The linearized plasmid was 5'-labeled using [γ-^{32}P]-ATP and T4 polynucleotide kinase and then digested with Bsm I. The resulting 978 base pair DNA fragment was purified on a 5% nondenaturing polyacrylamide gel. In a typical alkylation reaction, 40 000 cpm radioactive DNA material and 300 μM (bp) herring sperm DNA were mixed with LM (1, 2.5, or 5 μM) at 37 °C in sodium phosphate buffer (25 mM, pH 7.0), followed by addition of glutathione (20 equiv). The reactions were incubated for 2 h, followed by ethanol precipitation as described above. After piperidine work up as described above, DNA fragments were analyzed by electrophoresis on an 8% polyacrylamide sequencing gel. The gel was electrophoresed at 1000−1200 V, and samples were loaded at 1.5−2 h intervals for a total duration of 8 h. This staggered loading allowed us to resolve a
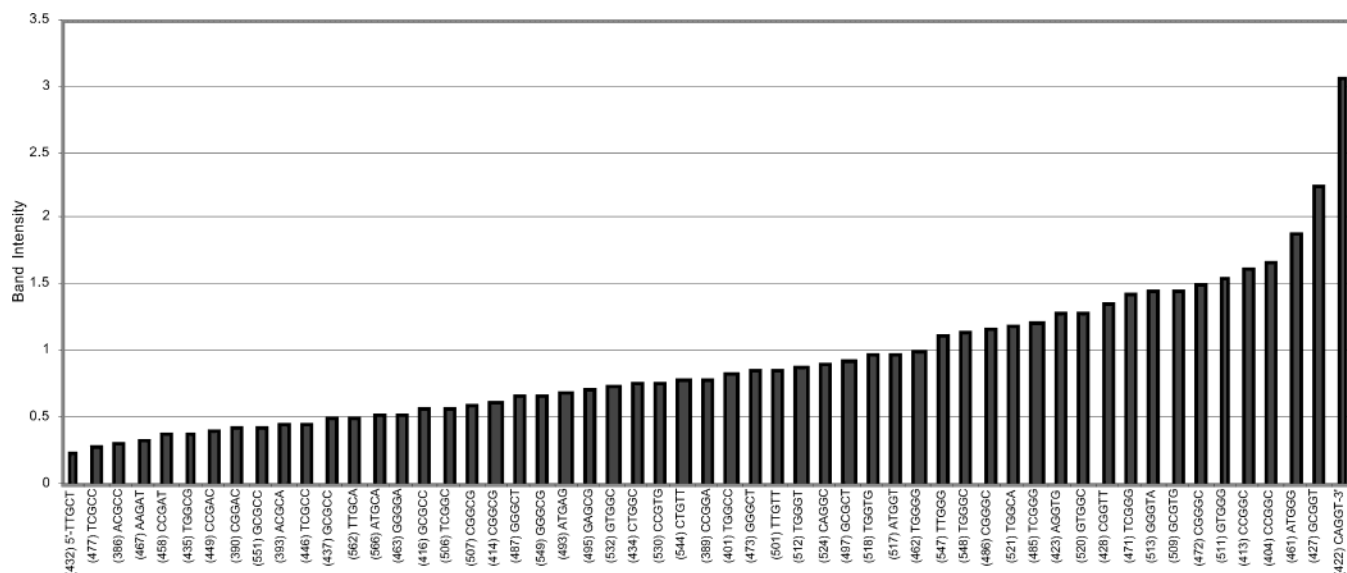
Figure 1. Sequence preferences for alkylation of the 978 base pair BamH I-Bsm I restriction fragment from pBR322 by activated LM (2.5 μM **1**, 10 equiv of glutathione, 50 mM NaClO$_4$, and 50 mM sodium phosphate buffer, pH 7, 37 °C, 2 h). The central G-residue in each sequence is the alkylation site. The numbers in parentheses denote the number of the central guanine residue in the numbering system of pBR322.

larger number of alkylation sites on the DNA fragment in a single gel. The yield of cleavage at each guanine residue was quantitatively analyzed by phosphorimaging as described above.

