Cytosine Methylation Enhances Mitomycin C Cross-Linking†

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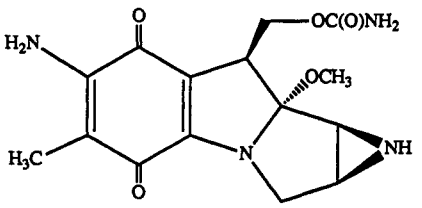
ABSTRACT: Mitomycin C (MC) is a powerful antitumor agent that targets the DNA sequence CpG. Because it is likely that this dinucleotide will contain 5-methylcytosine *in vivo,* we have compared the cross-linking efficiency of MC for DNA containing either 5-methylcytosine or normal cytosine embedded in random-sequence DNA oligomers. We have found that mitomycin C displays a small but significant preference for methylated DNA. Recognition of an abnormal methylation pattern in the DNA of transformed cells may therefore be one mechanism by which MC exerts its chemotherapeutic effects.

Mitomycin C (MC, **I**) is a toxic antibiotic widely used to treat cancer (Crooke & Bradner, 1976). Upon reductive activation, it forms DNA interstrand cross-links with a strong base-sequence specificity; interstrand cross-links are formed exclusively between the two guanines at CpG·CpG sequences and not at GpC·GpC sequences (Chawla et al., 1987; Weidner et al., 1989; Teng et al., 1989). The residues flanking this minimal core sequence also affect the reactivity of MC and modulate the overall reactivity of the CpG sequence (Millard et al., 1990; Borowy-Borowski et al., 1990).



I

In addition to being mitomycin C's target, CpG is exceptional among dinucleotide sequences in other ways. It is vastly under-represented in bulk vertebrate DNA, occurring overall at one-fifth its expected statistical frequency (Bird et al., 1985). Indeed, this doublet is significantly depleted in about 99% of the genome in higher eukaryotes (Holliday & Grigg, 1993), whereas the other 1% contains CpG at the expected frequency. These so-called CpG "islands" have an unknown function and appear to be associated with many genes (Bird, 1986).

Another distinguishing feature of CpG is that 60–90% of these sequences *in vivo* are methylated at the 5-position of cytosine, whereas only 3–5% of the total cytosine residues show this modification (Bird, 1986). Methylation is thought to be one mechanism for transcriptional regulation of genetic material (Cedar, 1988). For example, certain specific sites in the β-globin gene are less methylated in erythrocytes, where this gene is expressed, than in oviduct tissue, where it is not (McGhee & Ginder, 1979). Introduction of methylated genes into cells has confirmed that an inverse correlation between DNA methylation and transcriptional activity often exists (Stein et al., 1982; Busslinger et al., 1983; Keshet et al., 1985; Yisraeli et al., 1988). However, some tissue-specific genes do not follow this pattern because of their promoters' association with CpG islands, which generally remain free of detectable methylation (Antequera et al., 1990).

Cytosine methylation alters the local structure of duplex DNA (Hodges-Garcia & Hagerman, 1992) and thus may exert its regulatory effect through a DNA conformational change as well as through direct methyl group–protein interactions. Methylation may reduce gene expression by preventing the binding of protein activators or by facilitating the binding of protein inhibitors in different gene contexts. For example, methylation inhibits binding of a portion of c-Myc oncoprotein, which is thought to be involved in cell growth control (Prendergast & Ziff, 1991), yet certain down-regulating factors preferentially bind to methylated DNA (Pawlak et al., 1991; Meeham et al., 1989; Boyes & Bird, 1991). The exact role of methylation in the regulation of cell and gene function remains to be established.

Because MC's target sequence is likely to be methylated *in vivo,* we have examined its cross-linking preferences for CpG sequences containing either 5-methylcytosine or normal cytosine embedded in random-sequence DNA oligomers. Recognition of an aberrant methylation pattern of cancer cell DNA may be one mechanism by which MC exerts its chemotherapeutic effects. Both hypomethylation and hypermethylation are found in transformed cells (Jones & Buckley, 1990; Holliday & Grigg, 1993), and DNA methylase activity is abnormally high in some tumor cells (El-Deiry et al., 1991).

We used two different strategies to evaluate mitomycin C's preference for methylated versus normal DNA. One technique for quantifying the cross-linking efficiencies of different sequences has been to compare the cross-linking of oligomers containing these different sequences (Teng et al., 1989; Millard et al., 1990; Borowy-Borowski et al., 1990; Kirchner & Hopkins, 1991; Millard & White, 1993; Woo et al., 1993a,b). We therefore used Cerenkov counting of cross-linked products following denaturing polyacrylamide gel electrophoresis (DPAGE) isolation for different duplexes with either methylated or normal CpG sites. Our second technique was quantitative iron(II)-EDTA fragmentation to determine the partitioning of mitomycin C between a CpG site and a ZpG site (where Z is 5-methylcytosine) within the same duplex. This method has been used previously to locate the sites of interstrand cross-links in DNA fragments at single nucleotide resolution (Weidner et al., 1989; Millard et al., 1990; Weidner

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et al., 1990; Hopkins et al., 1991) and to quantitate isomeric cross-linked products differing in nucleotide connectivity not resolved by DPAGE (Millard et al., 1991). Our studies provide evidence for a 1.4–2-fold enhancement of the MC cross-linking efficiency of DNA fragments containing 5-methylcytosine versus normal cytosine.

