Cleavage by Calicheamicin γ_1^I of DNA in a Nucleosome Formed on the 5S RNA Gene of *Xenopus borealis*†

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ABSTRACT: The cleavage by calicheamicin γ_1^I (CLMγ_1^I) of a nucleosome formed on the 5S RNA gene of *Xenopus borealis* was studied *in vitro* as a first step toward the understanding of CLMγ_1^I−chromatin interactions within the cell. The drug does not cleave in the region of the dyad axis of the nucleosome. Outside of this region, double-stranded cleavage occurs with a periodicity of 10−11 bp. The sites of cleavage correspond to DNA sequences facing outward in the nucleosome. The drug shows some sequence preference of cleavage within these accessible sites. The predominant cleavage event within this nucleosome occurs at −1 helical turn from the dyad axis. This site constitutes a "hot spot" for CLMγ_1^I cleavage within the 5S nucleosome. We observe an overall footprinting effect whereby the drug preferentially cleaves DNA located outside the nucleosome core (analogous to the linker DNA of chromatin) as compared to cleavage within the nucleosome core. We discuss the importance of accessibility, structural deformations of DNA within the nucleosome, and steric constraints posed by sequence, in the recognition and cleavage of nucleosomal DNA by calicheamicin.

The calicheamicins (CLM) (Lee et al., 1991) belong to a growing family of enediyne antibiotics that include the neocarzinostatin chromophore (NCS) (Goldberg, 1991), the esperamicins (ESP) (Golik et al., 1987), dynemicin (Konishi et al., 1990), kedarcidin (Leet et al., 1992), C1027 chromophore (Xu et al., 1994), and maduropeptin (Schroeder et al., 1994). Calicheamicin γ_1^I (CLMγ_1^I) binds to the minor groove of duplex DNA in a sequence-selective manner, effecting predominantly double-stranded cleavage of DNA (Zein et al., 1988; Hangeland et al., 1992; Dedon et al., 1993).

The proposed mechanism for DNA cleavage by CLMγ_1^I consists of the formation of a diradical species, which, when positioned in the minor groove of DNA, abstracts hydrogen atoms from deoxyribose sugars on both strands of the DNA. This leads to the formation of carbon-centered radicals that are scavenged by oxygen to produce a variety of DNA cleavage products (De Voss et al., 1990a,b; Zein et al., 1989). The remarkable efficiency of this process has attracted growing interest in the use of enediyne antibiotics as agents for double-stranded cleavage of DNA, a lesion more difficult to repair (and therefore potentially more lethal) than the single-stranded cuts inflicted by other small-molecule DNA cleaving agents (Frankenberger-Schwager, 1990).

Experiments involving the use of oligonucleotides or restriction fragments containing various prokaryotic and eukaryotic DNA sequences have demonstrated several aspects of the process of DNA recognition, binding, and cleavage by CLMγ_1^I (De Voss et al., 1990b; Kishikawa et al., 1991.; Walker et al., 1992; Mah et al., 1994a,b). The large variety of sequences that are cut by CLM and the dependence of the extent of cutting on flanking sequence (Zein et al., 1988) clearly point to recognition of local DNA structure by the drug rather than specific reading of the DNA sequence. For example, cleavage experiments on bent DNA (Mah et al., 1994a) suggested that minor groove width and flexibility are important determinants in the recognition and cleavage of DNA by the drug. Nuclear magnetic resonance (NMR) studies revealed that the minor groove of an oligonucleotide widens upon CLM binding (Walker et al., 1993; Paloma et al., 1994).

Most experiments on calicheamicin reported to date have used purified DNA. However, in eukaryotic cells nuclear DNA is complexed with histones to form chromatin (van Holde, 1989). At the lowest level of compaction histones and DNA are organized in repeating units termed nucleosomes. Nuclease digestion of chromatin yields the nucleosome core particle, which consists of 146 base pairs (bp) of DNA wrapped in 1.75 turns of a left-handed superhelix around an octamer of histones (van Holde, 1989; Wolffe, 1992).

Complexation with the histone octamer gives rise to structural changes in DNA that include periodic narrowing and widening of the minor groove and the introduction of pronounced bends (or kinks) at various positions (Richmond et al., 1984). In addition, the helical repeat of the DNA is altered. Much of the nucleosomal DNA becomes overwound, while the central region around the dyad is underwound (Hayes et al., 1990). These differences in structure compared to free DNA would be expected to alter the cleavage of nucleosomal DNA by CLMγ_1^I. Moreover, the possible use of CLM as a chemotherapeutic agent requires careful characterization of the interaction of the drug with constituents of chromatin, the putative target of the drug *in vivo*.

