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The Histone Core Exerts a Dominant Constraint on the Structure of DNA in a Nucleosome†

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ABSTRACT: We have examined the structures of unique sequence, A/T-rich DNAs that are predicted to be relatively rigid [oligo(dA)·oligo(dT)], flexible [oligo[d(A-T)]], and curved, using the hydroxyl radical as a cleavage reagent. A 50-base-pair segment containing each of these distinct DNA sequences was placed adjacent to the T7 RNA polymerase promoter, a sequence that will strongly position nucleosomes. The final length of the DNA fragments was 142 bp, enough DNA to assemble a single nucleosome. Cleavage of DNA in solution, while bound to a calcium phosphate crystal, and after incorporation into a nucleosome is examined. We find that the distinct A/T-rich DNAs have very different structural features in solution and helical periodicities when bound to calcium phosphate. In contrast, the organization of the different DNA sequences when associated with a histone octamer is very similar. We conclude that the histone core exerts a dominant constraint on the structure of DNA in a nucleosome and that inclusion of these various unique sequences has only a very small effect on overall nucleosome stability and structure.

Nucleosomes are positioned in vivo with respect to DNA sequence (Simpson, 1991). Considerable effort has been made to understand what elements of DNA structure and histone composition within the nucleosome are responsible for the phenomenon of positioning and how nucleosome position might be influenced by trans-acting factors. Our most substantial insight into this problem concerns the influence of DNA structure on the rotational and translational position of the DNA helix with respect to the histone core (Travers, 1989).

Within a nucleosome core particle, 146 base pairs (bp) of DNA are wrapped in 1.8 turns around a histone core (Richmond et al., 1984). This constraint of DNA has two consequences. First, the histone core exhibits a preference for DNA that has an intrinsic curvature or anisotropic flexibility with a periodicity of 10.0 bp/turn (Schrader & Crothers, 1990). Second, rigid or straight DNA, such as tracts of oligo(dA)·oligo(dT) (>12 bp), is not favored for incorporation into a

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Chart I

oligo(dA)·oligo(dT)a

5′-GGATCCGGCCGAAAAAAAAAAAACAAAAAAAAAAAAAAAAACAAAAAAAAAAGCTAGCGATCC-3′

3′-CCTAGGCCGGCTTTTTTTTTTTTTGTTTTTTTTTTTTTTTTTGTTTTTTTTTTTCGATCGCTAGG-5′

oligo(dA)·oligo(dT)b

5′-GGATCGCTAGCTTTTTTTTTTTTGTTTTTTTTTTTTTTTTTGTTTTTTTTTTTTCGGCCGGATCC-3′

3′-CCTAGCGATCGAAAAAAAAAAACAAAAAAAAAAAAAAAAACAAAAAAAAAAAAGCCGGCCTAGG-5′

oligo[d(A-T)]

5′-GGATCCGGCCGATATATATATATCATATATATATATATATCATATATATATGCTAGCGATCC-3′

3′-CCTAGGCCGGCTATATATATATAGTATATATATATATATAGTATATATATACGATCGCTAGG-5′

curve A

5′-GGATCGCTAGCTAAAATGGCCTAAAATGGCCCTAAAATGGCCTAAAATGGCCCTAAGGATCC-3′

3′-CCTAGCGATCGATTTTACCGGATTTTACCGGGATTTTACCGGATTTTACCGGGATTCCTAGG-5′

nucleosome compared to curved DNA (Schrader & Crothers, 1990). Although these effects can influence nucleosome position with respect to DNA sequence (Prunell, 1982; Wolffe & Drew, 1989), the observed energetic preference for an ~160-bp fragment of DNA with a favored periodicity of anisotropic flexibility of 10.0 bp/turn compared to a piece of random sequence DNA is small (<3 kcal/mol). Therefore, the influence of DNA sequence on nucleosome position in vivo is not expected to be great.

In this report we have used the cleavage of DNA by the hydroxyl radical to examine the effect of association with a histone core on the conformation of three DNA sequences. We have used DNA fragments containing tracts of either oligo(dA)·oligo(dT), oligo[d(A-T)], or intrinsically curved DNA for reconstitution into nucleosomes. The total length of each of these unusual DNA sequences is individually approximately 50 bp (see Materials and Methods), although any individual stretch of oligo(dA)·oligo(dT) or oligo[d(A-T)] is at most 16 bp in length. These sequences represent the most extreme cases found in vivo and are consequently the most biologically relevant. We find that inclusion of these A/T-rich DNAs in a 142-bp DNA fragment has little effect on the energetics of nucleosome reconstitution. While we show that the structures of the distinct A/T-rich DNAs differ in solution, surprisingly we find that each of these unique DNA sequences adopts a similar conformation, as revealed by hydroxyl radical cleavage, within the nucleosome. The organization of DNA into a nucleosome depends on the presence of the T7 RNA polymerase promoter; this sequence exerts a dominant effect on nucleosome position within the 142-bp DNA fragment. These results support the hypothesis that the histone core exerts a dominant constraint on the structure of DNA in a nucleosome (Hayes et al., 1990).

