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Biochemistry

Thymine dimer formation as a probe of the path of DNA in and between nucleosomes in intact chromatin

(histones/DNA bending/linker DNA)

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ABSTRACT **Photo-induced thymine dimer formation was used to probe nucleosome structure in nuclei. The distribution of thymine dimers in the nucleosome and recent studies of the structure of thymine dimer-containing DNA suggest that the rate of thymine dimer formation is affected by the direction and degree of DNA bending. This premise was used to construct a model of the path of DNA in the nucleosome, which has the following features. (*i*) There are four regions of sharp bending, two which have been seen previously by x-ray crystallography of the core particle. (*ii*) The DNA in H1-containing nucleosomes deviates from its superhelical path near the midpoint; this is not seen with H1-stripped chromatin. (*iii*) The internucleosomal (linker) DNA appears to be relatively straight.**

Much of the knowledge about nucleosome structure comes from studies of isolated nucleosome core particles. Such studies provide only limited information about native chromatin structure since these particles lack histone H1, the internucleosomal (linker) DNA, and any constraints imposed by higher order chromatin structure. Therefore, it is valuable to develop methods to study nucleosome structure *in situ*. One such method is photodimerization of pyrimidines. Recently, the distribution of pyrimidine dimers (PDs) in core particles isolated from irradiated chromatin was described in careful detail (1, 2). Peaks of PD formation were found to occur with an average periodicity of 10.3 bases and were interpreted in terms of a footprint of histone–DNA interactions. Recent reports indicate that DNA in the immediate vicinity of a thymine dimer (TD) is bent toward the major groove (3, 4). Therefore, TDs should form preferentially wherever the DNA is already bent toward the major groove, and their distribution might reveal a great deal about the path of DNA in the nucleosome. Very little is known about the effect of H1 on the path of DNA in the nucleosome or about the path of DNA in the internucleosomal (linker) region. As this information is important for understanding higher order chromatin structure and the role of H1 in nucleosome structure, I have explored the possibility of using TD formation as an approach to gain such information.

MATERIALS AND METHODS

Synthesis of *N*-(*m*-Acetylbenzyl)-*N*,*N*-Dimethylethylenediammonium Dichloride (AcϕD). AcϕD was synthesized according to Meistrich *et al.* (5) from *m*-chloromethylacetophenone (6) and *N*,*N*-dimethyl-*N'*-acetylethylenediamine (7). Elemental analysis: 53.17% C; 7.59% H; 9.54% N. NMR (D_2O): 2.70δ (s, 3H)-$COCH_3$; 3.61δ (s, 6H)-$N(CH_3)$-; 3.87δ (m, 4H)-CH_2-CH_2-; 4.72δ (s, 2H) C_6H_4-CH_2-N; 7.73δ (t, 1H); 7.85δ (d, 1H); 8.12δ (s, 1H); 8.22δ (d, 1H). Melting point 165–175°C (dec). The NMR results agree with those reported by Meistrich *et al.* (5) except for the peak at 4.72δ; instead they reported a peak at 2.24δ. Also, the melting point differs from the one they reported, 283–284°C. However, the NMR spectrum and elemental analysis given here are consistent with the structure of AcϕD, and the compound was found to be effective in greatly stimulating the production of TDs in DNA irradiated with light >310 nm.

Isolation of Nuclei. All steps were carried out at 0–4°C unless otherwise stated. Frozen rat livers (Pel-Freez Biologicals) were homogenized with a Waring blender in buffer A [0.34 M mannitol/60 mM KCl/15 mM NaCl/0.15 mM spermine/0.5 mM spermidine/2 mM EDTA/0.5 mM EGTA/15 mM triethanolamine/1% thiodiglycol/0.25 mM phenylmethanesulfonyl fluoride, pH 7.3] and filtered through cheese cloth, and nuclei were isolated essentially as described (8).

H1 Extraction. Nuclei were suspended in buffer A at a DNA concentration of 0.5 mg/ml, and 400 μl was added to 10 ml of 0.6 M NaCl/10 mM triethanolamine/1 mM EDTA, pH 7.0. The chromatin was pelleted at 16,000 × *g* for 10 min, gently resuspended and washed in this buffer twice, suspended in buffer A containing 5 mM AcϕD, and left on ice for 15 min. The chromatin was pelleted and resuspended again in buffer A containing 5 mM AcϕD. This procedure removed 70–80% of the H1.