Dedon and co-workers recently reported a study of DNA damage in HeLa cell nuclei as well as nucleosome core

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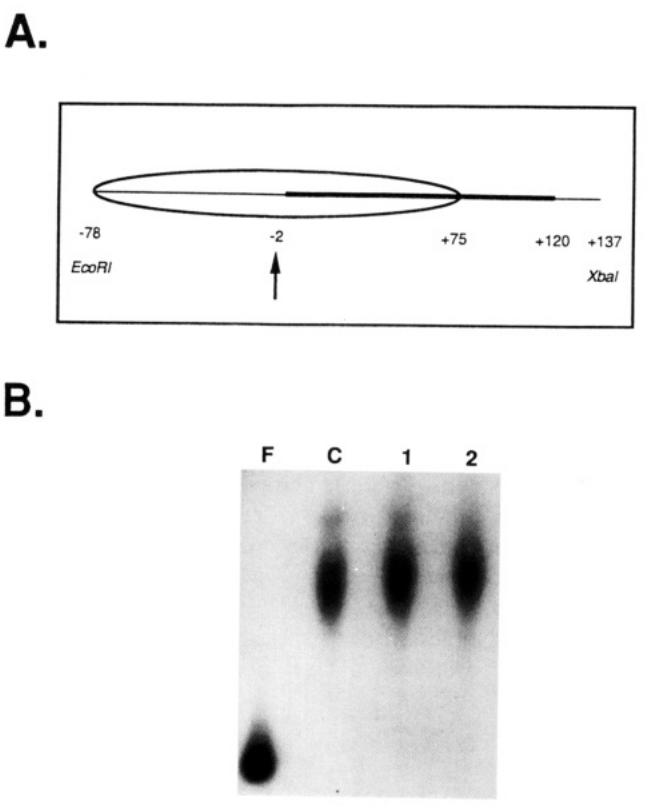

FIGURE 1: (A) Schematic representation of a reconstituted nucleosome on the *Eco*RI−*Xba*I fragment (−78 to +137). The 5S RNA gene is shown in bold (+1 to +120). The nucleosome is shown as an oval spanning positions −78 to +75. The dyad axis of the nucleosome is marked by the arrow. (B) Gel electrophoresis of the calicheamicin-treated 5S nucleosome. Shown is the autoradiograph of a native polyacrylamide nucleoprotein gel. Lanes: F, free 5S DNA; C, control 5S nucleosome (without added calicheamicin); 1 and 2, 5S nucleosome treated with 100 and 200 nM $CLM\gamma_1^I$, respectively.

particles mediated by several enediynes including NCS, ESP, and $CLM\gamma_1^I$ (Yu et al., 1994). These studies were performed on a heterogeneous mixture of nucleosomes from HeLa cells or on mixed-sequence nucleosome core particles from chicken erythrocytes. These workers found that NCS and ESP A1 predominantly cleaved the DNA linker regions that separate nucleosomes in chromatin, as well as the segments of DNA at the termini of the nucleosome. In contrast, both ESP C and $CLM\gamma_1^I$ cleaved within the nucleosome as well as in the linker region.

In this report we systematically classify the cleavage by $CLM\gamma_1^I$ of a uniquely positioned nucleosome that forms on the 5S ribosomal RNA gene from *Xenopus borealis* (Figure 1A) (Hayes et al., 1990). Both the 5S nucleosome and free 5S DNA have been used in previous studies of the interaction of minor groove-binding small molecules, such as distamycin (Churchill et al., 1990), netropsin (Portugal & Waring, 1987), and benzo[*a*]pyrenediol epoxide (BPDE) (Thrall et al., 1994). The advantage of this experimental system is that DNA of a particular sequence is complexed to the histone octamer in a well-defined translational and rotational setting. In contrast to the experiments using random sequence nucleosomes (Yu et al., 1994), the 5S nucleosome system allows us to compare the cleavage pattern of calicheamicin on free DNA with the cleavage pattern of the same DNA sequence bound to the histone octamer. Since the structure of the 5S nucleosome has been characterized extensively by biophysical methods (Hayes et al., 1990, 1991), we have the opportunity to identify specific structural features that are important in the recognition and cleavage of nucleosomal DNA by the drug.

EXPERIMENTAL PROCEDURES

Preparation and Radioactive Labeling of DNA Fragments. A 212 bp *Eco*RI−*Dde*I restriction fragment containing the *X. borealis* 5S RNA gene from the plasmid pXP-10 (Hayes et al., 1991) was PCR-cloned into plasmid pUC18 so as to add an *Xba*I site at the *Dde*I end of the fragment. A 215 bp *Eco*RI−*Xba*I fragment from the resulting plasmid pXP-11 was used in all the experiments. The restriction fragment was radiolabeled with ^{32}P at the 3′ end of the *Eco*RI site to label the coding strand, or 5′ end-labeled at the *Eco*RI site to radiolabel the noncoding strand. Labeling with ^{32}P at the 3′ or 5′ end was achieved by standard procedures (Maniatis et al., 1989). Radiolabeled DNA molecules were purified by nondenaturing polyacrylamide gel electrophoresis.

Nucleosome Preparation. Nucleosomes were prepared by salt/urea dialysis of radiolabeled DNA in the presence of purified chicken erythrocyte histones (Simon & Felsenfeld, 1979; Camerini-Otero et al., 1976). Histones were kindly provided by Dr. Alan Wolffe (Laboratory of Molecular Embryology, National Institutes of Health). Progress of nucleosome formation was monitored by gel electrophoresis (Wolffe, 1988). Other workers have established (Hayes et al., 1990) that the dyad axis of the 5S nucleosome prepared by this procedure lies about 75 bp from the radiolabeled end of the DNA fragment we use (see Figure 1A).