Alkylation of the Oligonucleotide Duplex 5′-ATA ATT TGT ATA GGG AGA GAA AGT TAA TAA-3′ by Activated LM, CEES, and DMS (Underlined Portion of the Sequence is Double-Stranded). Oligonucleotides were synthesized using an Applied Biosystems 3948 Nucleic Acid Synthesis and Purification System. The single-stranded 2′-deoxyoligonucleotide 5′-ATA ATT TGT ATA GGG AGA GAA AGT TAA TAA-3′ was labeled by treatment with [γ-^{32}P]ATP and T4 polynucleotide kinase. The resulting 5′-labeled oligonucleotide was purified on a denaturing 18% polyacrylamide gel. The purified single strand was mixed with 1.5 equiv of the complementary strand 5′-TTA TTA ACT TTC TCT CCC TAT-3′, heated at 90 °C for 5 min, and slowly cooled to room temperature over 4 h. The resulting oligonucleotide duplex was purified on a nondenaturing 18% polyacrylamide gel. In a typical alkylation reaction, a solution of LM (2 μL of a 0.5 mM solution in acetonitrile) was added to a mixture of labeled DNA and herring sperm carrier DNA in HEPES buffer and the reaction was initiated by addition of β-mercaptoethanol (10 equiv) (final concentrations: LM, 50 μM; labeled DNA, 40 000 cpm; herring sperm DNA, 80 μM bp; β-mercaptoethanol, 0.5 mM; HEPES, 50 mM, pH 7, final reaction volume 20 μL). Some reactions contained 100 mM NaClO$_4$. The resulting mixture was incubated at 25 °C for 6 h, followed by ethanol precipitation and piperidine workup as described above. For alkylation by the sulfur mustard **5**, all conditions were identical except that a 500 μM concentration of CEES was employed in place of LM and thiol. Analysis of the alkylation reactions was performed as described above except the DNA fragments resulting from Maxam−Gilbert workup were analyzed on a 20% denaturing polyacrylamide gel.

Results

Sequence Specificity for Alkylation of Restriction Fragments by Thiol-Activated LM. In this work, we surveyed the reactivity of activated LM with approximately 80 different guanine residues in double-stranded DNA restriction fragments and synthetic 2′-deoxyoligonucleotide duplexes. Alkylation reactions were conducted using ^{32}P-labeled DNA fragments under "single hit" conditions (low alkylation yields) (*21*). Following Maxam−Gilbert workup (*32, 33*) to convert the alkylation

sites into strand cleavages, we resolved the labeled DNA fragments using denaturing polyacrylamide gel electrophoresis and quantitatively determined the efficiency of DNA alkylation at each guanine residue by phosphorimaging. We find that activated LM displays distinct sequence preferences in its DNA alkylation chemistry. As shown in Figures 1−3, there is a greater than 10-fold difference between the most favored and the least favored alkylation sites in duplex DNA.

Analysis of the sequence preferences for alkylation of a 978 base pair fragment by activated LM (Figure 1) reveals that 15 of the 20 most favorable alkylation sites are 5′-GG sequences (where the underlined G is the alkylated base). Four of the remaining five most favored sequences in the top 20 are 5′-GT sequences. Fourteen of the 20 least favored sites are 5′-GC sequences, and four of the remaining six least favored sites in the bottom 20 are 5′-GA sequences. Overall, the results indicate that 5′-GG and 5′-GT sequences are favored alkylation sites, while 5′-GC and 5′-GA sequences are disfavored sites. Bases flanking these two base sequences clearly have an influence on the alkylation efficiency (for example, not all 5′-GG sequences are alkylated to an equal extent); however, we can perceive no clear trend in the favored (or disfavored) bases on the 5′-side of the alkylated base or the second-nearest neighbor on the 3′-side of the alkylation site. The overall trends gleaned from experiments with the 978 base pair restriction fragment (Figure 1) are further supported by the sequence preferences observed in a 377 base pair fragment of different sequence (Figure 2).