When done at 4°C, NaCl extraction of H1 should produce little or no nucleosome sliding (9, 10). If any sliding occurred during the extraction, it should not affect the results since it occurs before the formation of TDs. All subsequent steps with H1-stripped chromatin were carried out at 4°C to minimize nucleosome sliding. The remarkable similarity of the positions of high and low peaks (except at the middle of the nucleosome) in the patterns of TD formation obtained with H1-containing and H1-stripped chromatin indicates that sliding did not appreciably affect the results obtained with H1-stripped chromatin (see Fig. 2).

Irradiation. Purified nuclei or H1-stripped chromatin was suspended in buffer A containing 5 mM AcϕD and irradiated for 2 hr at 4°C between two Sylvania H37KB 250-W mercury lamps. A solution of thymidine at 10 mg/ml was used to block light <310 nm. In some cases nuclei were irradiated with 254-nm light using a germicidal lamp (dose, 350 J/m^2).

Nuclease Digestion of Irradiated Nuclei and Chromatin. After irradiation, the nuclei were washed twice in buffer A, suspended in 50 mM triethanolamine/25 mM KCl/1 mM $CaCl_2$/1 mM $MgCl_2$/1% thiodiglycol/0.25 mM phenylmethylsulfonyl fluoride, pH 7.5 (buffer B), at 1 mg/ml and digested for 5 min at 37°C with micrococcal nuclease (50 units/ml, the optimal amount depended on the batch). An S2 chromatin fraction was prepared from the digested nuclei as described (11). It was precipitated and digested with exonuclease III essentially as described (12) except the digestion was at 19°C.

After irradiation, the H1-stripped chromatin was resuspended twice in buffer A and once in buffer B. It was then

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Abbreviations: PD, pyrimidine dimer; TD, thymine dimer; AcϕD, *N*-(*m*-acetylbenzyl)-*N*,*N*-dimethylethylenediammonium dichloride.

suspended in 200 μl of buffer B and digested with 30 units of micrococcal nuclease at 4°C for 50 min, with periodic agitation with a glass rod. The sample was spun at 5000 × *g* for 5 min and the supernatant was collected. Fifteen micrograms of the soluble chromatin was digested at 4°C, with 1200 units of exonuclease III, for 45 min in 150 μl of buffer B containing 1.5 mM EGTA and 2 mM $MgCl_2$.

Mapping of Photoproducts. The exonuclease III digestion of chromatin was stopped with 0.5 ml of 0.2 Tris/0.1 M EDTA/1% SDS/proteinase K (0.1 mg/ml), pH 8.5, and incubated for 2 hr at 37°C. The DNA was purified by phenol extraction, precipitated with ethanol, dissolved in 0.5 ml of 0.5% glycerol/0.25 M NaCl/1 mM $ZnSO_4$/30 mM sodium acetate, pH 4.5, containing 150 units of nuclease S1, and digested for 30 min at 19°C. The reaction was stopped by adding 100 μl of 1 M Tris/0.1 M EDTA, pH 8.5. One hundred units of calf intestinal alkaline phosphatase was added, the sample was incubated for 1 hr at 37°C, and DNA was purified as before. One to 4 μg of this DNA in 30 μl of 50 mM Tris/10 mM $MgCl_2$/5 mM dithiothreitol/1 mM spermidine, pH 7.8, containing 10 units of polynucleotide kinase and 50 μCi of [γ-^{32}P]ATP (1 Ci = 37 GBq) was incubated at 37°C for 1 hr to label the 5′ ends. The labeled DNA was digested with RNases A and T1 (100 μg/ml) for 2 hr at 37°C, precipitated with ethanol, dissolved in 90% formamide containing TBE (50 mM Tris base/50 mM boric acid/1 mM EDTA), heated to 90°C, and run in a 5% polyacrylamide gel (1:27.5 acrylamide: bisacrylamide) containing 7 M urea and TBE. Bands of the desired size, detected by autoradiography, were excised, and the DNA was eluted with 0.6 ml of 10 mM Tris/100 mM NaCl/0.4 mM EDTA, pH 7.2, at 37°C. The eluted DNA was extracted with phenol, precipitated with ethanol, and then digested with T4 DNA polymerase as described (13). The reaction was terminated and DNA was purified as before. The digests were run on a denaturing polyacrylamide gel containing a high level of bisacrylamide (1:8) to suppress the effect of sequence on electrophoretic mobility (14, 15). The gel was dried and autoradiographed without an intensifying screen. Fragment lengths were determined by comparison to standards produced from irradiated restriction fragments digested with T4 DNA polymerase (1).