$CLM\gamma_1^I$ Cleavage Reactions. $CLM\gamma_1^I$ was kindly provided by Dr. G. A. Ellestad (Lederle Laboratories) and was stored in solution at −20 °C at a concentration of 40 μM in 100% methanol. This solution was used to make further dilutions in 100% methanol.

$CLM\gamma_1^I$ (at a final concentration of 100 nM) was allowed to react with the 5S nucleosome in a buffer consisting of 10 mM Tris·HCl and 1 mM ethylenediaminetetraacetic acid (EDTA) (pH 8.0) for 3 min at room temperature. The reaction mixture contained 0.5−1.0 pmol of radiolabeled 5S DNA (complexed with the histone octamer), 0.3 μg of sonicated calf thymus DNA, and 6 mM 2-mercaptoethanol. After termination of the reaction by the addition of glycerol to 5% (v/v), the mixture was immediately loaded onto a 4.5% nucleoprotein polyacrylamide gel (Hayes et al., 1991) in order to separate the treated nucleosome core particles from free DNA. Radiolabeled DNA in the band containing nucleosome core particles was isolated by the crush and soak procedure (Maniatis et al., 1989), followed by precipitation and lyophilization.

Radiolabeled free DNA was subjected to cleavage by $CLM\gamma_1^I$ under identical reaction conditions, followed by precipitation and lyophilization.

To convert the 5′-aldehyde nucleoside ends produced by calicheamicin cleavage to 5′- phosphates, 3′ end-labeled DNA fragments were further passed through QIAquick-spin columns (Qiagen) to remove residual acrylamide, treated with 100 μL of 1.0 M NaOH, and placed at 90 °C for 5 min. The DNA was once again precipitated and lyophilized.

Hydroxyl Radical Footprinting. Reconstituted nucleosomes were treated with hydroxyl radical, and the DNA was

isolated, precipitated, and lyophilized as described (Dixon et al., 1991).

Gel Electrophoresis and Analysis. DNA was resuspended in formamide loading buffer, heated to 90 °C, placed on ice, and then loaded onto an 8% denaturing polyacrylamide gel. Counts of radioactivity were equalized for each lane before loading. Products of the guanine-specific Maxam−Gilbert sequencing reaction (Maxam & Gilbert, 1980) were run in parallel to act as size markers.

Alternatively, DNA was resuspended in native loading buffer and run on a 14% nondenaturing polyacrylamide gel. DNA restriction fragments radiolabeled at the 5′ end were run in parallel as size markers.

Gels were dried and exposed to film (Kodak XAR-5) at room temperature. Scans of particular lanes were obtained by exposure of the dried gel to a storage phosphor plate (Molecular Dynamics) and analyzed with a Model 400E PhosphorImager (Molecular Dynamics) using the included ImageQuant software. Plots of scans were made using Microsoft Excel.

RESULTS

Dialysis of radiolabeled 5S DNA with purified chicken erythrocyte histones yields primarily nucleosome core particles and some free DNA (Hayes et al., 1990). In order to characterize DNA cleavage by calicheamicin *within the nucleosome*, the cleavage reaction mixture was subjected to electrophoresis on a nondenaturing "nucleoprotein" gel (Hayes et al., 1991). Drug-treated nucleosomal DNA was isolated from the band containing nucleosome core particles, thus ensuring that the calicheamicin-treated DNA we analyzed was still associated with the histone octamer. A wide range of drug concentrations (10 nM−1 μM) was tested. Under "single-hit" (Goodisman & Dabrowiak, 1985) conditions (which we determined to be [CLMγ_1^I] < 300 nM), the integrity of the nucleosome core particle is not significantly altered as seen on a nucleoprotein gel (Figure 1B).

Figures 2 and 3 show the cleavage data for the coding (3′ end-labeled) and noncoding (5′ end-labeled) strands, respectively. A comparison of the cleavage pattern for free DNA with that of nucleosomal DNA (lanes 1 and 3 on Figure 2 and lanes 1 and 2 on Figure 3) reveals several interesting features. First, the region from positions −10 to +10 around the dyad axis of the nucleosome (marked by the arrow) is hardly cleaved in nucleosomal DNA. This is not surprising since free DNA also suffers very little cleavage by calicheamicin in this region (Mah et al., 1994a). On either side of the dyad region, CLMγ_1^I cleaves DNA in the nucleosome at sites that are spaced at regular intervals, roughly 10−11 bp apart. While *free* DNA also shows a rather periodic cleavage pattern [lanes 1, Figures 2 and 3; also see Mah et al. (1994a)], the pattern is more regular for DNA associated with the histone octamer (compare lanes 1 and 3, Figure 2, and lanes 1 and 2, Figure 3).

Examination of the scans in Figures 4 and 5 shows that calicheamicin cleaves the nucleosome at sites that also are strongly cleaved by the hydroxyl radical. In other words, calicheamicin cleavage occurs at sequences in the nucleosome that face outward (away from the histone octamer) (Hayes et al., 1990). Other sequences that are cleaved by the drug in free DNA (for example, see Figure 3, lanes 1 and 2, and Figure 5, positions +24 to +27) are not cleaved in nucleosomal DNA. According to the hydroxyl radical cleavage experiment, these are sequences that are directed inward toward the histone octamer. Thus accessibility of outward-facing sequences to the drug results in the highly periodic cleavage observed on either side of the dyad. Inward-facing sequences are probably occluded by histone−DNA interactions.