In addition, we examined sequence specific DNA alkylation by LM using the synthetic 2′-deoxyoligonucleotide duplex: 5′-ATA ATT TGT ATA GGG AGA GAA AGT TAA TAA-3′ (Figure 3). This duplex has been used previously by others as a substrate to examine electrostatic effects in DNA alkylation reactions (*23*) and contains six base-paired guanine residues in different sequence contexts along with one guanine residue in a single strand setting (the underlined region in the sequence shown above is double-stranded). First, we
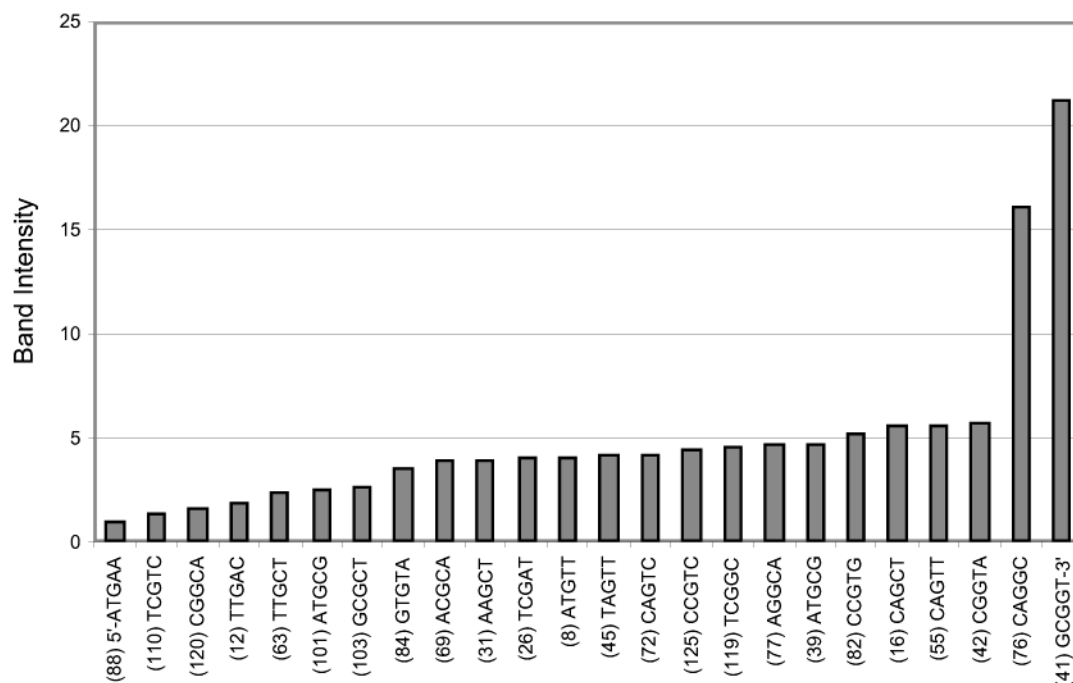
Figure 2. Sequence preferences for alkylation of the 377 base pair EcoR I-BamH I restriction fragment from pBR322 by activated LM (2.5 μM **1**, 10 equiv of glutathione, 50 mM NaClO$_4$, and 10 mM HEPES buffer, pH 7, 37 °C, 2 h). The central G-residue in each sequence is the alkylation site. The numbers in parentheses denote the number of the central guanine residue in the numbering system of pBR322.
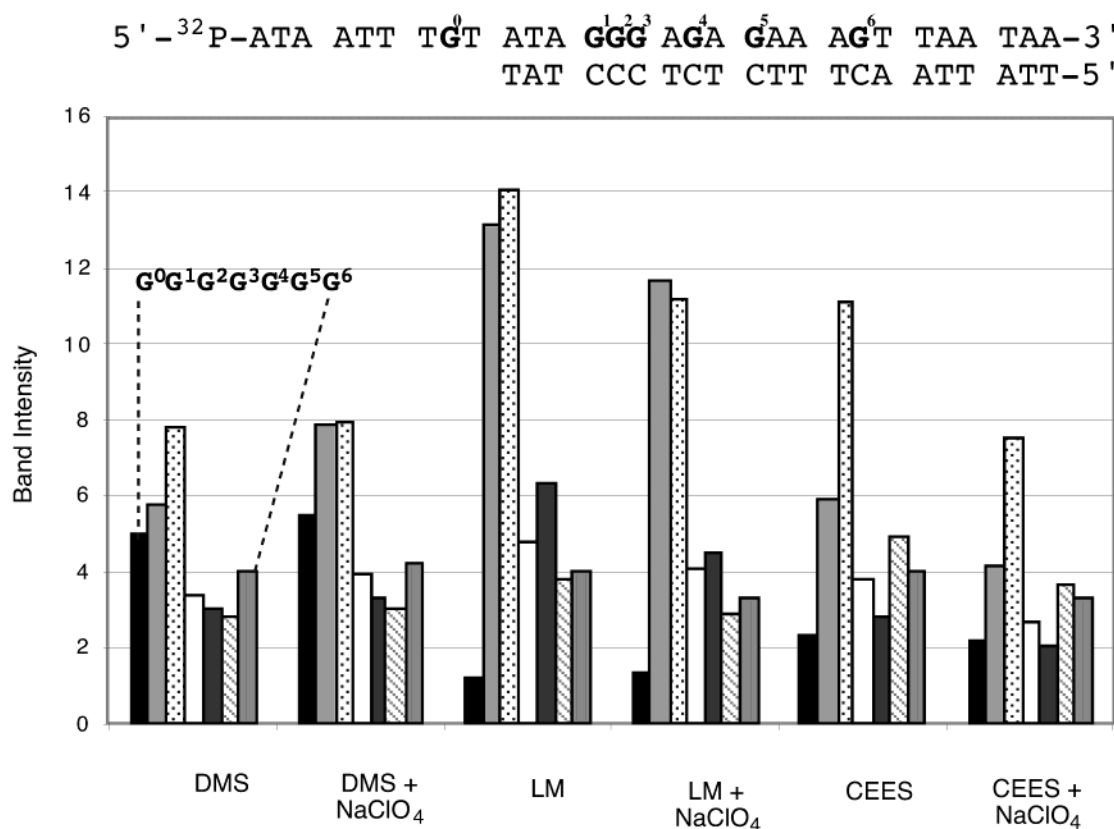