Scans of Autoradiographs. Autoradiographs were scanned, and the digitalized scans were transformed to linear plots by use of an algorithm developed by Samuel Litwin (Fox Chase Cancer Center, Philadelphia). The details of this program will be published elsewhere.

RESULTS

Isolated rat liver nuclei or H1-stripped chromatin were irradiated with 313-nm light in the presence of AcϕD to produce TDs (5). After irradiation, an S2 fraction, which comprises most of the H1-containing nucleosomes (11), was prepared from the nuclei, and mononucleosomes were prepared from the H1-stripped chromatin. These nucleosome preparations were trimmed with nucleases to yield DNA fragments of ≈162 bases for H1-containing mononucleosomes from nuclei and ≈146 bases for H1-stripped mononucleosomes (see *Materials and Methods*). The locations of TDs in these fragments were determined by digestion with the 3′ exonuclease of T4 DNA polymerase, which cannot proceed past a TD (13), and electrophoresis of the resultant fragments.

The formation of TDs in H1-containing and H1-stripped nucleosomes occurred with a periodicity of ≈10 bases (Fig. 1), as shown for PD formation by UV light alone by Gale *et al.* (1). This periodicity was not seen when protein-free nucleosomal DNA was irradiated under identical conditions, showing that it is due to nucleosome structure rather than the distribution of potentially reactive sites. The TD distribution produced with naked nucleosomal DNA was almost random

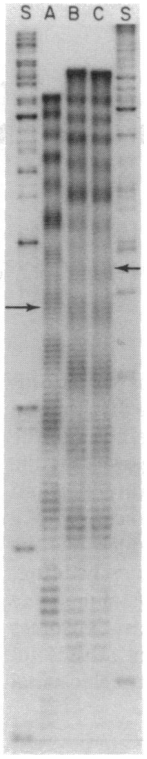

FIG. 1. 3′-Exonucleolytic digests of TD-containing nucleosomal DNA. Trimmed mononucleosomal DNA, labeled on its 5′ end, was digested with T4 DNA polymerase and the digests were electrophoresed in a denaturing polyacrylamide gel, which was analyzed by autoradiography. Sources of nucleosomal DNA: H1-stripped chromatin irradiated in the presence of AcϕD (lane A); H1-containing chromatin, from nuclei irradiated in the presence of AcϕD (lane B); H1-containing chromatin, from nuclei irradiated with 254-nm light (lane C); standards, used to determine fragment lengths (lanes S).

with only a few small peaks that did not correspond to peaks seen with nucleosomes (see Fig. 2, plot D).

To compare the patterns, the autoradiograms were scanned and the relationship of electrophoretic mobility to fragment length was linearized using an algorithm developed by Samuel Litwin. Alignment of the resultant plots was achieved at most sites by shifting the H1-stripped pattern up as seen in Fig. 2. The magnitude of the required shift varied from 8 bases, as seen for this experiment, to 11 bases (this variability is probably due to variations in the trimming of the ends of the nucleosomal DNA). This confirmed that H1 protects ≈10 base pairs (bp) at each end of nucleosomal DNA from nuclease, as suggested from results obtained with DNase I (16). The positions of the peaks of TD formation are diagramed in Fig. 3. The symmetric structure of nucleosomes lacking H1 should make both DNA strands equivalent. Although H1 could in theory confer asymmetry on nucleosomal DNA, the relative positions and intensities of the peaks in the H1-containing nucleosomes are the same as seen with H1-stripped nucleosomes except for the peaks near the middle (see below). In the presence or absence of H1, sites approximately +2, −2, +4, and −4 helical turns from the midpoint of the nucleosome are more reactive to TD formation (Figs. 2 and 3).

In H1-containing nucleosomes, the 10-base periodicity is disrupted in the middle of the nucleosome: instead of the single peak seen just 3′ of the middle of H1-stripped nucleosomes, there are two smaller peaks at 80 and 87 bases (Figs. 2 and 3). Neither of these corresponds to the peak seen with H1-stripped nucleosomes. Sometimes nucleosomes from H1-stripped chromatin produce a very small peak at 79 bases, corresponding to 87 bases in H1-containing nucleosomes. This is most likely due to the fact that the removal of H1 was usually incomplete, so that a small fraction of nucleosomes contained H1 during the irradiation; these nucleosomes can lose H1 during the micrococcal nuclease digestion and produce 146-bp fragments (17).