Materials and Methods

DNA Fragments. Plasmid derivatives of T7CAT were used (Figure 1; Wolffe & Drew, 1989) in which the oligonucleotides shown in Chart I were inserted at the *Bam*HI site.

Plasmid DNA was restricted with *Hin*dIII, and the linearized DNA was then radiolabeled with [α-^{32}P]dATP and [α-^{32}P]dCTP before restriction with *Dde*I. Labeled DNA fragments, 142 bp in length, were purified on nondenaturing 4% polyacrylamide gels and extracted by electroelution.

Hydroxyl Radical Footprinting of Reconstituted Nucleosomes. Chicken erythrocyte histones H2A/H2B and H3/H4 were prepared by chromatography on hydroxyapatite (Simon & Felsenfeld, 1979) and dialysis into 10 mM Tris-HCl, pH 7.5/1 mM Na_3EDTA/0.25 mM phenylmethanesulfonyl fluoride. The concentrations of the histone fractions were determined by measurement of absorbance at 230 nm using an extinction coefficient of 4.2 cm^{-2}·mg^{-1} (Stein, 1979). Histones were reconstituted onto the radiolabeled DNA fragments by salt/urea dialysis (Camerini-Otero et al., 1976). The efficiency of reconstitution was monitored by electrophoresis (Wolffe, 1988). Cleavage of DNA in nucleosomes was accomplished as described (Hayes et al., 1990). For quantitative analysis, autoradiographs were scanned with a Joyce-Loebl Chromoscan 3 densitometer fitted with an aperture wide enough to allow measurement of the optical density across a whole lane. The area of each band was determined with use of software included in the densitometer. Integrals of bands from the control (free DNA) samples were subtracted from the integrals of corresponding bands in the nucleosome samples. The resulting values, which represent the amount of cleavage at each nucleotide in the nucleosome, were then smoothed by performing a three-bond running average throughout the entire data set. Data sets were fitted with a sine function by using the program PASSAGE on a Macintosh computer. Data sets were also analyzed by Fourier transformation. The errors quoted in the text are $\pm 2\sigma$.

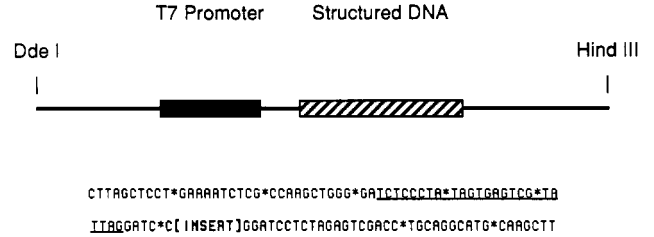

FIGURE 1: Schematic representation of the four 142-bp DNA fragments used in this study. The DNA fragments were identical except for a 50-bp insert of A/T-rich DNA of various sequence (structured DNA, see Materials and Methods). The positions of the T7 promoter, the DNA insert, and the restriction enzyme sites used to obtain the fragments from the parent plasmid, pT7CAT, are indicated (Wolffe & Drew, 1989). The DNA sequence common to all of the constructs is also shown, with the T7 promoter region underlined.

