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Effects of DNA looping on pyrimidine dimer formation

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ABSTRACT

We have assessed the effects of DNA curvature on pyrimidine dimer (PD) formation by examining the pattern of PD formation in DNA held in a loop by lambda repressor. The loop region was composed of diverse DNA sequences such that potential PD sites occurred throughout the loop. PD formation in the loop occurred with peaks at approximately 10 base intervals, just 3′ of where the bending of the DNA was inferred to be toward the major groove. This relationship between the peaks and the DNA curvature is essentially identical to that observed in the nucleosome. This indicates that DNA curvature is the major source of the periodicity of PD formation in the nucleosome, and supports an earlier model of the conformation of nucleosomal DNA based on PD formation. DNA loops containing diverse sequences should be of general value for assessing the effects of DNA curvature on DNA modification by other agents used to probe DNA-protein interactions and DNA conformation.

INTRODUCTION

Pyrimidine dimer (PD) formation has been used to probe protein-DNA interactions *in vivo* and *in vitro*, a method referred to as photofootprinting (1–5). However, it is not always clear if effects on PD formation are the result of protein-DNA contacts, or indirect effects, such as distortion of the conformation of neighboring DNA (2,3,5). In the nucleosome, PD formation occurs with a periodicity of approximately 10 bases, with the peaks located just 3′ of where the major groove faces toward the nucleosome core (6,7). One interpretation of this pattern has been as a photofootprint of histone-DNA interactions (6). However, another possible source of the periodicity is the curvature of nucleosomal DNA, with PD formation being more rapid when the bending of the DNA is toward the major groove (7). The latter hypothesis was based on an energy minimization model of DNA containing a thymine dimer (8), and has been used to construct a model of the path of DNA in intact chromatin (7). To test the hypothesis that DNA curvature can produce periodicity of PD formation, we have examined the pattern of PD formation in a DNA loop.

MATERIALS AND METHODS

Preparation of DNA

A mixture of 108-base oligonucleotides of sequence, gggcaagct-taccacggcttatcaccgccagaggtacc(n_{33})tatcaccgccagaggtaaaccgggc-tagaattcgggc, were synthesized, using an Applied Biosystems DNA synthesizer and phosphoramidite chemistry (9). The 33 bases marked n were synthesized using equal amounts of all four bases. The product was 5′ end labeled, and run on a 5% denaturing polyacrylamide gel (7). The band corresponding to the full length product, located by autoradiography, was excised, and DNA eluted with 0.4 ml of 10 mM Tricine, 100 mM NaCl, 0.4 mM EDTA, pH 7.2. This DNA was amplified using polymerase chain reaction (PCR) with primers gggcaagcttacca-cggc and gcccgaattctagcccgg (10). The PCR products migrated as 108-base fragments in a denaturing gel, but migrated aberrantly on a native gel. A possible explanation for this is that, as the products increase in concentration during PCR, they tend to anneal to each other rather than to the primers, producing DNA molecules that cannot be replicated and are not complementary in the diverse sequence region. Therefore, the PCR products were cloned in the pBluescript II SK$^-$ plasmid (Stratagene) using the *Eco*RI and *Hind*III restriction nuclease sites at the ends of the PCR products; this produced a 94 bp insert. A mixture of clones, containing approximately 5×10^4 independent sequences, was grown overnight and plasmid DNA purified using Qiagen plasmid purification kit (Qiagen Inc.).

The plasmids were cut with *Eco*RI, treated with calf intestinal alkaline phosphatase, and labeled on the 5′ end using T4 polynucleotide kinase (7). Then they were cut with *Hind*III, and the 94 bp fragments purified by electrophoresis in a native gel (5% acrylamide, 1:20 bisacrylamide) containing 50 mM Tris–base, 50 mM boric Acid, 1 mM EDTA (TBE).