Another interesting feature of H1-containing nucleosomes is the absence of an obvious peak corresponding to positions +7 and −7 helical turns from the midpoint—i.e., 10 bp from

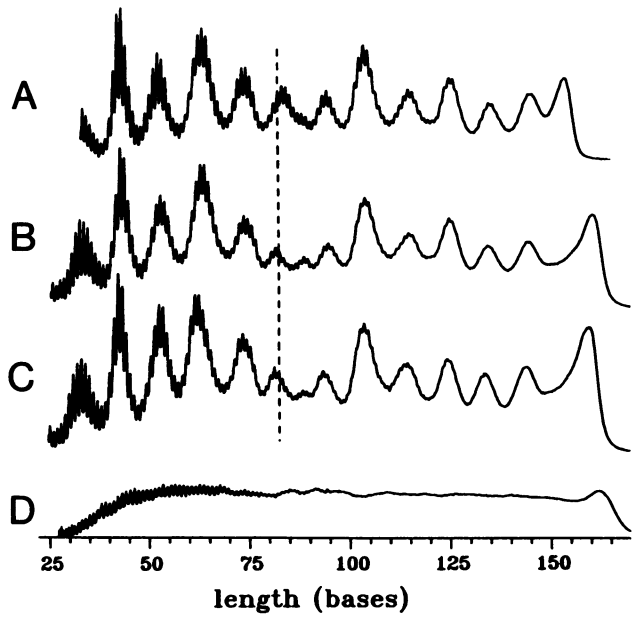

FIG. 2. Comparison of linearized scans. Scans of autoradiograms were linearized, and the resulting plots were aligned at their midpoints, which required that the plot from H1-stripped nucleosomes be shifted up by 8 bases relative to those from H1-containing nucleosomes. Source of nucleosomal DNA: H1-stripped chromatin irradiated in the presence of AcϕD (plot A); H1-containing chromatin, from nuclei irradiated in the presence of AcϕD (plot B); H1-containing chromatin, from nuclei irradiated with 254-nm light (plot C). Plot D shows the result obtained when protein-free DNA from H1-containing nucleosomes was irradiated in the presence of AcϕD. Eight bases must be substracted to obtain the length of fragments in plot A.

the ends. However, if a small peak were present near the 3′ end, it might be obscured by the nearby artifactual peak due to undigested full-length molecules. The pattern near the 5′ end of the DNA is not seen in this analysis, because these small fragments were not efficiently precipitated.

To obtain information about linker DNA, dinucleosomal DNA from irradiated nuclei was examined. Dinucleosomal length DNA was obtained from very thin slices of the first gel (see *Materials and Methods*) in order to minimize heterogeneity of linker length. The TD pattern in DNA obtained from one such experiment is shown in Fig. 4. A complete nucleosome pattern can be seen toward the 3′ end of the DNA (the right side of Fig. 4). This pattern, which reflects the distribution of TDs in both nucleosomes of the dinucleosome, is like that obtained with H1-containing mononucleosomes, having a small peak (at ≈272 bases) corresponding in relative position and size to the peak at 80 bases in H1-containing mononucleosomes; note the 5′ displacement of the peak from the periodicity of the other peaks in both cases (Figs. 2, plot B, and 4). A peak corresponding to the peak at 87 bases of

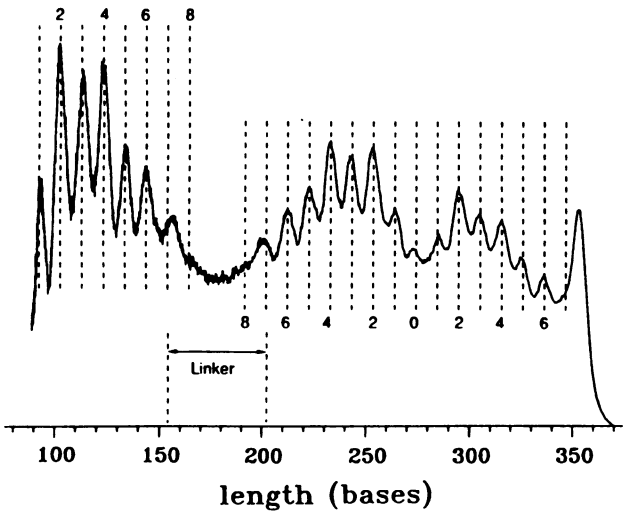

FIG. 4. Distribution of TDs in dinucleosomes. Dinucleosomal DNA from photosensitized nuclei was analyzed for the distribution of TDs, and the resulting autoradiogram was scanned and linearized as before. Assuming that the average spacing between peaks represents the helical repeat of nucleosomal DNA, the dashed lines indicate the number of helical turns of DNA from the center of the nucleosome.