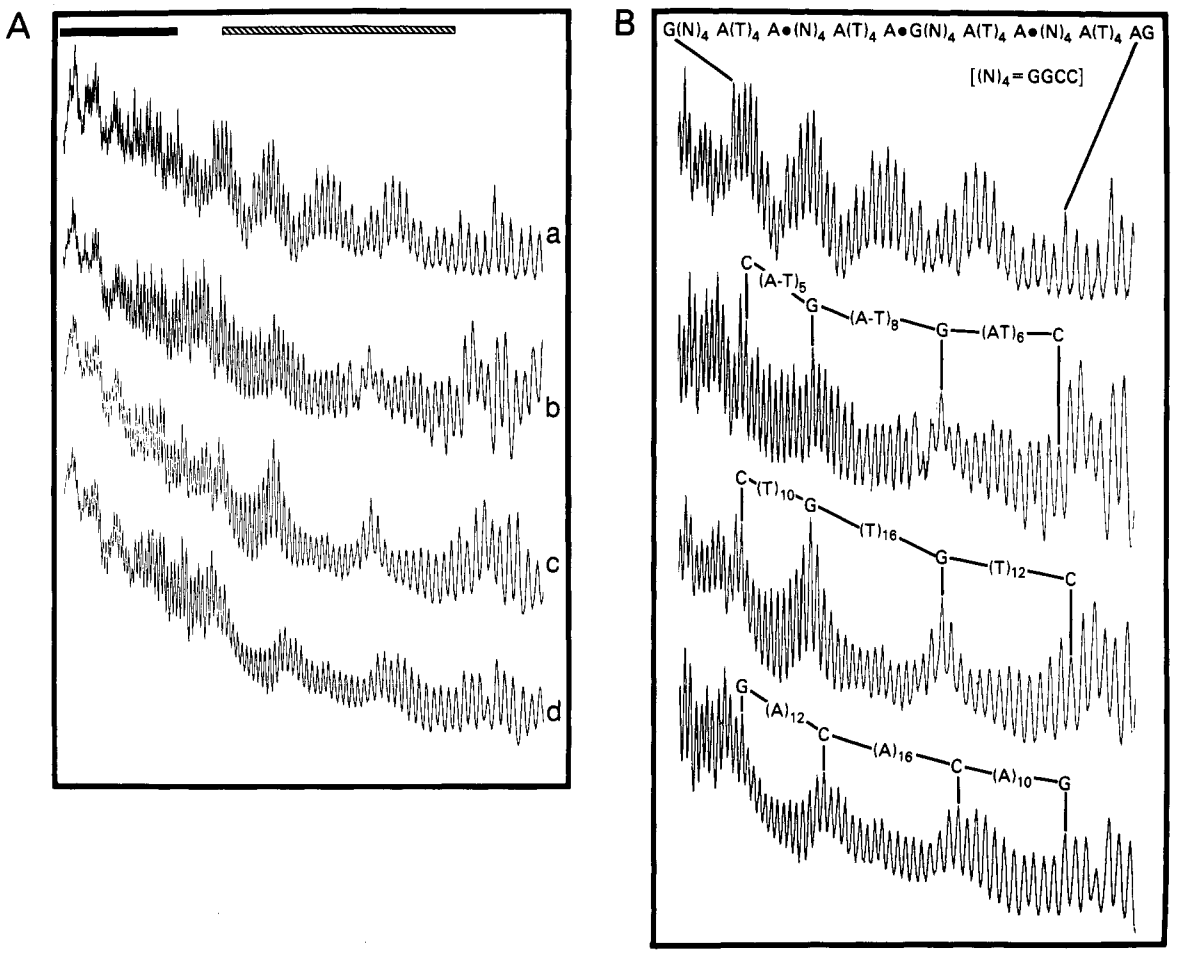

FIGURE 2: Densitometric analysis of the hydroxyl radical cleavage patterns of the oligo[d(A-T)], oligo(dA)·oligo(dT), and curved-DNA-containing fragments. (A) The cleavage pattern of the entire sequence is shown. The regions covered by the T7 promoter (present in all fragments, solid bar) and the A/T-rich DNA (different in all fragments, hatched bar) are indicated: (a) curve A; (b) oligo[d(A-T)]; (c) oligo(dA)·oligo(dT)b; (d) oligo(dA)·oligo(dT)a. (B) Detail of the hydroxyl radical cleavage pattern in the region of A/T-rich DNA is shown.

Competitive Reconstitution. We followed the procedures described by Jayasena and Behe (1989), with minor modification. Radiolabeled DNA (1 ng) fragments were incubated at approximately 0.75 M salt with chicken erythrocyte (2 μg of DNA) chromatin, stripped of linker histones. After incubation at 37 °C for 30 min to allow equilibration of histones between labeled and unlabeled DNA fragments (Jayasena & Behe, 1989; Schrader & Crothers, 1989), the exchange was quenched by lowering the ionic strength with three additions of low-salt buffer (10 mM Tris-HCl, pH 8.0 1 mM EDTA). This procedure results in intermediate salt strengths of 0.19 M, 0.107 M, and 75 mM. Reconstitution efficiencies were determined by quantifying the relative amounts of the labeled fragment in the nucleosomal and free DNA bands in 4.5% polyacrylamide gel.