MATERIALS AND METHODS

Preparation of Radiolabeled DNA Duplexes. Methylated and control oligonucleotides were purchased from Operon Technologies, Inc., and purified through denaturing polyacrylamide gel electrophoresis (DPAGE) as described previously (Millard & White, 1993). Self-complementary DNAs were 5′-end-radiolabeled with [γ-^{32}P]ATP (3000 Ci/mmol, from Amersham) and T4 polynucleotide kinase (New England Biolabs) under standard conditions (Sambrook et al., 1989). For non-self-complementary DNAs, 0.5 OD_{260} of single strand was annealed with 0.5 OD_{260} of the complementary strand prior to 3′-end-labeling with [α-^{32}P]dATP (3000 Ci/mmol, from Amersham) and Klenow (New England Biolabs) under standard conditions (Sambrook et al., 1989). Radiolabeling was followed by ethanol precipitation using 3 M NaOAc, an 85% ethanol wash, and lyophilization. Self-complementary duplexes are illustrated below as having a single radiolabeled strand for convenience only. Radiolabeling of one, both, or a mixture of one and both strands would not affect the interpretation of the data.

Mitomycin C Cross-Linking. Mitomycin C cross-linked DNAs were prepared as previously described (Millard & Hopkins, 1993). Mitomycin C (from a fresh stock in 33% aqueous methanol) was added to 1 OD_{260} of radiolabeled duplex DNA in 15 mM Tris (pH 7.5) to effect a 2.5:1 drug-to-base pair ratio. Samples were incubated at 37 °C for 2 h, purged with argon for 15 min, and put on ice. The MC was reductively activated through three subsequent additions at 15-min intervals of 1 equiv of fresh sodium dithionite. Cross-linking was followed by ethanol precipitation and lyophilization. To calculate cross-linking efficiencies the resulting pellet was dissolved in 100 μL of purified water, and a 5-μL aliquot was removed for Cerenkov counting to determine total cpm in the reaction. The remaining sample was lyophilized for DPAGE.

Denaturing Polyacrylamide Gel Electrophoresis. Cross-linked samples were resuspended in 5 M aqueous urea/0.1% xylene cyanole and loaded onto a 20% polyacrylamide gel (19:1 acrylamide/bisacrylamide, 50% urea, 0.35 mm thick, 41 × 37 cm), which was run on a Hoefer thermojacketed Poker Face gel stand at ca. 65 W and 65 °C. Autoradiography was used to visualize the single-stranded and cross-linked DNA, which had roughly one-half the mobility of the corresponding single strands. Gel slices of dimensions approximately 1 × 2 cm were excised from the appropriate region of the gel and Cerenkov counted. The percentage of cross-linked DNA was determined from the ratio of counts in the gel slices to the total counts in the reaction. Experiments were repeated and standard deviations calculated as reported.

Hydroxyl Radical Fragmentation. Iron(II)-EDTA cleavage reactions were performed on approximately 50 000 cpm (Geiger counter) DNA in 5 mM Tris (pH 7.5) with 50 μM $(NH_4)_2Fe(SO_4)_2$/100 μM EDTA, 1 mM sodium ascorbate, and 10 mM H_2O_2 in a total volume of 10 μL for 2 min at 25 °C. These cleavage reagents were added to the side of an Eppendorf tube, and the reaction was initiated by brief centrifugation. Reactions were stopped with thiourea (1 μL of a 10 mM solution, followed by vortexing), lyophilized, and sequentially resuspended in 10 μL of loading buffer, heat-denatured, chilled, and loaded onto a 25% polyacrylamide gel, which was run as described above for the 20% gels. The gel was dried (Hoefer Drygel Sr.) onto Whatman 3MM paper and autoradiographed for about 2 h.

Densitometry. Densitometry (Hoefer GS-300, interfaced to a Macintosh II computer) data were smoothed, plotted, and integrated (Hoefer Scientific GS370 Densitometry Program, Version 2.0) to obtain plots of cleavage intensity for each nucleotide. Bands were assigned by reference to a Maxam–Gilbert guanine-specific sequencing reaction (Maxam & Gilbert, 1980) on radiolabeled native duplex DNA. Iron(II)-EDTA cleavage data were normalized by dividing each integrated peak area by the average peak area for all fragments derived from cleavage one residue or more to the radiolabeled side of the first anticipated site of cross-linking (e.g., residues T4–C6/Z6 in Figure 5).

Table I: DNA Duplex Fragments Used in These Studies[a]

nucleotide sequence	descriptor
5′*AATATATTCGAATAT3′ 3′TATAAGCTTATATAA5′	**1C**
5′*AATATATTZGAATAT3′ 3′TATAAGZTTATATAA5′	**1Z**
5′AAT-TTATGCGCATGCGCATT3′ 3′A*AATACGCGTACGCGTAA5′ 4 7 13	**2CC**
5′AAT-TTATGCGCATGZGZATT3′ 3′A*AATACGCGTAZGZGTAA5′ 4 7 13	**2CZ**
5′AAT-TTATGZGZATGZGZATT3′ 3′A*AATAZGZGTAZGZGTAA5′ 4 7 13	**2ZZ**

[a] An asterisk (*) indicates the position of the ^{32}P-radiolabel; Z = 5-methylcytosine. Numbering scheme is as shown.