DNase I digestion

The labeled 94 bp DNA fragments (final concentration < 10pm) were mixed with lambda repressor (final concentration 1.1 μg/ml) in 125 μl of digestion buffer (10 mM Tris pH 8.0, 20 mM KCl, 2.5 mM $MgCl_2$, 1 mM $CaCl_2$, 25 μg/ml bovine serum albumin, 10 μg/ml chicken blood DNA) and incubated at room temperature for 30 min. In control experiments repressor was omitted. (Lambda repressor was purified from X90 cells containing plasmid pMH236 (11) using a modification of the CM-

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Sephadex/hydroxylapatite method described by Johnson et al.(12,13).) Beef pancreas DNase I was added (35 ng in 10 μl of digestion buffer) and the mixture incubated at room temperature for 30 min. Following the digestion, 15 μl of 0.5 M EDTA was added and the DNA fragments were precipitated with 300 μl of ethanol. The precipitates were run in a denaturing polyacrylamide gel (8% acrylamide, 1:15 bisacrylamide, 7 M urea, TBE). After electrophoresis the gel was fixed, dried and autoradiographed with an intensifying screen.

Irradiation of DNA and mapping of photoproducts

See Figure 1 for a diagram of the method. The labeled DNA fragments were dissolved in 8 ml of digestion buffer containing 0.2 M mannitol and 1% thiodiglycol, and lambda repressor was added to a final concentration of 1.1 μg/ml. The concentration of DNA fragments was kept low, < 10pm, to minimize pairing of DNA molecules through repressor interactions (14). The mixture was incubated at room temperature for 30 min, and then placed on ice and irradiated with 254 nm light using a germicidal lamp (dose 500 J/m^2). In control experiments lambda repressor was omitted. Following irradiation, SDS was added to 1%, EDTA to 50 mM, and the mixture was extracted twice with phenol. The DNA was precipitated with ethanol, dissolved in 90% formamide containing TBE, heated briefly to 95°C, and run in a denaturing polyacrylamide gel (5% acrylamide, 1:15 bisacrylamide, 7 M urea, TBE). The 94-base fragments were recovered from the gel as described above, and digested with T4 DNA polymerase, which degrades the fragments from their 3′ ends until it reaches a PD (15). The digest was run on a denaturing polyacrylamide gel (8% acrylamide, 1:15 bisacrylamide, 7 M urea, TBE), and the gel was fixed, dried and autoradiographed with an intensifying screen. The autoradiographs were scanned with an LKB XL enhanced laser densitometer.

The predominant form of DNA damage produced by 254 nm irradiation is the cyclobutane PD. Although other photoproducts are also formed and detected by these methods, their contribution is generally relatively minor (16,17). In mixed DNA sequences, such as bulk nucleosomal DNA, moreover, it has been demonstrated that the pattern of photoproducts detected by this method is dominated by cyclobutane PDs, and that other photoproducts are minor and randomly located throughout the nucleosome (18). Also, we previously found that the pattern of photoproducts formed in nucleosomal DNA by photosensitization, a process that specifically forms cyclobutane thymine dimers, was virtually identical to that produced by 254 nm irradiation (7).

RESULTS AND DISCUSSION

The studies of Hochschild and Ptashne (19), and Griffith, Hochschild and Ptashne (20) showed that DNA molecules containing two binding sites for lambda repressor separated by an integral number of helical turns of DNA will form loops held together by interactions between the two bound repressor dimers. We have used such loops to examine the effects of DNA curvature on PD formation. However, since a loop containing a single DNA sequence could form PDs only where there are adjacent pyrimidines, we used a population of DNA molecules in which the two binding sites for lambda repressor were separated by 5 helical turns of DNA (52bp from the center of one binding site to the center of the other) containing a 33 bp region of diverse DNA sequences (see top of Figure 1, and Figure 3). This was prepared using a combination of DNA synthesis, PCR, and DNA cloning. In the presence of lambda repressor these molecules should form DNA loops uniform in size, but heterogeneous in base sequence. A diagnostic property of a looped structure is a 10 base periodicity in sensitivity to DNase I (19). In the presence of purified lambda repressor, the digestion of the diverse sequence region showed a periodicity of approximately 10 bases as expected for looped molecules (Figure 2, top scan) (the peak on the left is distorted by a hypersensitive site; it is also truncated, most likely due to steric hindrance by the repressor protein). The large amplitude of the periodicity indicates that most of the molecules are looped, and therefore, that loops can be formed with a wide variety of DNA sequences. In the absence of lambda repressor, DNase I digestion was approximately uniform throughout the diverse sequence region (Figure 2, second scan).