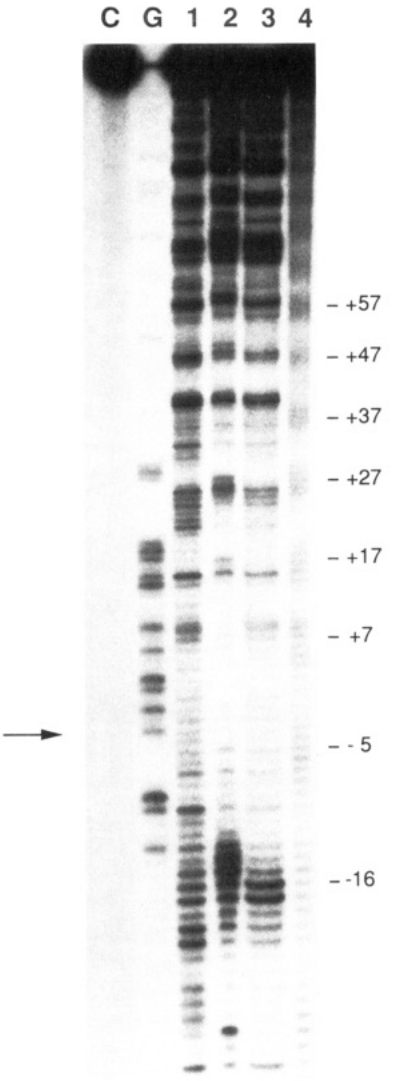

FIGURE 2: Calicheamicin cleavage of the coding strand of the 5S nucleosome. Shown is the autoradiograph of a DNA sequencing gel on which was separated the cleavage products of the coding strand of the *Eco*RI−*Xba*I fragment of pXP-11, 3′ end-labeled at the *Eco*RI site. Lanes: C, uncut DNA; G, products of the Maxam−Gilbert guanine-specific sequencing reaction; 1, products of CLMγ_1^I cleavage of free DNA; 2, products of CLMγ_1^I cleavage of the 5S nucleosome with subsequent isolation of treated nucleosomal DNA by nondenaturing electrophoresis on a nucleoprotein gel; 3, products of CLMγ_1^I cleavage of the 5S nucleosome isolated as described for lane 2 followed by NaOH treatment; 4, products of hydroxyl radical cleavage of the 5S nucleosome. The numbers denote nucleotide positions, and the arrow marks the dyad axis of the nucleosome.

In addition to the periodic cleavage at outward-facing sequences, nucleosomal DNA is also cleaved at some inward-facing sequences around positions +40 and +60 (Figure 4). These cleavage sites are found in both free and nucleosomal DNA. This result suggests that inward-facing sequences become more accessible to the drug the farther they are from the dyad axis of symmetry of the nucleosome.

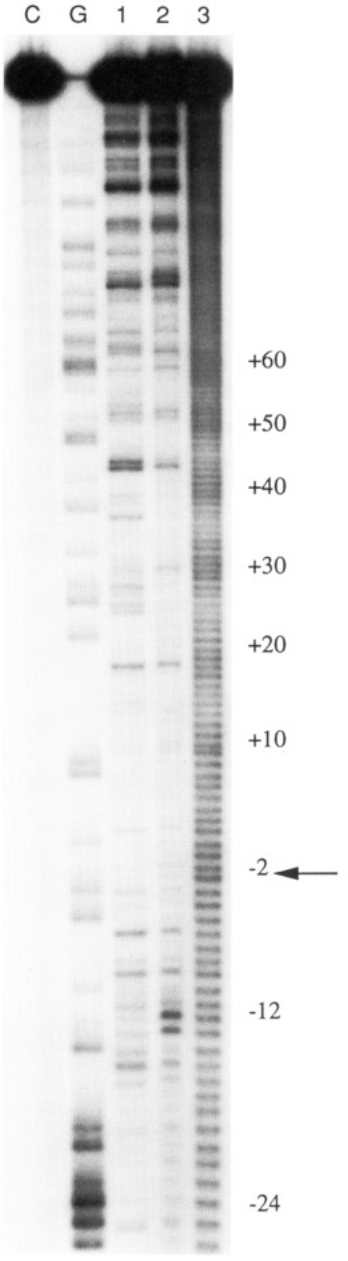

FIGURE 3: Calicheamicin cleavage of the noncoding strand of the 5S nucleosome. Shown is the autoradiograph of a DNA sequencing gel on which was separated the cleavage products of the noncoding strand of the *Eco*RI−*Xba*I fragment of pXP-11, 5′ end-labeled at the *Eco*RI site. Lanes: C, uncut DNA; G, products of the Maxam−Gilbert guanine-specific sequencing reaction; 1, products of $CLM\gamma_1^I$ cleavage of free DNA; 2, products of $CLM\gamma_1^I$ cleavage of the 5S nucleosome with subsequent isolation of treated nucleosomal DNA by nondenaturing electrophoresis on a nucleoprotein gel; 3, products of hydroxyl radical cleavage of the 5S nucleosome. The numbers denote nucleotide positions, and the arrow marks the dyad axis of the nucleosome.