H1-containing mononucleosome cannot be seen and is probably obscured because of a higher background in the dinucleosome analysis. The likely cause of this background is heterogeneity of the parent fragments, which cannot be as well controlled as when mononucleosomes are analyzed. However, such heterogeneity must be relatively small since all other aspects of the pattern are similar to those obtained with H1-containing mononucleosomes.

Also seen in Fig. 4 is part of the nucleosome pattern toward the 5′ end of the DNA, the remainder having run off the gel. The positions of the high and low peaks in this pattern are consistent with an H1-containing nucleosome. The nucleosome repeat length for these dinucleosomes, calculated by measuring the distance from a peak in the 5′ nucleosome pattern to the homologous peak in the 3′ pattern, was 192 bp.

The dinucleosome pattern revealed two small peaks (at ≈157 bases and at ≈200 bases) that could not be detected with mononucleosomes because of their proximity to the ends of the chromatosome DNA. If the average distance between the main peaks represents the helical repeat of nucleosomal DNA, then, as can be seen in Fig. 4, each of these small peaks occurs about a quarter turn of DNA from the position expected from the periodicity of the other peaks. No other peaks were evident in the linker region of these dinucleosomes (Fig. 4); however, in some experiments with different length dinucleosomes, extremely small peaks were present (not shown).

To determine if inaccessibility of nucleosomal DNA to AcϕD contributed to the pattern of TD formation, nuclei

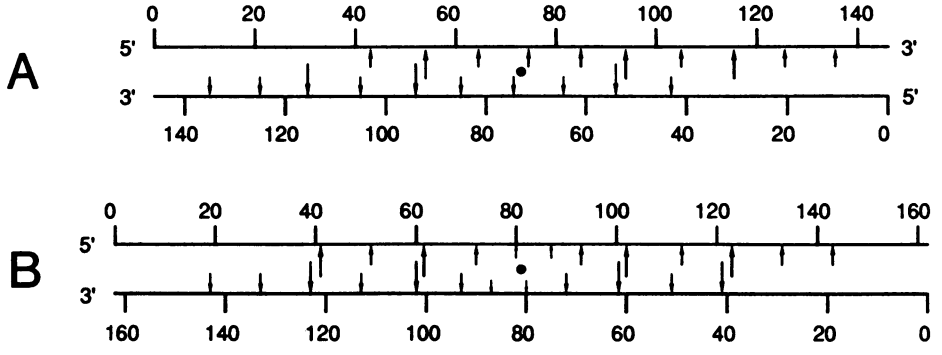

FIG. 3. Sites of peak TD formation in nucleosomal DNA. Peaks of TD formation determined from linearized plots are indicated by arrows. The size of the arrow approximates the size of the peak. The dot marks the middle of the DNA, and the two strands were assumed to be symmetric. (*A*) H1-stripped chromatin irradiated in the presence of AcϕD (full-length fragment, 146 ± 1 bases). (*B*) H1-containing nucleosomes, from nuclei irradiated in the presence of AcϕD (full-length fragment, 162 ± 1 bases).

were irradiated with 254-nm light in the absence of a photosensitizer. This produces predominantly cyclobutane ring PDs but is not as specific as photosensitization for the production of TDs (18). H1-containing nucleosomes were prepared and analyzed in the usual way. The pattern of PD formation was very similar to the pattern of TD formation seen with photosensitization except that the reactivity at position 87 was reduced relative to that seen with photosensitization (Fig. 2). The reason for this difference is not clear but may be due to differences in the photochemistry of photosensitization and 254-nm irradiation (18) or to a preferential binding of the photosensitizer to this region of the nucleosome. The close similarity of the patterns obtained with 254-nm irradiation and photosensitization indicates that inaccessibility of nucleosomal DNA to AcϕD is not responsible for the effect that H1 has on the pattern of TD formation near the middle. It also indicates that the formation of TDs and other cyclobutane ring PDs is modulated in a similar way by nucleosome structure.