Figure 3. Alkylation of a 2′-deoxyoligonucleotide duplex by activated LM, CEES, and DMS, with and without added salt (100 mM NaClO$_4$). To facilitate visual interpretation of salt effects, the height of the band for G^6 in each of the "no salt" data sets is normalized to four.

observe that the sequence preferences observed for alkylation of this substrate by activated LM are consistent with those seen in the restriction fragments (Figures 1 and 2). That is, the two 5′-GG sites within the oligonucleotide duplex are hotspots for alkylation by activated LM.

Although 5′-GT sequences are generally favored, the 5′-AGT site featured in this oligonucleotide duplex is not a particularly good alkylation site, mirroring the results seen for the 5′-AGT site in the BamH I-Bsm I restriction fragment shown in Figure 2. It is noteworthy that LM
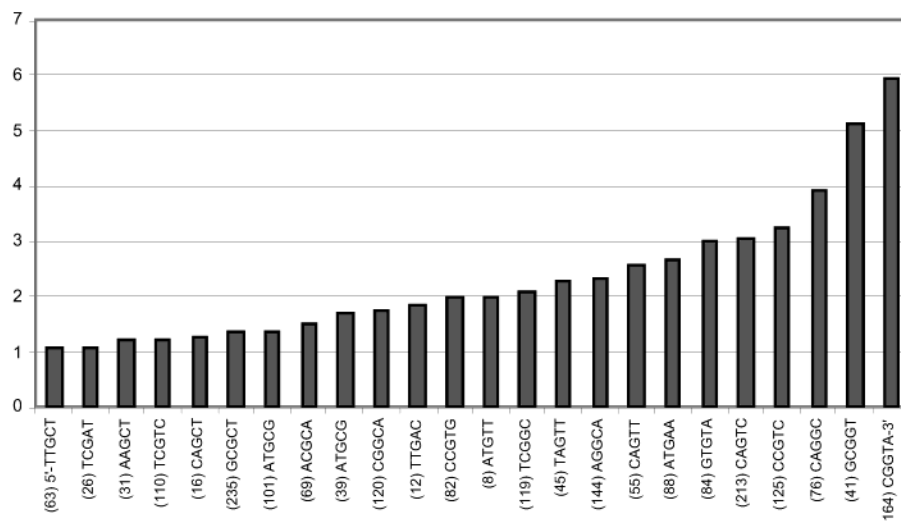
Figure 4. Sequence preferences for alkylation of the 377 base pair EcoR I-BamH I restriction fragment from pBR322 by CEES (50 μM **5**, 50 mM NaClO$_4$, and 10 mM HEPES buffer, pH 7, 37 °C, 2 h). The central G-residue in each sequence is the alkylation site. The numbers in parentheses denote the number of the central guanine residue in the numbering system of pBR322.

does not efficiently alkylate the guanine residue in the single-stranded region of the oligonucleotide. In contrast, this guanine residue is alkylated effectively by both DMS and CEES. It has previously been noted that LM does not efficiently alkylate 2′-deoxyguanosine nucleosides or single-stranded DNA, although reaction conditions were not reported (*5*). Importantly, the requirement that guanine residues reside within a double helical environment to be efficiently alkylated by LM provides a strong indication that noncovalent association of the antibiotic with the DNA duplex is necessary to achieve efficient reaction.

Effects of Added Salt on Sequence Specificity and Alkylation Efficiency of LM, CEES, and DMS. As noted in the Introduction, flanking bases can exert a marked effect on the reaction of guanine residues in DNA with positively charged electrophiles such as diazonium ions and aziridinium ions (*21−23*). In fact, the observed sequence specificity for these particular positively charged alkylating agents often correlates quite well with calculated sequence-dependent variations in the molecular electrostatic potential (MEP) at the N^7-atom of guanine residues in duplex DNA (*21, 22, 24*). MEP values for N^7 of guanine residues in various sequence contexts are calculated by summing the electrostatic effects of the 3′- and 5′-neighboring bases given in Table 2 of ref *24*. Reactions at residues with larger negative potentials are favored due to electrostatic attraction between the charged electrophile and the DNA nucleophile (*22, 34−36*). In cases where this type of electrostatic interaction is thought to play a role in determining sequence specificity, it has been observed that addition of salts (e.g., NaCl, MgCl$_2$, and NaClO$_4$) or cationic DNA-binding ligands such as ethidium bromide or distamycin inhibits DNA alkylation (*35, 37*). It is suggested that these additives screen electrostatic interactions between the alkylating agent and the N^7-position of guanine residues in DNA (*21−23, 34, 35, 37*).