Lastly, a homopyrimidine/homopurine tract that extends from positions −15 to −27 is cleaved extensively in free DNA (Mah et al., 1994a). In comparison, the cleavage pattern of this region in nucleosomal DNA is altered dramatically. On the pyrimidine-rich strand (the coding strand) free DNA exhibits a fairly uniform cleavage pattern over positions −10 to −20 (Figure 4). However, the cleavage of nucleosomal DNA shows a pronounced footprinting effect which corresponds closely to the hydroxyl radical footprint of the nucleosome in this region. Positions −16 and −17, where the minor groove faces outward, are cleaved to the greatest extent by calicheamicin; the cleavage pattern fades away on either side. Comparison of lanes 2 and 3 in Figure 2 also shows that cleavage at these positions produces 5′-aldehyde ends as seen from the change in mobility after base treatment (Kappen et al., 1983; Mah et al., 1994a). On the complementary, homopurine strand, free 5S DNA suffers strong cleavage at positions −7, −10, and −16 (positions that would face inward in the nucleosome); other positions show little cleavage (Figure 5). With nucleosomal DNA, the most intensely cleaved sites now appear at positions −13 and −14. Since these positions correspond to sites of strong cleavage by the hydroxyl radical, they must face outward from the surface of the nucleosome. The positions of intense calicheamicin-induced cleavage of the two strands of nucleosomal DNA are offset by three nucleotides in the 3′ direction, which is indicative of minor groove binding by the drug (Mah et al., 1994a).

$CLM\gamma_1^I$ is known to make primarily double-stranded cuts in DNA; very little single-stranded cleavage occurs (Zein et al., 1988; Kishikawa et al., 1991). In order to test whether the cleavage we observe in nucleosomal DNA is indeed double stranded, we ran the DNA cleavage products on a nondenaturing polyacrylamide gel (Figure 6). A double-stranded cut at the site of intense cleavage in the homopyrimidine/homopurine tract would yield a DNA fragment 65 bp in length. Lanes 2, 3, and 4 clearly show very intense bands around 65 bp in size, not seen in the cleavage products of free DNA (lanes 5 and 6). Once again, absence of cleavage at the dyad and a highly periodic cleavage pattern spaced at intervals of 10−11 bp can be seen. Comparison of the cleavage products in this gel to the cleavage patterns seen in the denaturing gels (Figures 2 and 3) shows that the periodic cleavage of DNA in the nucleosome by $CLM\gamma_1^I$ is primarily double stranded. This result is in agreement with those of Dedon and co-workers, who used mixed-sequence nucleosome core particles in their calicheamicin cleavage experiments (Yu et al., 1994).

Figure 6 illustrates two other important features of the cleavage event within the nucleosome. First, a comparison of the cleavage of free and nucleosomal DNA at the same concentration of drug (lanes 3 and 5) reveals an overall footprinting effect. The 215 bp fragment used here positions the nucleosome with its dyad axis 75 bp from the label (Figure 1A). This gives an overhang of about 60 bp which would be analogous to the linker region in higher order chromatin structures. It can be seen clearly in Figure 6 (lanes 3 and 5) that cleavage of sequences within the nucleosome (that is, cuts that give rise to products shorter than 147 bp) is less than that for the same sequences in free DNA. However, for products greater than 147 bp in length cleavage is more intense for nucleosomal DNA than for free DNA. This demonstrates that sites in the 60 bp overhang (the analog in our system of the linker region in chromatin) are cleaved preferentially to those within the nucleosome core.

Second, the intense cleavage seen one turn away from the dyad axis (producing a product of around 65 bp in this case) is the predominant double-stranded cleavage event within the nucleosome core. Free DNA shows more intense cleavage at sites other than the homopyrimidine/homopurine tract, whereas within the nucleosome the most intense cleavage is centered at a site −1 turn from the dyad axis within the homopyrimidine/homopurine tract. We have