DISCUSSION

Several factors may contribute to the observed periodic modulation of TD formation by the nucleosome. Accessibility of the DNA to AcϕD does not appear to be a significant factor since a very similar pattern is obtained with 254-nm irradiation (Fig. 2). This conclusion is consistent with DNA modification experiments that showed that the major groove, and probably the minor groove, of nucleosomal DNA are largely free of histone interactions (19, 20).

To account for the periodicity of PD formation, Gale *et al.* (1) suggested that the binding of histones to DNA restricts movement of the DNA backbone at the sites where it is closest to the histone core, thereby inhibiting PD formation. However, the available data suggest that the mobility of DNA in nucleosome core particles is not greatly restricted compared to free DNA, except for some motions of DNA sugars (reviewed in ref. 21). Furthermore, if histone interactions inhibit TD formation by limiting conformational flexibility, then it would be expected that the linker DNA, being relatively unprotected as judged by sensitivity to nucleases, should be more reactive for TD formation than the DNA in the core of the nucleosome. However, my results with dinucleosomes (Fig. 4) indicate that TD formation in the linker is low relative to the peaks seen in the core region. Thus, at present, it would appear that DNA conformation rather than conformational flexibility is the major factor modulating TD formation in the nucleosome, although it is obviously not possible to rule out some effects of the latter.

A conformational feature of nucleosomal DNA that could produce periodicity in TD formation is curvature. According to a model constructed using energy minimization, DNA in the immediate vicinity of a TD is bent toward the major groove (3), a conclusion supported by recent experiments using gel electrophoresis of TD-containing DNA fragments (4). (Although no information exists about the effect of other cyclobutane ring PDs on DNA structure, it seems likely that it is similar to that of TDs.) Therefore, a bend in the DNA toward the major groove should be favorable for TD dimer formation. In the nucleosome, where the DNA is wrapped around the core histones, TD formation should peak where the bending of the DNA is toward the major groove—i.e., at ≈10-bp intervals. In the crystal structure of the core particle, the bending of the DNA is toward the major groove at the middle (22), and, as seen in Figs. 2, plot A, and 3*A*, a peak of TD formation occurs about 1.5 bases 3′ of the middle of H1-stripped nucleosomes. This is consistent with the model of Pearlman *et al.* (3), in which the TD is located immediately 3′ of the maximum bending toward the major groove.

According to this interpretation, it should be possible to gain information about the path of the DNA in the nucleosome from the positions and sizes of the peaks of TD formation. Although other features of DNA conformation may make significant contributions to the rate of TD formation, it seems worthwhile to construct a model of nucleosomal DNA based on two simple assumptions: (*i*) that peaks of TD formation occur immediately 3′ of where the DNA is bent toward the major groove; and (*ii*) that peak height reflects the degree of bending—i.e., being higher where the DNA bending toward the major groove is closer to the 27–30° predicted for a TD (3, 4).

The highest peaks of TD formation occur at positions +2, −2, +4, and −4 helical turns from the center of the nucleosome in the presence or absence of H1 (Figs. 2 and 3), indicating that the DNA is bent more sharply near these regions (see Fig. 5*A*). The exact position of sharpest bending in these regions cannot be located because of the influence of the direction of bending (e.g., bends toward the minor groove will not be detected). The intermediate-resolution x-ray crystallographic structure of the core particle (22) indicated that the DNA is more sharply bent at positions +1, −1, +4, and −4. The bending at +4 and −4 agrees with the present model based on TD formation, but TD formation indicates sharp bends near +2 and −2 rather than +1 and −1. Recent work by Hogan *et al.* (23) using reaction with either singlet oxygen or methylene blue indicated sharp bends or kinks at positions +1.5 and −1.5 (these bends would not be detected by TD formation since they are toward the minor groove). It would seem likely from these various findings that the distortion of DNA conformation in this region is not simple. Higher resolution crystal structures (24) will hopefully clarify this matter.