We investigated the effect of added salts on DNA alkylation by LM using the oligonucleotide duplex shown in Figure 3. For the purposes of comparison, we also examined DNA alkylation by the simple episulfonium ion derived from CEES (**5**), which alkylates DNA via the episulfonium ion **6** (Scheme 2) (*38, 39*). Addition of 100

Scheme 2. Generation of an Episulfonium Ion from CEES

CH$_3$CH$_2$S$\diagdown\diagup$Cl $\longrightarrow$ CH$_3$CH$_2$S$^+\diagdown$

     5   **6**
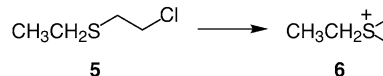
mM NaClO$_4$ results in an 18% decrease in the overall yield of DNA alkylation by activated LM (this calculation ignores the poorly alkylated guanine in the single-stranded region of the oligo). Addition of salt causes a 27% overall inhibition of DNA alkylation by the episulfonium ion **6** derived from CEES. Addition of salt does not alter sequence specificity for either the simple sulfur mustard **6** or the LM. As expected (*21, 22, 35*), we find that addition of NaClO$_4$ (100 mM) does not inhibit DNA alkylation by the uncharged alkylating agent DMS. These findings suggest that electrostatic interactions between the activated LM and the N^7-position of guanine residues facilitate efficient DNA alkylation.

Does Sequence Specificity Correlate with the MEP of the N^7-Position of Guanine Residues In Duplex DNA? The effects of flanking DNA sequence on the MEP at the N^7-position of guanine residues in double-stranded DNA has been calculated