RESULTS

The self-complementary DNA duplexes **1C** and **1Z** (Table I), containing a central TCGA site, were 5′-end-labeled and cross-linked with mitomycin C. These two duplexes differed in that they contained either normal cytosine (**1C**) or 5-methylcytosine (**1Z**) in the putative cross-linking site. DNA was isolated by precipitation and analyzed by 20% DPAGE. The resulting autoradiogram is shown in Figure 1. In addition to recovering single strands, and presumably monoadducts (band 1), a less mobile interstrand cross-linked product was also formed in each MC reaction (band 2). Immediately apparent was the seemingly higher yield of cross-link for the duplex containing the methylated site. We therefore attempted to quantify the methylation-induced increase in mitomycin C cross-linking.

A total of 17 MC cross-linking trials for the methylated duplex **1Z** were compared to 18 trials for the control duplex **1C**. Reactions were run on sets of three of each DNA (one sample was lost during gel loading). Following cross-linking and DPAGE, autoradiography was used to locate band 2, which was excised for Cerenkov counting and subsequent

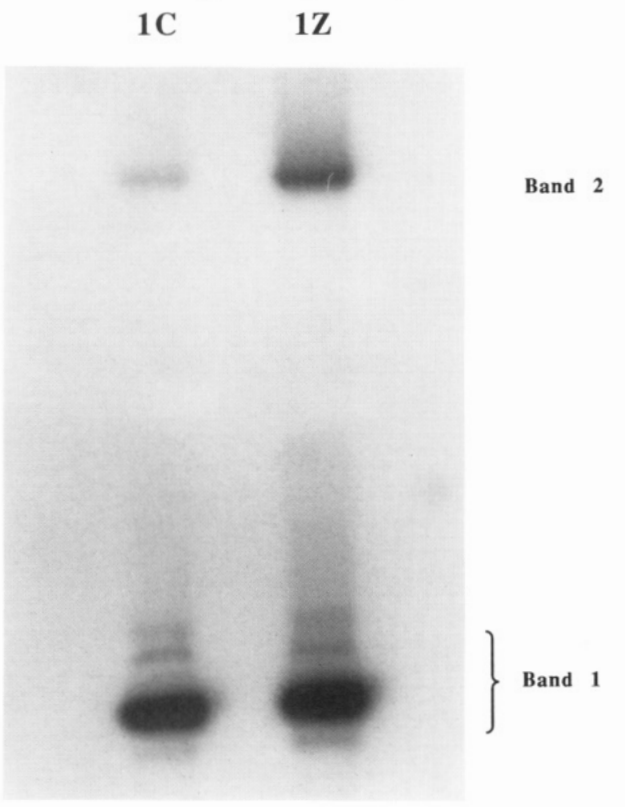

FIGURE 1: DPAGE analysis of mitomycin C cross-linking reactions with duplexes **1C** (normal) and **1Z** (methylated). Cross-linked DNA (band 2) has a lower mobility than native and monoadducted DNA (band 1).

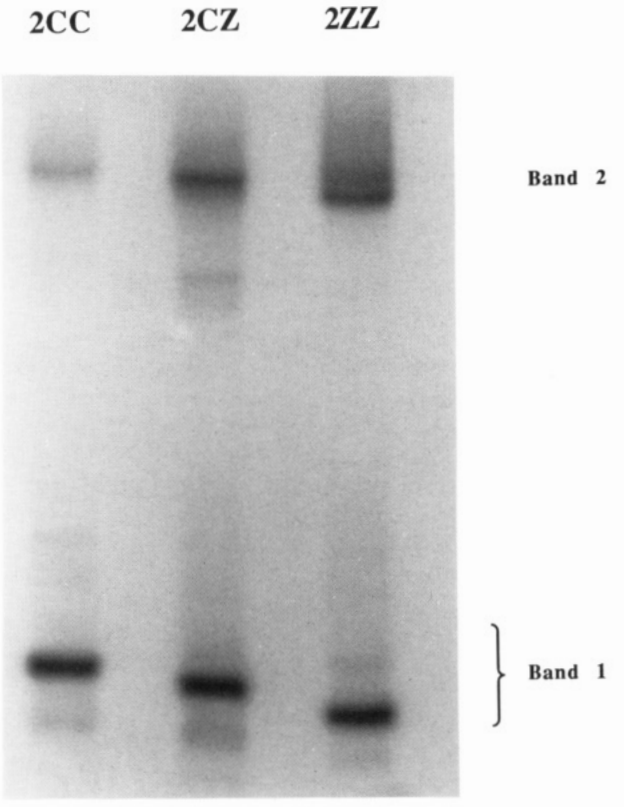

FIGURE 2: DPAGE analysis of mitomycin C cross-linking reactions on two-site duplexes: lane 1, **2CC** (normal); lane 2, **2CZ** (one normal, one methylated site); lane 3, **2ZZ** (both methylated).