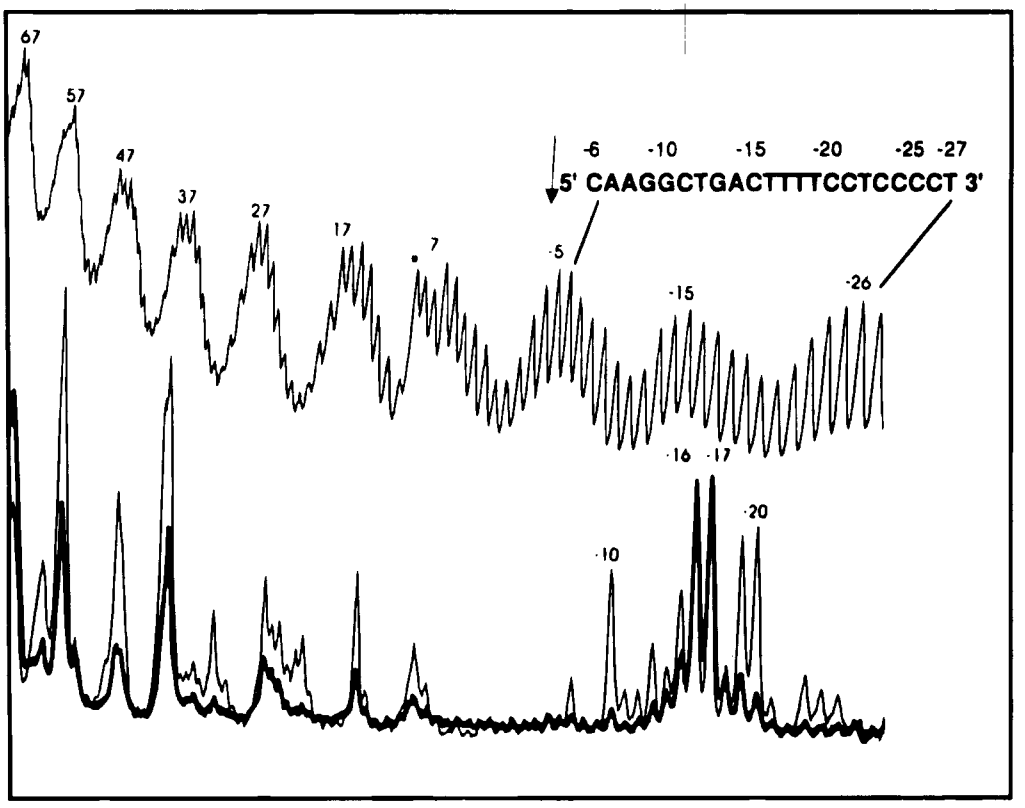

FIGURE 4: Calicheamicin and hydroxyl radical cleavage patterns for the coding strand of the 5S nucleosome. One-dimensional scans were made from the gel shown in Figure 2. The top scan is of the lane containing products of hydroxyl radical cleavage of the nucleosome. At the bottom is shown an overlay of CLMγ_1^I cleavage patterns for 5S nucleosomal DNA (heavy line) and for free 5S DNA (light line). The numbers denote nucleotide positions, and the arrow marks the dyad axis of the nucleosome. The asterisk marks a gel-running artifact where two nucleotides are represented by one peak. The nucleotide sequence of the oligopyrimidine region at −1 turn (see text) is indicated on the scan. In each case, counts of radioactivity were equalized before loading.

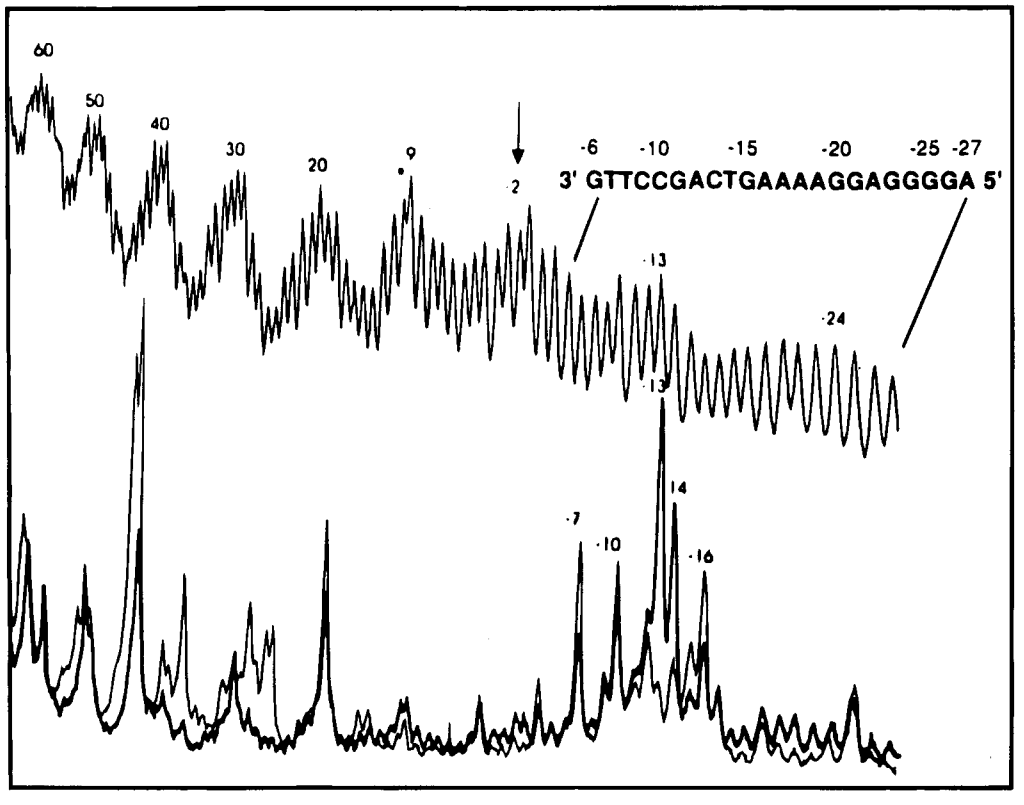

FIGURE 5: Calicheamicin and hydroxyl radical cleavage patterns for the noncoding strand of the 5S nucleosome. One-dimensional scans were made from the gel shown in Figure 3. The other details are as in Figure 4.

observed this to be the most intensely cleaved site over a range of CLMγ_1^I concentrations from 10 nM to 1 μM (data not shown). This site of most intense cleavage constitutes a "hot spot" for CLMγ_1^I cleavage within the 5S nucleosome.