Results and Discussion

DNA Structure in Solution. The various A/T-rich DNA sequences have been previously shown to have very different structures in solution (see below). We wished to confirm that the hydroxyl radical cleavage reagent could detect the predicted structural features. These observations will serve as reference for any changes in DNA structure following association with either calcium phosphate or the histone octamer. Oligo(dA)·oligo(dT) tracts are predicted to be straight and rigid (Nelson et al., 1987) with a fairly constant narrow minor groove width. X-ray crystal structures of oligonucleotides containing such tracts reveal large positive propellar twists of the base pairs and an ordered water structure ("spine of hydration") in the minor groove (Fratini et al, 1982; Drew & Dickerson, 1981; Nelson et al., 1987; Coll et al., 1987). Changes in sequence that affect these structural features should lead to widening of the minor groove. For example, guanine, with its 2-amino group in the minor groove, would interfere with the spine of hydration. Our cleavage data in solution using hydroxyl radical confirm the predictions from X-ray studies (Figure 2). The presence of a G-C base pair in the midst of an oligo(dA)·oligo(dT) tract increases hydroxyl radical cleavage significantly (Figure 2B), indicating a locally widened minor groove (Burkhoff & Tullius, 1987).

In contrast to oligo(dA)·oligo(dT), oligo[d(A-T)] tracts are predicted to be conformationally flexible. This flexibility is presumed to be a consequence of the instability of the TpA dinucleotide step (Klug et al., 1979; Drew et al., 1981; Travers & Klug 1987). The hydroxyl radical cleavage pattern of the oligo[d(A-T)] sequence is clearly different from the cleavage pattern of the oligo(dA)·oligo(dT) sequence (Figure 2B). Theoretical studies (Chupina, 1985; Calladine, 1982) indicate that the TpA step is incompatible with high propellar twist, and hence the minor groove is forced to be wider. This observation is substantiated by the alternating character of the hydroxyl radical cleavage pattern of the oligo[d(A-T)] tract. Cleavage at the thymidine is slightly greater than at the adenine in the oligo[d(A-T)] sequence (Figure 2B).

We have previously examined the hydroxyl radical cleavage patterns of naturally curved kinetoplast DNA and synthetic oligonucleotides containing short oligo(dA)·oligo(dT) tracts (Burkhoff & Tullius, 1987, 1988). Cleavage of the short

phased oligo(dA)·oligo(dT) tracts in curve A is consistent with these earlier observations (Figure 2B). Our previous observations support the significance of structural perturbations at the junction between a segment of B-DNA and the structure adopted by the oligo(dA)·oligo(dT) tract (Koo et al., 1986) leading to a periodic change in minor groove width (Burkhoff & Tullius, 1987). However, the frequency of hydroxyl radical cleavage is not uniform along an oligo(dA)·oligo(dT) tract, suggesting that the structural perturbation is distributed over several base pairs.

We conclude that the three A/T-rich DNA sequences that we have studied have structural properties revealed by hydroxyl radical cleavage that are consistent with previous observations. In addition, these studies serve to confirm the utility of the hydroxyl radical reagent in determining microheterogeneities in DNA structure at single-base-pair resolution.

Helical Periodicities of A/T-Rich DNA in Solution. We next wished to further characterize these DNAs by determining the helical periodicities of these sequences. This is done by occluding one face of the DNA molecule from a cleavage reagent with use of a calcium phosphate crystal. Where the DNA is exposed, it will be nicked or modified; the frequency of nicking or modification along the DNA helix allows helical periodicity to be determined. The hydroxyl radical reagent has been successfully used for this purpose with mixed and unique sequence DNA fragments (Tullius & Dombroski, 1985; Hayes et al., 1990).

Rhodes and Klug (1981), using DNase I as the cleavage agent, determined that the helical periodicity of poly[d(A-T)] when bound to calcium phosphate was 10.6 ± 0.1 bp/turn. Our quantitative analysis (Figure 3A) of the hydroxyl radical cleavage pattern of the DNA fragment containing this sequence yields an average helical periodicity of 10.5 ± 0.1 bp/turn, in substantial agreement with previous observations. The modulation in hydroxyl radical cleavage for this DNA fragment is very clear. Since oligo[d(A-T)] tracts are believed to be flexible, we presume that the unique orientation of this DNA molecule relative to the crystal surface is a consequence of the influence of flanking sequences on crystal binding.