The 10-base periodicity of TD formation is disrupted in the middle of H1-containing nucleosomes, suggesting a change in the direction of bending in this region. The direction of bending in this region, however, cannot be as simply inferred as in other regions, because in this case the data suggest that the two strands behave differently. Recalling that the peak of TD formation occurs ≈1.5 bases 3′ of where the bending toward the major groove is expected to be maximal, the peak at 80 bases suggests the DNA is bent toward the major groove ≈2.5 bases from the middle of the nucleosome (the middle of the DNA for these fragments is at 81 bases, see Fig. 3*B*). Since at this site the major groove faces sideways rather than toward the histone octamer (22), this peak suggests a small sideways bend. However, using the same logic, the smaller peak at 87 bases, which represents the other strand of DNA (see Fig. 3*B*), indicates a small bend away from the histone

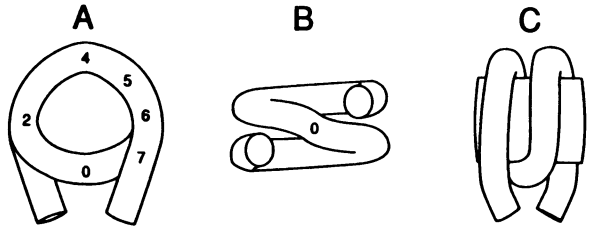

FIG. 5. Model of the path of DNA in H1-containing nucleosome based on TD formation. The numbers represent the approximate number of helical turns (≈10 bp each) from the middle of the DNA. Three different views are shown. (*A*) Increased bending at approximately +2, −2, +4, and −4 helical turns from the middle. (*B*) Sideways bends near the middle of the DNA. As discussed in the text, the pattern of TD formation near the middle does not lead to any simple prediction of the path of the DNA, and the sideways bend shown here is only one possible interpretation. (*C*) Sideways bends near the ends. The histone core is shown in this view to aid the perspective of the drawing.

octamer. The lack of agreement in the indicated direction of bending in this region (80 sideways and 87 away from the octamer) suggests that the conformations of the two strands are not being altered in the same way, which does not seem unlikely if the direction of bending is changing. The simplest model for the path of the DNA in this region is a sideways bend (Fig. 5*B*), but more complicated paths are clearly not ruled out. It is also possible that factors other than DNA bending, such as changes in the twist of the DNA or a direct effect of H1 binding, are at least partially responsible for the pattern of TD formation in this region.

Other evidence for a change in the direction of bending near the middle of the nucleosome comes from the results of Satchwell *et al.* (25). They sequenced 177 cloned DNA fragments derived from isolated core particles and found that the sequences AA and TT tended to occur preferentially at sites where, in the nucleosome, the DNA is bent toward the minor groove, suggesting constraints on nucleosome position related to DNA bending. They found that the phase of this periodicity was disrupted near the center of the core DNA, indicating that the direction of bending changes in this region of the nucleosome. Whether these data reflect the *in situ* distribution of these sequences in H1-containing nucleosomes, from which most of the core particles in their study were derived, is not certain since some nucleosome sliding probably occurred during their preparation of core particles. To the extent that this result represents H1-containing nucleosomes, it agrees with the results presented in this paper. Note that the sites of preferred TT location are not where the DNA is bent toward the major groove and, thus, are not responsible for the peaks in TD formation.

In contrast to the present data, no effect of H1 was seen on the pattern of PD formation observed by Gale *et al.* (1), which was essentially like that seen in the present study with H1-stripped chromatin. Their method differed from that used in the present work in certain respects, which may account for this difference. They analyzed core particle DNA produced from long chromatin fragments that had been irradiated with 254-nm light. It is likely that some of these core particles were derived from H1-free nucleosomes, which, if present in sufficient quantity, would produce a PD pattern in the middle that would obscure the larger of the two small peaks characteristic of H1-containing nucleosomes—i.e., the one corresponding to 80 bases in the chromatosome. The other peak characteristic of H1-containing nucleosomes (at 87 bases in the chromatosome), in my experience, is very small when 254-nm irradiation is used (Fig. 2, plot C) and would be further diminished by the presence of core particles derived from H1-free nucleosomes.

The TD pattern near the ends of the nucleosomal DNA, observed in the dinucleosome analysis, suggests that the DNA superhelix does not complete two full turns around the histone core. The peaks seen in this region are small and are located about a quarter turn of DNA from the position expected from the periodicity of the other peaks (Fig. 4). This indicates that there is only a small amount of bending in this region and that this bending is sideways with respect to the histone core (Fig. 5). Electric dichroism studies of isolated nucleosomal particles also indicate that the DNA straightens as it exits the nucleosome (26).