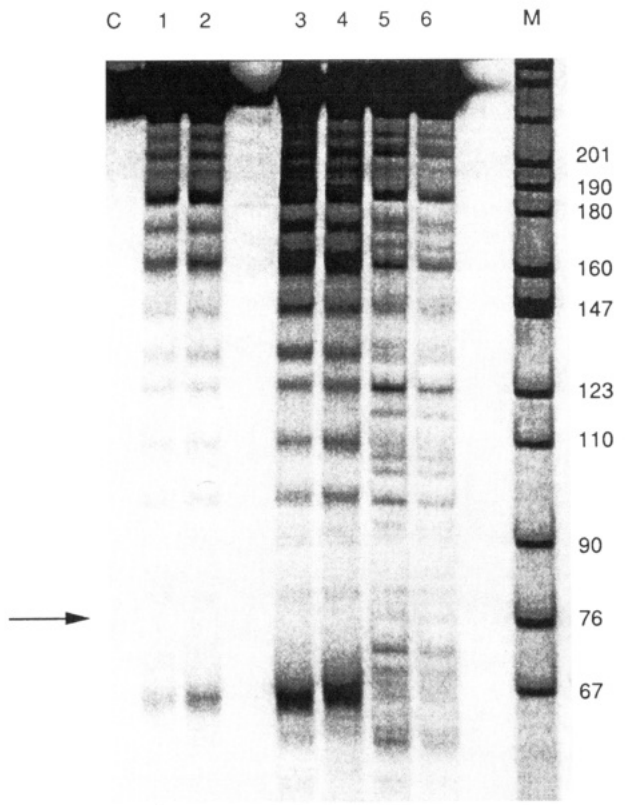

FIGURE 6: Double-stranded cleavage by calicheamicin of DNA in the 5S nucleosome. Shown is the autoradiograph of a native polyacrylamide gel on which was run (lane C) uncut DNA, (lanes 1−4) cleavage products of nucleosomes treated with 50, 100, 200, and 300 nM CLMγ_1^I, respectively, followed by separation of nucleosomal DNA on a nucleoprotein gel, (lanes 5 and 6) products of free DNA treated with 200 and 100 nM CLMγ_1^I, respectively, and (lane M) 5′ end-labeled size markers. For this experiment the *Eco*RI−*Xba*I fragment from plasmid pXP-11 was 5′ end-labeled at the *Eco*RI site. The numbers to the side denote the size (in base pairs) of the marker fragments in lane M; the arrow marks the dyad axis of the nucleosome.

DISCUSSION

Our study of the interaction of CLMγ_1^I with a nucleosome reconstituted on the 5S RNA gene from *X. borealis* demonstrates several interesting features in the recognition and cleavage of nucleosomal DNA by the drug. The dyad region of the nucleosome (positions −10 to +10) is not cleaved, either in free DNA or in the nucleosome. On either side of the dyad region, the drug cleaves nucleosomal DNA with a periodicity of 10−11 bp at sites that face outward in the nucleosome. The striking result seen in our work is the cleavage pattern within the homopyrimidine/homopurine tract situated one helical turn away from the dyad. The predominant cleavage event within the nucleosome occurs at positions −13 and −14 on the noncoding strand and at positions −16 and −17 on the coding strand, yielding the expected 3 bp offset. The observed 5′-aldehyde termini at this site on the pyrimidine-rich coding strand are indicative of 5′ hydrogen abstraction. This site faces outward from the surface of the nucleosome. In free DNA there exist other sites that are more intensely cleaved by the drug, implying that it is not just the sequence that makes this a highly favorable site for cleavage. Our results show instead that within the context of the nucleosome this site becomes a hot spot for cleavage by CLMγ_1^I.

Two effects, steric and structural, are likely to be behind the change in the calicheamicin cleavage pattern between free 5S DNA and the 5S nucleosome. The clear 10−11 bp periodicity of CLMγ_1^I cutting at sites facing outward from the nucleosome clearly points to the importance of the accessibility of the minor groove of the DNA within the nucleosome. Similar periodicity has been observed in the case of the minor groove-binding drug BPDE (Thrall et al., 1994) and the enzyme HIV integrase (Pruss et al., 1994). Inward-facing sequences are probably occluded by the compressed minor groove (Drew & Travers, 1984) and by histone−DNA interactions. However, this steric effect alone cannot explain the lack of cleavage at the dyad and +1 turn, as well as the hot spot at −1 turn.

It is imperative to examine the mode of CLMγ_1^I binding to DNA as well as the structure of DNA within the nucleosome before attempting an analysis of these results. NMR experiments have shown that the binding of calicheamicin to an oligonucleotide causes conformational changes in the DNA but little change in the structure of the drug (Walker et al., 1993; Paloma et al., 1994). The NMR results of Kahne and co-workers (Walker et al., 1993) indicate that there is greater distortion for the pyrimidine-rich strand than the purine-rich strand. These workers conclude that the steric pressure exerted by the drug causes a widening of the DNA minor groove. They propose that this widening occurs *via* partial unstacking of the bases on the pyrimidine strand, and therefore CLM binds selectively to sequences that can accommodate the conformational changes required for drug binding (Walker et al., 1993). Results from circular dichroism measurements (Krishnamurthy et al., 1993) and molecular dynamics computer simulation (Langley et al., 1993) are in agreement with this conclusion.