Table II: MC Cross-Linking Efficiencies of Different DNAs

DNA[a]	av % cross-linking	N^b	sd[c]	p^d
1C	1.21	18	0.65	
1Z	1.88	17	1.56	0.05 (**1Z** > **1C**)
2CC	5.78	8	1.67	
2CZ	10.06	7	3.26	0.003 (**2CZ** > **2CC**)
2ZZ	16.39	3	2.18	0.008 (**2ZZ** > **2CZ**)

[a] See Table I. [b] Number of replicates. [c] Standard deviation. [d] One-tailed student's *t*-test comparing the indicated mean cross-linking efficiencies.

determination of cross-linking efficiency for each reaction. Table II summarizes the resulting cross-linking efficiencies of these trials. The average percent cross-linking was 1.21 ± 0.65 for the control DNA versus 1.88 ± 1.56 for the methylated DNA. Although our efficiencies are dramatically lower than those reported by Borowy-Borowski et al. (1990), they are comparable to those found by Teng et al. (1989) and Millard et al. (1991). Why these efficiencies differ is unknown since similar cross-linking conditions were used.

Although the total cross-linking yields differed by as much as a factor of 2 from one set of experiments to the next, values for the same duplex within a series were much more reproducible. Because these data were variable, a Student *t*-test was used for analysis. A one-tailed test demonstrated that the MC cross-linking efficiency for the methylated DNA was greater than that of the control DNA, with $p = 0.05$. Although the standard deviations for these measurements are quite large, mitomycin C apparently shows a small but significant preference for cross-linking methylated over normal DNA. This difference is about 1.5-fold in this duplex.

To verify that this preference was not a function of the TCGA site in particular, we also studied duplexes **2CC**, **2CZ**, and **2ZZ** (Table I), all containing two MC sites with flanking sequences different from those of duplex **1**. The GCGC site of these duplexes has previously been demonstrated to be cross-linked more efficiently than the TCGA site (Millard et al., 1990; Borowy-Borowski et al., 1990; Millard et al., 1991). Duplex **2CC** contained only normal cytosine residues, duplex **2CZ** contained normal cytosine in both strands at the first cross-linking site and 5-methylcytosine in both strands at the second cross-linking sites and duplex **2ZZ** contained only 5-methylcytosine. Figure 2 shows a typical autoradiogram of MC cross-linking reactions for these duplexes, which again shows both native (band 1) and cross-linked (band 2) DNA. Interestingly, the mobilities of the native DNA's differ depending on the degree of methylation. Electrophoretic mobilities of methylated fragments previously have been shown to differ from those of unmethylated molecules having the same base sequence (Hodges-Garcia & Hagerman, 1992). In addition to being noticeably more intense for the methylated duplexes, band 2 also shows some heterogeneity. Two chemically distinct molecules with slightly different mobilities result from cross-linking this asymmetric duplex. The entire band corresponding to cross-linked product was excised from the gel for quantification.

Table II summarizes the MC cross-linking efficiencies for several trials on the two-site DNA's. The average percent cross-linking was 5.78 ± 1.67 for **2CC** (no methylation) versus 10.06 ± 3.26 for **2CZ** (one site methylated) versus 16.39 ± 2.18 for **2ZZ** (both sites methylated). These data further support the hypothesis that cytosine methylation enhances mitomycin C cross-linking. Substitution of one methylated site for a nonmethylated one increases cross-linking by 1.6–1.8-fold ($p = 0.003$ with a one-tailed *t*-test for **2CC** and **2CZ**; $p = 0.008$ for **2CZ** and **2ZZ**). Likewise, substitution of two methylated sites for two nonmethylated ones increases cross-linking by 2.8-fold or an average of about 1.4-fold per site.