The use of diverse sequences in the loop region allowed us to obtained a DNase I cutting pattern of much higher resolution than that obtained when single sequence loops were used (14,19). The locations of the peaks relative to the direction of DNA curvature can be estimated by measuring their distances from the center of the repressor binding sites; since repressor binds as a dimer centered over the minor groove (21), and since the loops form only when the binding sites are separated by an integral number of helical turns (19), we can reasonably assume that the minor groove faces the inside of the loop at the centers of the binding sites (see model in reference 19). The sites of highest sensitivity to DNase I occur 16, 26 and 37 bp from the center the binding site on the *Eco*RI side of the loop (Figure 2, top scan), i.e., just 3′ of where the bending of the DNA is predicted to be toward the major groove (see Figure 3). This is essentially the same conclusion reached by Hochschild and Ptashne using single sequence loops (19). It is also consistent with the crystal structure of DNase I complexed with DNA,

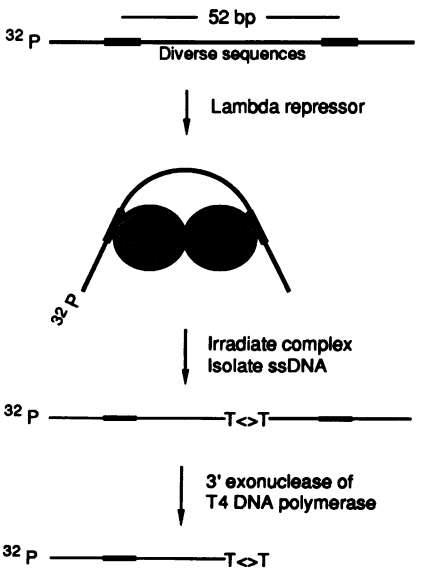

Figure 1. Outline of method used to examine the effects of DNA looping on PD formation. Lambda repressor binding sites are indicated by the thick regions. Lambda repressor is shown as a grey circle. The complex is shown for illustrative purposes only; the amount of DNA curvature is unknown and may be more or less than shown in this figure. T< >T indicates a cyclobutane thymine dimer, the most common PD formed by irradiation with 254 nm light (16,17).

which shows that DNase I bends the DNA toward the major groove, and therefore, is expected to prefer to cut DNA that is already bent in that direction (22).

The effects of DNA looping on PD formation was then examined (see Figure 1 for diagram of method). In the presence of repressor, three peaks of PD formation occurred with a periodicity of approximately 10 bases (Figure 2, third scan). In the absence of lambda repressor, the frequency of PD formation was nearly uniform throughout the diverse sequence region (Figure 3, bottom scan). Since no contacts between the repressor and the DNA outside of the binding sites have been observed by either x-ray crystallography (21) or DNase I footprinting (19), the periodicity presumably results from DNA curvature alone. Peaks of PD formation occur 16, 27 and 37 bp from the center the binding site on the *Eco*RI side of the loop (Figure 2, third scan). Their locations are nearly identical to the sites of maximum sensitivity to DNase I; i.e., just 3′ of where the bending of the DNA is predicted to be toward the major groove.

The 10-base periodicity of PD formation seen in these DNA loops is reminiscent of that seen in the nucleosome (6,7), but the amplitude of the modulation is less. However, it can be expected that the amplitude depends on the degree of curvature; therefore, if the curvature in the loops is less than that in the nucleosome, as seems possible based on crude models of the loop (19), then the effects on PD formation can be expected to be less. Also, it is likely that not all of the molecular species of DNA form stable loops. For example, the studies of Drew and Travers indicate that the dinucleotide sequence TT favors bending away from the major groove (23). Therefore, in these experiments, those DNA molecules with TT sequences located near the observed peaks of PD formation will tend to resist being bent in the direction of the loop. Since TT sequences are favored sites of PD formation (16,17), the population of looped molecules may have a reduced occurrence of potential PD sites in the vicinity of the observed peaks of PD formation, leading to a diminution of the periodicity. The extent of this effect is not easily assessed, although the amplitude of the DNase I digestion pattern (Figure 2) indicates that most molecules are looped.