It is particularly interesting to note that some of these distortions exist naturally in DNA within the nucleosome. It has been established that there is a periodic narrowing and widening of the minor and major grooves of nucleosomal DNA. Sequences facing outward have a widened minor groove whereas the minor groove is compressed in sequences facing inward (Drew & Travers, 1984). Structural studies (Hayes et al., 1990) have shown that the helical repeat is 10.70 bp/turn over about three turns of the DNA helix centered on the nucleosome dyad, and therefore, this region is underwound with respect to free DNA. The rest of the nucleosome was found to have a helical repeat of 10.02 bp/turn, overwound with respect to free DNA.

Although there is an overall footprinting effect within the nucleosome, the widened minor groove at sites facing outward (on either side of the dyad region) does allow binding and cleavage by the drug, and this is in agreement with the NMR studies discussed earlier (Walker et al., 1993). The absence of cleavage in the dyad region at sites facing outward may reflect the difference in helical periodicity or perhaps the inability of the DNA in this region to structurally deform (Arents et al., 1991).

The hot spot for calicheamicin cleavage that we found in the homopyrimidine/homopurine sequence of the 5S nucleosome is situated at the junction of the two regions of different helical periodicity, where other experiments show that the DNA is deformed. For example, X-ray crystallographic work on the nucleosome provided evidence for a sharp bend at −1.0 turn (Richmond et al., 1984), which implies that the minor groove at −1 turn from the dyad axis is unusually

wide. Changes in base stacking so as to open up base planes toward the major groove have been suggested to occur in this region (Hogan et al., 1987; Crick & Klug, 1975). Integration of an oligonucleotide into the 5S nucleosome by the enzyme HIV integrase shows a clear preference for the major groove of nucleosomal DNA located 1.5 and 3.5 turns from the dyad (Pruss et al., 1994). This region of the nucleosome also has been shown to be hypersensitive to attack by singlet oxygen (Hogan et al., 1987). The combination of an oligopyrimidine/oligopurine run, which is the preferred site of CLMγ_1^I cleavage (Zein et al., 1988; Mah et al., 1994a), and an already existing distortion in nucleosomal DNA are consistent with the preferential binding and cleavage by the drug that we observe.

An intriguing point is that the symmetry of the nucleosome would seem to require that the site at +1 turn from the dyad axis be similarly a cleavage hot spot, if DNA distortion is the only factor in enhancing selectivity of the drug. The striking absence of cleavage at +1 turn (Figure 6) points to differences in sequence between the two sites as being at least of equal importance. The sequence at +1 turn from the dyad axis that faces outward is 5′GGCCGT (expected site of cleavage underlined). Cleavage of the homopyrimidine sequence CCCC recently was demonstrated to be significantly disfavored relative to the sequences TTTT and TCCT (Chatterjee et al., 1994). Modeling studies by Beveridge and co-workers indicated that, relative to TCCT, the sequence CCCT would be unfavorable for calicheamicin binding due to the presence of an additional 2-amino moiety from the third guanine (Langley et al., 1993). We and others have reported that the presence of guanine at specific locations in a site decreases drug binding and cleavage (Li et al., 1994; Chatterjee et al., 1994; M. Chatterjee, S. C. Mah, T. D. Tullius, and C. A. Townsend, manuscript submitted). This could account for the lack of cleavage at +1 turn despite the expected structural distortions in the DNA at this site.

Our analysis shows that the cleavage of DNA within the nucleosome by calicheamicin is not just a simple function of accessibility. Structural differences within the nucleosome, such as helical periodicity, minor groove width, structural flexibility, bending and other associated conformational changes, and steric constraints imposed by the sequence, also are important factors in determining the extent of cleavage at various sites in the nucleosome.

In the course of this work we observed that under single-hit conditions of cleavage by the drug a single double-stranded cut made by CLMγ_1^I within the nucleosome does not seriously affect the integrity of the nucleosome core (as demonstrated by nucleoprotein polyacrylamide gel electrophoresis in Figure 1B). This could have important ramifications for repair processes within the cell following CLMγ_1^I cleavage, as well as for the stability of the nucleosome core itself. When higher concentrations of drug were used (>300 nM), we found that the nucleosome dissociated, and periodic cleavage was seen throughout the nucleosome. These results are similar to those reported by Dedon and co-workers, who used higher drug concentrations on mixed-sequence nucleosomes (Yu et al., 1994). We also observed that the calicheamicin cleavage pattern of a mixture of nucleosomal DNA and free DNA was the sum of the cleavage patterns obtained in separate experiments on free and on nucleosomal DNA. This clearly indicates that the use of nucleoprotein gel electrophoresis after cleavage does not introduce significant bias in our experiments.

Further work is in progress to elucidate to what extent accessibility, structural distortion, and sequence are responsible for CLMγ_1^I cleavage within the nucleosome.

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