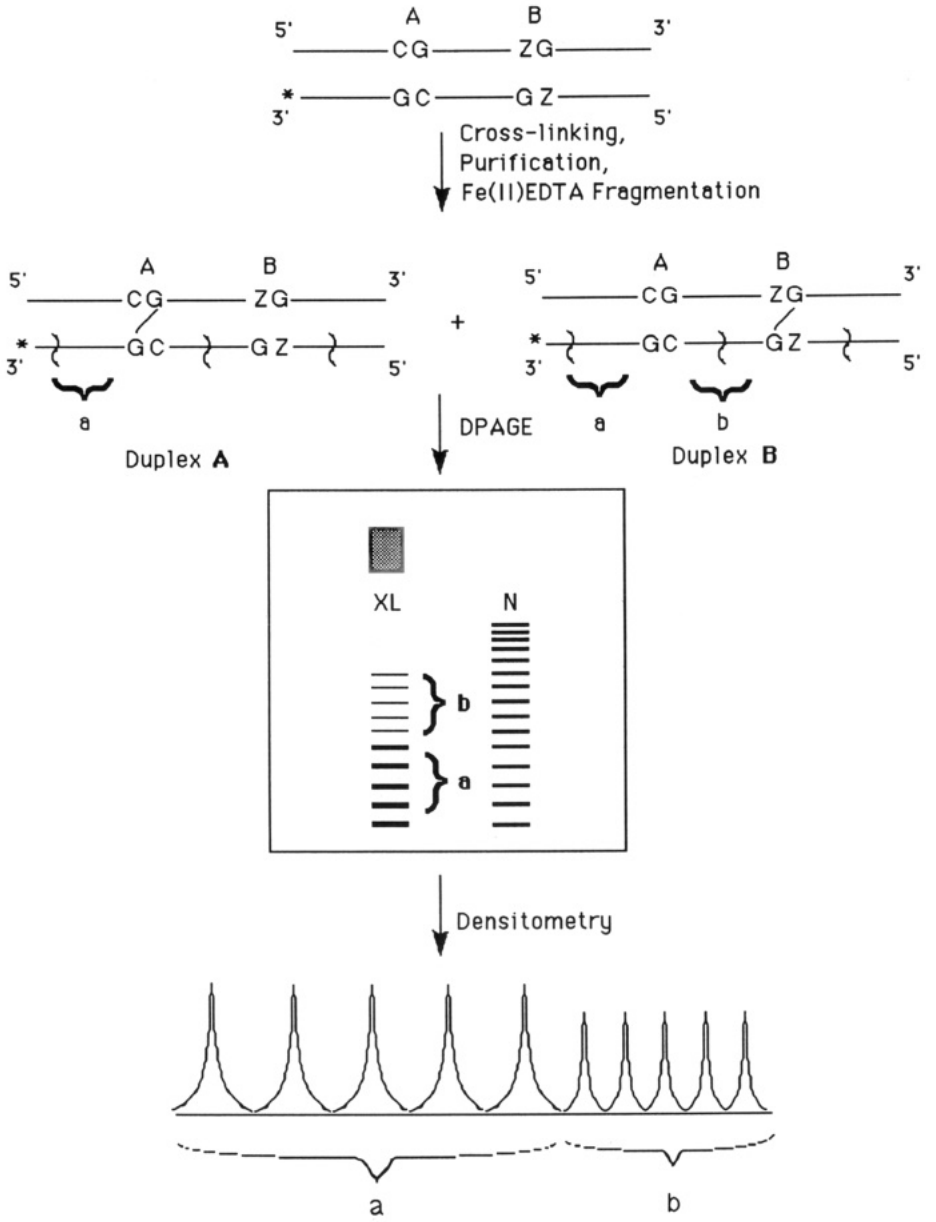

FIGURE 3: Steps involved in quantitative iron(II)-EDTA fragmentation on cross-linked (XL) and native (N) DNA. Asterisks denote ^{32}P radiolabels; curved lines represent chemical cleavage, which occurs with single-hit kinetics under our conditions.

To determine the partitioning of mitomycin C between a methylated and a nonmethylated site within the same duplex, we subjected purified cross-link to random single-hit fragmentation using iron(II)-EDTA (Tullius et al., 1987). Only fragmentation between the radiolabel and the cross-linked nucleotide affords fragments shorter than the starting single strand. Thus, the abundance of various fragment sizes, as analyzed by single-nucleotide-resolving DPAGE, reveals sites and efficiencies of cross-linking (Weidner et al., 1989; Millard et al., 1990, 1991; Hopkins et al., 1991). Random single-hit fragmentation of native DNA, in contrast, yields an equimolar mixture of all fragment sizes up to and including the full-length single strand.

Figure 3 illustrates this principle. Consider the DNA duplex depicted containing the normal site A and the methylated site B. Cross-linking will produce a mixture of the cross-linked duplexes **A** and **B**, containing a single cross-link at sites A and B, respectively. Random fragmentation of both **A** and **B** yields radiolabeled fragments up to the A site, producing fragments denoted **a**. However, only cleavage of duplex **B** produces short fragments (denoted **b** on the autoradiogram) upon fragmentation between sites A and B. Cleavage of **A** after the cross-linked site A renders radiolabeled fragments much larger than the reference single strand because of the covalent attachment of the nonlabeled strand via the cross-linking agent. Neither duplex produces short fragments upon cleavage after site B. Separation of the resulting products through single-base-resolving DPAGE produces a distinct pattern for the cross-linked mixture relative to the control native DNA (N). The drops in cleavage intensity are diagnostic for the sites of cross-linking.

This analysis predicts that the yield of fragments **b** will be lower than that of fragments **a** and that this decrease will correspond to the relative abundance of duplexes **A** and **B**. The relative amounts of fragments **a** and **b** can be obtained through integration of the densitometry data, as shown at the bottom of Figure 3. If the average area of the peaks corresponding to the **b** fragments is equal to 50% that of the average area of the **a** peaks, then duplexes **A** and **B** are present in equal amounts and neither site is preferred by the cross-linking agent. On the other hand, if the average area of the **b** peaks is greater than 50% of the average for **a** (as depicted),