previously (*24*). Many positively charged alkylating agents reacting at the N^7-position of guanine residues show a very good correlation between MEP and preferred alkylation sites, with efficient alkylation occurring at guanine residues within sequences that foster a larger negative electrostatic potential at N^7-G (*14, 21, 22, 24*). In the reaction of many nitrogen mustards with DNA, the MEP appears to be a primary determinant of sequence specificity (*21, 22*). Similar findings have recently been reported for the alkylation of guanine residues by the mitomycin C metabolite, 2,7-diaminomitosene (*14*).

Our results showing that DNA alkylation by activated LM is inhibited by added salts indicate that there may be an electrostatic component to the reaction. Thus, we investigated whether the sequence specificity observed for the alkylation of guanine residues in double-stranded DNA by LM correlates strongly with MEP. For purposes of comparison, we also examined the relationship between the MEP and the sequence specificity of DNA alkylation by the simple episulfonium ion **6** (Scheme 2
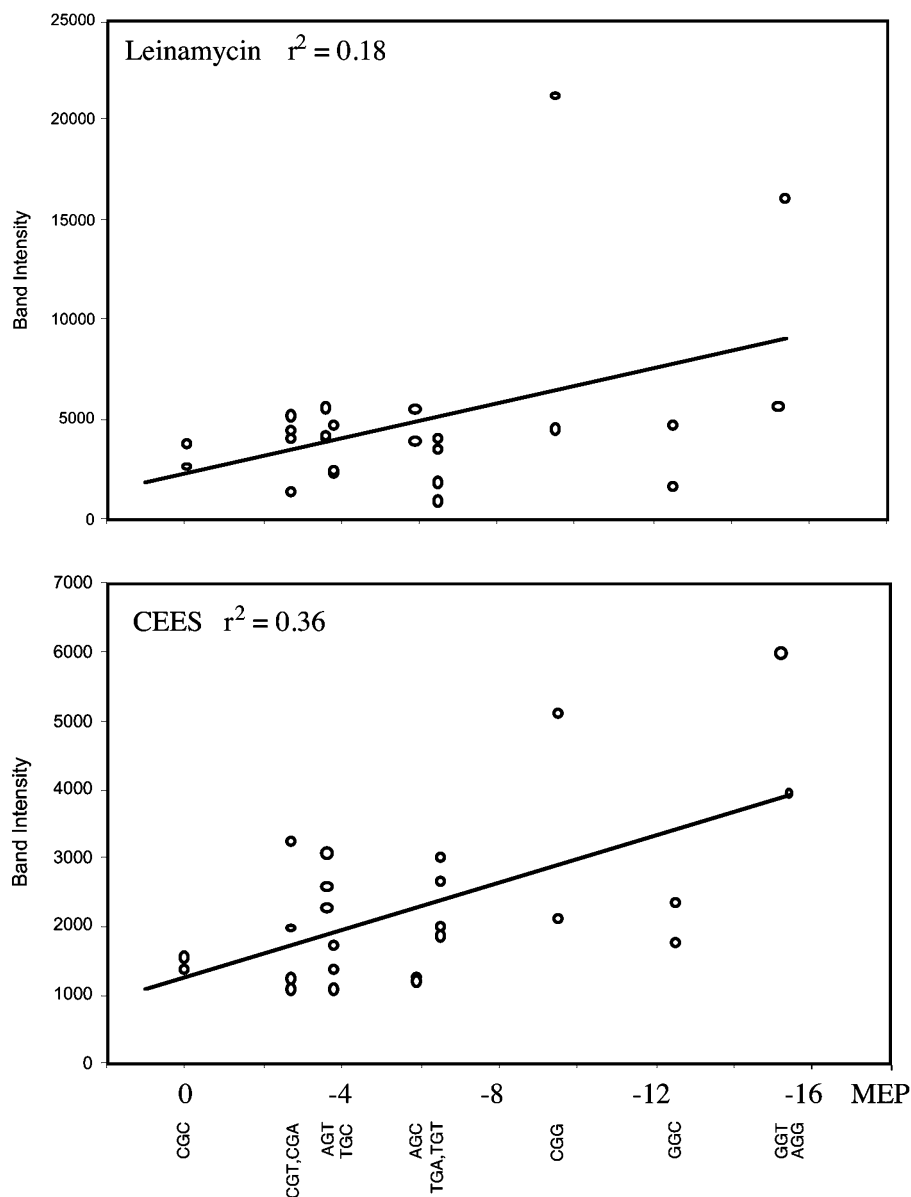
Figure 5. Plots of alkylation efficiency vs calculated (*25*) MEP for individual guanine residues in the 377 base pair EcoR I-BamH I restriction fragment from pBR322 (analysis uses alkylation data shown in Figures 2 and 3). The upper panel is DNA alkylation by thiol-activated LM, and the lower panel is DNA alkylation by CEES (**5**).