The remainder of the linker region had a relatively uniform low level of TD formation (Fig. 4). This indicates that this region of the linker contains very little bending, at least for the subset of dinucleosomes with this linker length. Two alternate explanations, that linker DNA exists in conformations whose patterns of TD formation cancel each other out or that the structure of linker DNA is unconstrained, seem unlikely. Very little previous information about the structure of the linker is available. Mitra *et al.* (27) found that in chromatin fibers the photochemical dichroism of the linker differed from that of the rest of the nucleosome, indicating that the linker DNA does not continue the superhelix of the core. However, they were unable to determine the orientation of the linker with respect to the fiber axis, without information about the curvature of linker DNA. Some models of higher order chromatin structure have the linker DNA relatively straight, whereas others have it continue the superhelix of the nucleosome or otherwise strongly bent (reviewed in ref. 21). The results reported here are consistent with a straight linker, although it will be necessary to examine other linker lengths before this result can be generalized.

TD formation is a useful method to probe chromatin structure. It can be used on isolated nuclei or living cells and does not produce any gross alterations of chromatin folding *in vitro* (28). In the nucleosome it appears to be a sensitive probe of DNA bending, which should make it useful for studying nucleosome conformation in a wide variety of circumstances. The apparent sensitivity of the method to DNA bending may also make it useful for examining other nucleoprotein complexes. However, it seems likely that proteins that interact in the grooves of the DNA, such as regulatory proteins, will affect TD formation in ways independent of DNA bending.

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1. Gale, J. M., Nissen, K. A. & Smerdon, M. J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6644–6648.
2. Gale, J. M. & Smerdon, M. J. (1988) *J. Mol. Biol.* **204**, 949–958.
3. Pearlman, D. A., Holbrook, S. R., Pirkle, D. H. & Kim, S. (1985) *Science* **227**, 1304–1308.
4. Husain, I., Griffith, J. & Sancar, A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2558–2562.
5. Meistrich, M. L., Lamola, A. A. & Gabbay, E. (1970) *Photochem. Photobiol.* **11**, 169–178.
6. Goldfarb, Y. L. (1978) *Khim. Geterotsikl. Soedin.* **11**, 1474–1476.
7. Price, C., Kabas, G. & Nakata, I. (1965) *J. Med. Chem.* **8**, 650–655.
8. Pehrson, J. R. & Cohen, L. H. (1984) *Biochemistry* **23**, 6761–6764.
9. Spadafora, C., Oudet, P. & Chambon, P. (1979) *Eur. J. Biochem.* **100**, 225–235.
10. Watkins, J. F. & Smerdon, M. J. (1985) *Biochemistry* **24**, 7279–7287.
11. Rose, S. M. & Garrard, W. T. (1984) *J. Biol. Chem.* **259**, 8534–8544.
12. Strauss, F. & Prunell, A. (1982) *Nucleic Acids Res.* **10**, 2275–2293.
13. Doetsch, P. W., Chan, G. L. & Haseltine, W. A. (1985) *Nucleic Acids Res.* **13**, 3285–3304.
14. Lutter, L. C. (1979) *Nucleic Acids Res.* **6**, 41–56.
15. Morgan, J. E., Calkins, C. C. & Matthews, H. R. (1989) *Biochemistry* **28**, 5095–5106.
16. Simpson, R. T. (1978) *Biochemistry* **17**, 5524–5531.
17. Noll, M. & Kornberg, R. D. (1977) *J. Mol. Biol.* **109**, 393–404.
18. Meistrich, M. L. & Lamola, A. A. (1972) *J. Mol. Biol.* **66**, 83–95.
19. McGhee, J. D. & Felsenfeld, G. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2133–2137.
20. McGhee, J. D. & Felsenfeld, G. (1982) *J. Mol. Biol.* **158**, 685–698.
21. van Holde, K. E. (1988) *Chromatin* (Springer, New York).
22. Richmond, T. J., Finch, J. T., Rushton, B., Rhodes, D. & Klug, A. (1984) *Nature (London)* **311**, 532–537.
23. Hogan, M. E., Rooney, T. F. & Austin, R. H. (1987) *Nature (London)* **328**, 554–557.
24. Richmond, T. J., Searles, M. S. & Simpson, R. T. (1988) *J. Mol. Biol.* **199**, 161–170.
25. Satchwell, S. C., Drew, H. R. & Travers, A. A. (1986) *J. Mol. Biol.* **191**, 659–675.
26. Crothers, D. M., Dattagupta, N., Hogan, M., Klevan, L. & Lee, K. S. (1978) *Biochemistry* **17**, 4525–4533.
27. Mitra, S., Sen, D. & Crothers, D. M. (1984) *Nature (London)* **308**, 247–250.
28. Gale, J. M. & Smerdon, M. J. (1988) *Biochemistry* **27**, 7197–7205.