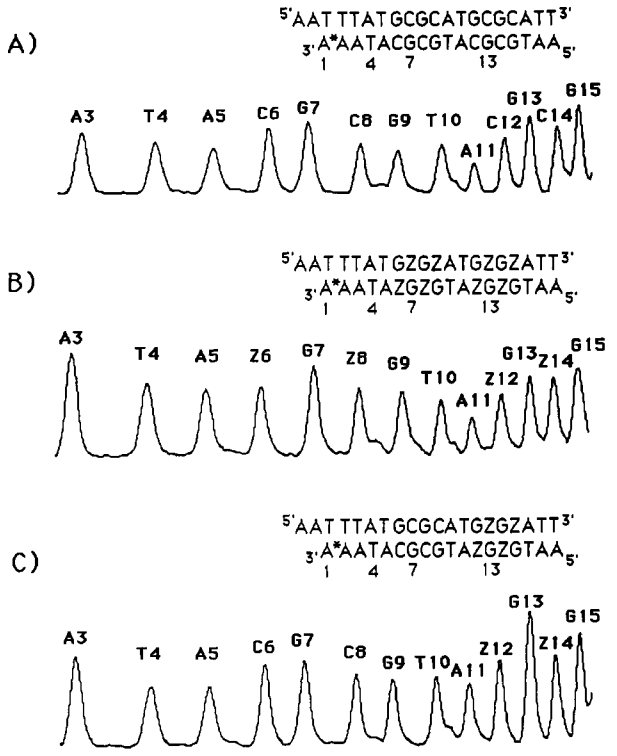

FIGURE 4: Partial fragmentation pattern for 3'-end-radiolabeled (* indicates ^{32}P) native duplexes using iron(II)-EDTA: (A) **2CC**; (B) **2ZZ**; (C) **2CZ**. Lettering denotes the residues cleaved.

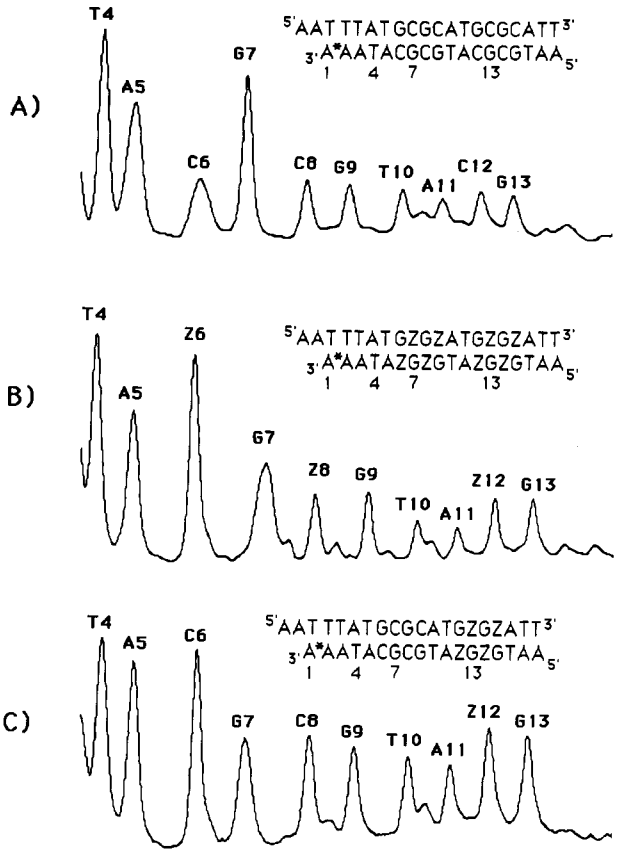

FIGURE 5: Partial fragmentation pattern for 3'-end-radiolabeled (* indicates ^{32}P) mitomycin C cross-linked duplexes using iron(II)-EDTA: (A) **2CC**; (B) **2ZZ**; (C) **2CZ**. Lettering denotes the residue cleaved.

then the B site, or the methylated site in this case, is preferred. Likewise, if the average area of **b** is less than 50% that of **a**, then the nonmethylated site is preferred.

This assay was used to quantify the cross-linking of the two-site DNA (**2**) containing either two normal sites (**2CC**), one normal and one methylated site (**2CZ**), or two methylated sites (**2ZZ**). Each duplex was independently 3'-end-radiolabeled and reacted with mitomycin C. The resulting cross-linked material was then isolated through DPAGE. Native and cross-linked samples were subjected sequentially to iron(II)-EDTA fragmentation, DPAGE, and autoradiography. The cleavage patterns of the control native DNAs were virtually indistinguishable, with approximately equal cleavage at each residue (Figure 4). However, for all three cross-linked samples, the yield of fragments corresponding to cleavage at residues C8/Z8–G13 was diminished relative to fragments from T4–G7 (Figure 5). In addition, there was a virtual absence of fragments between C14/Z14 and A18. A population of molecules containing some that are cross-linked at G7 and the rest at G13 should give exactly this result. Furthermore, the decreases were comparable for the duplexes containing two identical sites (**2CC** and **2ZZ**), yet different for the duplex containing two distinct sites (**2CZ**). Figure 6 shows the normalized fragment abundance, calculated through integration of the densitometry data, for the three duplexes. Aberrant reactivity of residues in the vicinity of the MC cross-link has been noted previously (Millard et al., 1991), presumably because of local structural deformations (Jolles et al., 1993). For this reason, residues G7 and G13 were excluded from the analysis. Normalization of the average of cleavage at T4–C6/Z6 to 100% results in an average relative yield for C8/Z8–C12/Z12 of 50% for the **2CC** duplex, 45% for the **2ZZ** duplex, and 68% for the **2CZ** duplex. These results are consistent with a 1:1 mixture of cross-links at G7 and G13 in the two duplexes with identical sites and about a 1:2 ratio in the duplex with distinct sites. That is, the methylated site is preferred by about a 2:1 ratio over the nonmethylated site within the same duplex. These results concur with those previously presented for duplex **1**.