The relative locations of the peaks of PD formation in the loops, just 3′ of where the curvature of the DNA is toward the major groove, is essentially identical to that previously obtained with nucleosomes, consistent with DNA curvature being the principal factor in the periodicity of PD formation in the nucleosome. Although some contributions of histone-DNA contacts cannot be entirely ruled out, an attempt to detect periodicity of PD formation with DNA bound to calcium phosphate crystals has been reported to be negative (6).

In addition to providing support for the use of PD formation to probe the conformation of nucleosomal DNA (7), our results serve as a caution regarding the use of PD formation to examine interactions of DNA with proteins that recognize specific DNA sequences. Although direct contacts between some proteins and specific bases do affect PD formation (1,4,5), not all of the effects of such proteins can necessarily be taken as evidence of such contacts (present study and 2,5). This caution also clearly applies to DNase I and probably other DNA modifying reagents used to probe protein-DNA interactions. Looped molecules would be valuable in assessing the effects of DNA curvature on such reactions. In cases where the reaction shows significant sequence specificity, the use of diverse sequences in the loop region, as in the present study, would facilitate analysis of the results.

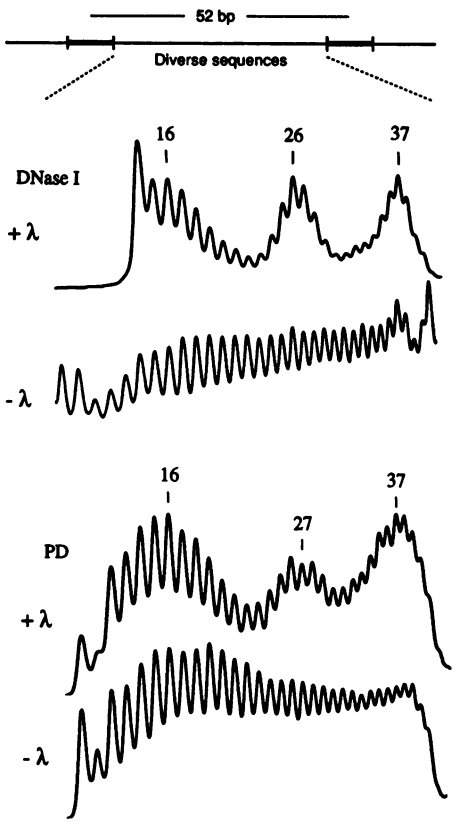

Figure 2. Effects of DNA looping on DNase I digestion and PD formation. Schematic of the DNA molecules used in these studies is shown at top; the thick regions mark the lambda repressor binding sites. The top two scans are of autoradiograms showing the distribution of DNA fragments produced by digestion with DNase I. Below this are scans of autoradiograms showing the distribution of DNA fragments produced from UV irradiated DNA molecules as outlined in Figure 1. + λ indicates lambda repressor was present before and during the DNase I digestion or UV irradiation; − λ indicates it was omitted. The scans show the entire diverse sequence region. The numbers above the peaks indicate the numbers of bases from the middle of the lambda repressor binding site on the *Eco*RI side of the fragments (see Figure 3). The depression in the peak at 27 bases, seen in the UV irradiation experiment, was not always observed and its cause is not known.

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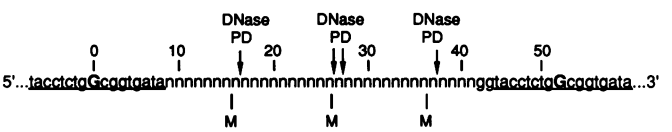

Figure 3. Locations of the peaks of DNase I sensitivity and PD formation in the DNA loop. The strand that was labeled is shown. The binding sites for lambda repressor are underlined with the central G capitalized. The bases are numbered from the center of the binding site on the *Eco*RI side of the molecule, i.e. the 5′ end. The locations of the peaks are indicated by the arrows and are the same for DNase I cutting and PD formation except for the middle ones, which occurs at 26 bases for DNase I and 27 bases for PD formation. M marks the sites where the bending of the DNA in the loop is inferred to be toward the major groove (see text). The 33 bases (marked n) in the middle of the molecules are the diverse sequence region.

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