and Figure 4). The sequence specificity of DNA alkylation by LM correlates poorly with MEP (the correlation coefficient, r^2, is approximately 0.2; Figure 5). The sequence preferences for DNA alkylation by the sulfur mustard **6** show a slightly more significant correlation with MEP (r^2 approximately 0.4; Figure 5). Similar results can be gleaned from the data published by Guengerich and co-workers regarding the sequence specificity of DNA alkylation by the episulfonium ion derived from *S*-(2-chloroethyl)glutathione (*40*). In contrast, the sequence preferences of many nitrogen mustards correlate much more strongly with MEP, showing r^2 values of 0.75−0.9 (*21*). Analysis of our data indicates that MEP is not the primary determinant of sequence specificity for DNA alkylation by LM.

Discussion

We find that LM shows significant (>10-fold) preferences for alkylation at certain sequences in duplex DNA

(Figures 1−3). Analysis of DNA alkylation by LM in several different DNA restriction fragments and 2′-deoxyoligonucleotide duplexes reveals that 5′-GG is the most preferred alkylation site, followed by 5′-GT sequences. Reaction at 5′-GC and 5′-GA sites is disfavored. Although bases surrounding these two base sequences clearly influence alkylation efficiency (this is indicated, for example, by the fact that all 5′-GG sequences are not alkylated with equal efficiency), we cannot detect a clear trend in the favored (or disfavored) bases on the 5′-side of the alkylated base or the second-nearest neighbor on the 3′-side of the alkylation site.