DISCUSSION

Mitomycin C is a highly toxic antibiotic that clinically is most effective for colorectal, lung, head and neck, breast, stomach, and pancreatic carcinomas (Moore et al., 1968; Crooke & Bradner, 1976). Its biological activity is thought to derive from its DNA interstrand cross-linking ability. Because the dinucleotide target of mitomycin C, CpG, is vastly overmethylated *in vivo*, we examined the effect of cytosine methylation on the drug's cross-linking efficiency. Interestingly, El-Deiry et al. (1991) have reported a 200-fold increase in methyltransferase activity in human colonic carcinomas, and Baylin et al. (1986) have found regions of increased methylation in human small cell lung carcinomas, indicating hypermethylation in some of the very cancers toward which MC shows activity. We have found that cytosine methylation does indeed enhance the efficiency of mitomycin C cross-linking.

Initially, we compared the cross-linking of two duplexes each containing a TCGA central sequence and differing only in the substitution of a 5-methyl-CpG site (**1Z**) for a normal CpG site (**1C**). Purified cross-linked material was Cerenkov counted and compared to the total radioactivity in the initial sample to determine cross-linking efficiencies. This technique has been used to determine sequence preferences of other cross-linking agents such as nitrous acid (Kirchner & Hopkins, 1991), diepoxybutane (Millard & White, 1993), and several pyrrole-based mitomycin C analogs (Weidner et al., 1990).

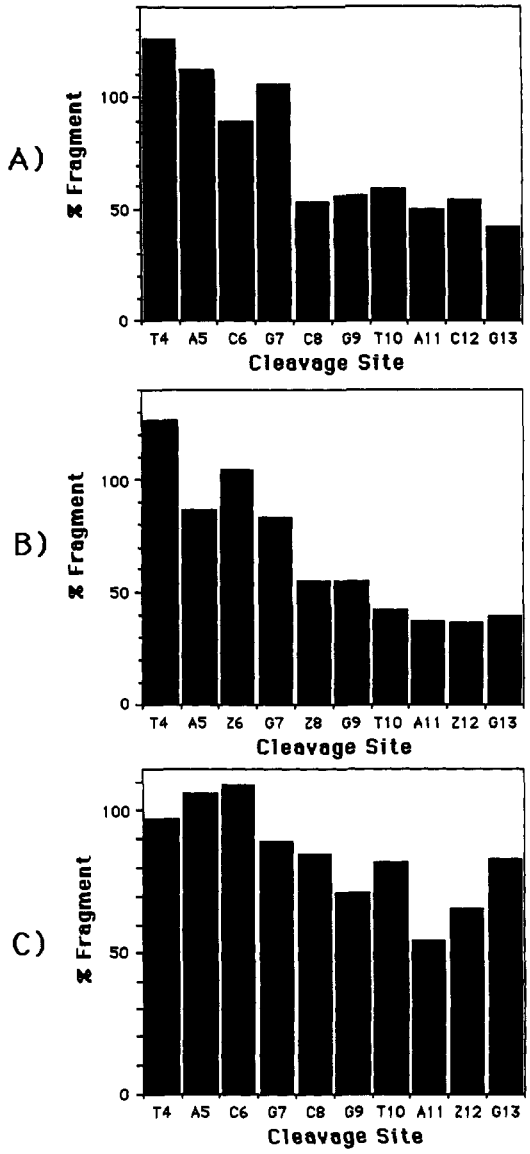

FIGURE 6: Normalized fragment abundance of MC-cross-linked DNA as a function of cleavage site: (A) **2CC**; (B) **2ZZ**; (C) **2CZ**.

The quantification of several trials led us to conclude that mitomycin C shows about a 1.5-fold preference for the methylated TCGA site over the control.

This preference of MC for 5-methylcytosine-containing sites is not unique to duplex **1**: other DNAs displayed this same effect. The parent duplex **2** (Table I) contained a GCGC mitomycin C core sequence instead of the TCGA core of oligomer **1**. In addition, duplex **2** contained two potential sites for MC cross-linking. The cross-linking efficiencies of the members of this family increased with the number of methylated sites present. Duplex **2CC**, containing two normal GCGC sites, was cross-linked the least, whereas duplex **2ZZ**, containing two methylated GCGC sites, was cross-linked the most. Duplex **2CZ**, containing one normal and one methylated site, was intermediate. Replacement of a normal site by a methylated one enhanced cross-linking efficiency by about 1.4–1.8-fold in this series.

Duplex **2CZ** allowed the comparison of a methylated and normal site within the same molecule. This was particularly crucial because of the small effect at hand. Random chemical cleavage followed by the separation of resulting fragments was performed for both a native and a cross-linked sample. This technique has been used previously to determine the impact of neighboring residues on the mitomycin C cross-linking of CpG sites (Millard et al., 1990, 1991). Our control duplexes, containing two identical sites (**2CC** and **2ZZ**), both showed approximately equal cross-linking of the two CpG sites within the same molecule. However, fragmentation of the duplex with distinct sites indicated that the molecule containing a cross-link at the methylated CpG site is present in about a 2-fold excess over that containing a cross-link at the normal CpG site. Thus, when presented with a choice of two sites within the same duplex that differ only in their methylation patterns, mitomycin C is twice as likely to cross-link the methylated site.