We find that DNA alkylation by LM is inhibited by addition of salt (100 mM NaClO$_4$). Such an effect is typical for positively charged alkylating agents (*21, 22, 35*) and indicates that despite the fact that activated LM exists predominantly as the negatively charged epoxide **3** and alkylates DNA via the zwitterionic episulfonium ion **2**, this agent nonetheless displays properties characteristic of cationic alkylating agents. The data suggest

that electrostatic interactions between the activated LM and the N[7]-position of guanine residues in DNA facilitate the alkylation reaction. However, we find that the observed sequence preferences for DNA alkylation by LM do not correlate strongly with calculated sequence-dependent variations in the MEP at the N[7]-atom in guanine residues in duplex DNA (Figure 5). Thus, electrostatic interactions between activated LM and DNA do not appear to be the primary determinant for sequence specificity of DNA alkylation by this natural product.

The sequence specificities seen for DNA alkylation by the simple episulfonium ion **6** and activated LM (**2**) show marked differences (Figures 1, 3, and 4). For example, the 5′-A<u>G</u>G site shown in Figure 3 is an alkylation hotspot for activated LM but is not particularly favored by **6**. Thus, it is clear that the sequence preferences seen for LM are not simply those expected for a "generic" episulfonium ion. It seems unlikely that the differences in the sequence specificity of LM and **6** are due only to steric effects in the reaction of the larger LM-derived episulfonium ion with DNA. This conclusion is based on the fact that alkylation at the sterically unencumbered central guanine in 5′-GGG sequences is not favored over reaction at more sterically hindered sites such as 5′-NGT or 5′-TGN. In fact, some of the relatively hindered 5′-GT sequences examined in our study are favored alkylation sites for activated LM (for discussion of sequence-dependent steric accessibility in duplex DNA, see refs *24* and *25*).

Overall, it seems likely that sequence-specific noncovalent interactions of LM with the DNA duplex on the 3′-side of the alkylated guanine residue play a major role in determining the preferred alkylation sites. As part of these studies, we have provided data supporting the notion that LM does, in fact, bind noncovalently to the DNA duplex. Specifically, the observation that LM does not efficiently alkylate single-stranded DNA under conditions where double-stranded DNA is efficiently modified (Figure 3) provides evidence that noncovalent association of activated LM with the double helix facilitates efficient DNA alkylation. Similar findings provided early evidence that the noncovalent binding of aflatoxin B$_1$ epoxide to duplex DNA is required to achieve efficient alkylation at guanine residues (*41*). Indeed, subsequent studies demonstrated that aflatoxin epoxide binds to DNA via intercalation prior to reaction (*42*). Further circumstantial evidence for noncovalent binding of LM to DNA is provided by our observation that DNA alkylation by the LM-derived episulfonium ion is markedly more efficient than that by the simple episulfonium ion **6**. It is well-known that noncovalent association of reactive species to the double helix can markedly facilitate covalent DNA modification (*11, 26−30, 43−45*).

Interestingly, the sequence preferences displayed by activated LM (favored 5′-<u>G</u>G, disfavored 5′-<u>G</u>C) are similar to those seen previously for a quinacrine mustard that is thought to bind noncovalently to DNA via intercalation on the 3′-side of the alkylated guanine residue (*22*). Similarly, the favored 5′-<u>G</u>G alkylation site observed for LM has recently been suggested by Saito and co-workers as a preferred alkylation site for neutral, DNA intercalating epoxides (*46*). On the basis of these literature precedents, it is tempting to speculate that the planar (*47*) thiazole-2,4-dienone fragment on the left hand side of LM serves as an unusual type of DNA intercalator, where nonaromatic conjugated π-bonds are

part of the intercalating system. This general phenomenon has been seen previously in a few instances (*48−50*).

In summary, our results suggest that electrostatic interactions between the charged episulfonium moiety of activated LM and the N[7]-position of guanine residues in DNA facilitate efficient DNA alkylation reactions but that the observed sequence specificity for this natural product arises primarily through noncovalent interactions with the double helix on the 3′-side of the alkylated base. Further studies are required to characterize the manner in which LM interacts with duplex DNA.

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