In addition to the absolute core specificity of mitomycin C for the dinucleotide sequence CpG, the identity of neighboring residues has a significant impact on the relative reactivity of these sites (Millard et al., 1990; Borowy-Borowski et al., 1990). The results reported herein suggest that mitomycin C is also affected by the presence of 5-methylcytosine in the cross-link site. We have found that the dinucleotide 5-methyl-CpG is cross-linked in a 1.4–2-fold preference to normal CpG in the duplexes examined. This difference may arise from local charge effects, rendering the N2 of the reactive guanine more nucleophilic, or from a local conformational change, rendering it more accessible. Further investigation into the origin of this effect clearly is warranted. Recognition of an aberrant DNA methylation pattern may be one of the factors accounting for the somewhat selective nature of mitomycin C's cross-linking toward transformed cells and for its resulting chemotherapeutic value.

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REFERENCES

Antequera, F., Boyes, J., & Bird, A. (1990) *Cell 62*, 503.
Baylin, S. B., Hoppener, J. W. M., de Bustros, A., Steenbergh, P. H., Lips, C. J. M., & Nelkin, B. (1986) *Cancer Res. 46*, 2917.
Bird, A. R. (1986) *Nature 321*, 2095.
Bird, A., Taggart, M., Frommer, M., Miller, O. J., & Macleod, D. (1985) *Cell 40*, 91.
Borowy-Borowski, H., Lipman, R., & Tomasz, M. (1990) *Biochemistry 29*, 2999.
Boyes, J., & Bird, A. (1991) *Cell 64*, 1123.
Busslinger, M., Hurst, J., & Flavell, R. A. (1983) *Cell 34*, 197.
Cedar, H. (1988) *Cell 53*, 3.
Chawla, A. K., Lipman, R., & Tomasz, M. (1987) in *Structure and Expression, Volume 2: DNA and Its Drug Complexes* (Sarma, R. H., & Sarma, M. H., Eds.) p 305, Adenine Press, Guilderland, NY.
Crooke, S. T., & Bradner, W. T. (1976) *Cancer Treat. Rev. 3*, 121.
El-Deiry, W. S., Nelkin, B. D., Celano, P., Yen, R.-W. C., Falco, J. P., Hamilton, S. R., & Baylin, S. B. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 3470.
Hodges-Garcia, Y., & Hagerman, P. J. (1992) *Biochemistry 31*, 7595.
Holliday, R., & Grigg, G. W. (1993) *Mutat. Res. 285*, 61.
Hopkins, P. B., Millard, J. T., Woo, J., Weidner, M. F., Kirchner, J. J., Sigurdsson, S. T., & Raucher, S. (1991) *Tetrahedron 47*, 2475.
Jolles, B., Laigle, A., Liquier, J., & Chinsky, L. (1993) *Biophys. Chem. 46*, 179.
Jones, P. A., & Buckley, J. D. (1990) *Adv. Cancer Res. 54*, 1.

Keshet, I., Yisraeli, J., & Cedar, H. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 2560.
Kirchner, J. J., & Hopkins, P. B. (1991) *J. Am. Chem. Soc. 113*, 4681.
Maxam, A. M., & Gilbert, W. (1980) *Methods Enzymol. 65*, 499.
McGhee, J. D., & Ginder, G. D. (1979) *Nature (London) 280*, 419.
Meeham, R. R., Lewis, J. D., McKay, S., Kleiner, E. L., & Bird, A. P. (1989) *Cell 58*, 499.
Millard, J. T., & Hopkins, P. B. (1993) *Mutat. Res. 285*, 165.
Millard, J. T., & White, M. M. (1993) *Biochemistry 32*, 2120.
Millard, J. T., Weidner, M. F., Raucher, S., & Hopkins, P. B. (1990) *J. Am. Chem. Soc. 112*, 3637.
Millard, J. T., Weidner, M. F., Kirchner, J. J., Ribeiro, S., & Hopkins, P. B. (1991) *Nucleic Acids Res. 19*, 1885.
Moore, G. E., Bross, I. D. J., Ausman, R., Nadler, S., Jones, R., Slack, N., & Rimm, A. A. (1968) *Cancer Chemother. Rep. 52*, 675.
Pawlak, A., Bryans, M., & Jost, J.-P. (1991) *Nucleic Acids Res. 19*, 1029.
Prendergast, G. C., & Ziff, E. B. (1991) *Science 251*, 186.
Sambrook, J., Fritsch, E. G., & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Approach*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Stein, R., Razin, A., & Cedar, H. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 3418.
Teng, S. P., Woodson, S. A., & Crothers, D. M. (1989) *Biochemistry 28*, 3901.
Tullius, T. D., Dombroski, B. A., Churchill, M. E. A., & Kam, L. (1987) *Methods Enzymol. 155*, 537.
Weidner, M. F., Millard, J. T., & Hopkins, P. B. (1989) *J. Am. Chem. Soc. 111*, 9270.
Weidner, M. F., Sigurdsson, S. T., & Hopkins, P. B. (1990) *Biochemistry 29*, 9225.
Woo, J., Sigurdsson, S. T., & Hopkins, P. B. (1993a) *J. Am. Chem. Soc. 115*, 1199.
Woo, J., Sigurdsson, S. T., & Hopkins, P. B. (1993b) *J. Am. Chem. Soc. 115*, 3407.
Yisraeli, J., Frank, D., Razin, A., & Cedar, H. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 4638.