More insight into what determines the orientation of a DNA fragment on a crystal surface comes from the analysis of the curved DNA fragment (Figure 3B). Binding to the crystal might be expected to maximize charge neutralization along the DNA backbone. Our quantitative analysis suggests that regions along the double helix that have a narrow minor groove width are oriented toward the crystal surface. The relative positions of maxima and minima in hydroxyl radical cleavage, when this fragment is associated with the crystal surface, clearly differ from those of the other DNA fragments. This demonstrates that local sequence features other than the nature of the ends of a linear fragment can determine how a DNA molecule associates with calcium phosphate. This result may be contrasted with the similar structures adopted by these sequences upon association with a histone core (Figure 4, see below).

Rhodes and Klug (1981) also used DNase I to determine a helical periodicity of 10.0 ± 0.1 bp/turn for poly(dA)·poly(dT). Our quantitative analysis (Figure 3C) using an oligo(dA)·oligo(dT) tract that is interrupted with two G-C base pairs indicates that the average helical periodicity for this DNA fragment is 10.4 ± 0.1 bp/turn. This is the expected result as the interruptions in the oligo(dA)·oligo(dT) tract lead to the presence of substantial regions of B-form-like structure that should have a greater number of base pairs per turn (~10.5). It is interesting to note that the regions predicted

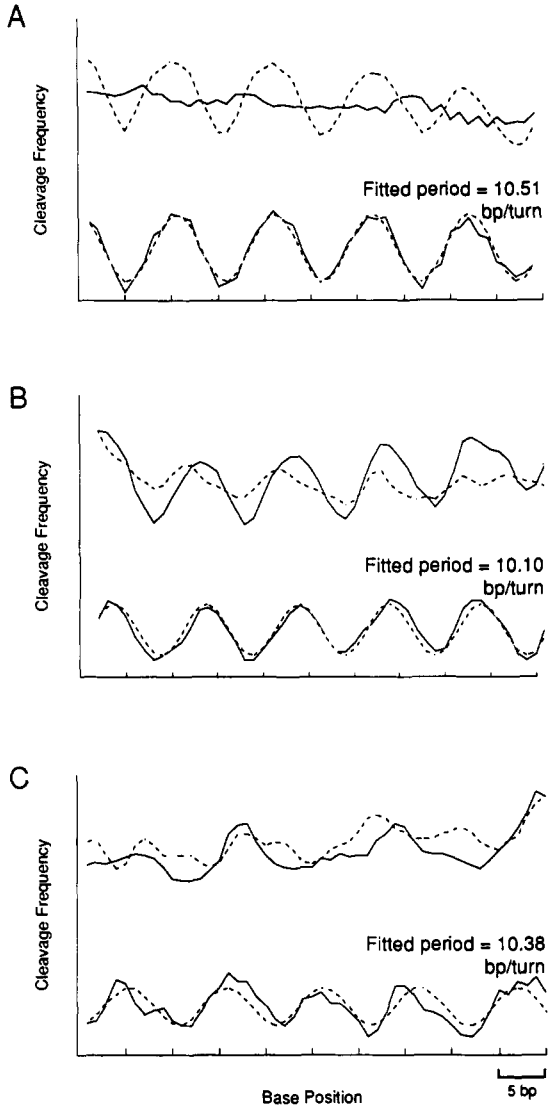

FIGURE 3: Helical periodicity of A/T-rich DNA inserts. Quantitated cleavage data for the region in each fragment containing oligo[d(A-T)], curved DNA, and oligo(dA)·oligo(dT) free in solution (upper broken line) and adsorbed onto a calcium phosphate crystal surface (upper solid line) are shown in panels A, B, and C, respectively. The difference between these two data sets (the lower solid line) and a sine curve fitted to the difference plot (lower broken line) are also shown. The fitted helical period is indicated.

to have wide minor grooves are the regions of preferred hydroxyl radical cleavage on the crystal surface, suggesting that they are the most exposed. Regions of narrow minor groove width face toward the crystal surface. This feature is similar to that seen for the curved DNA fragment.

DNA Structure in the Nucleosome. At this point, we have established that the three different A/T-rich DNA sequences used in our experiments have the expected structural peculiarities in solution (Figure 2) and that they behave differently following binding to a crystal surface (Figure 3). Recent experiments have shown that structural features of a unique sequence of DNA in a nucleosome (Hayes et al., 1990) are identical with those of DNA within mixed sequence nucleosome core particles (Hayes et al., 1991). A prediction from this work is that all DNA sequences, once incorporated into a nucleosome, will have similar conformations. The energetic requirement necessary to deform DNA into the nucleosome would presumably determine the translational and rotational position with respect to the histone core. The configuration of lowest energy will determine the most favored position. The

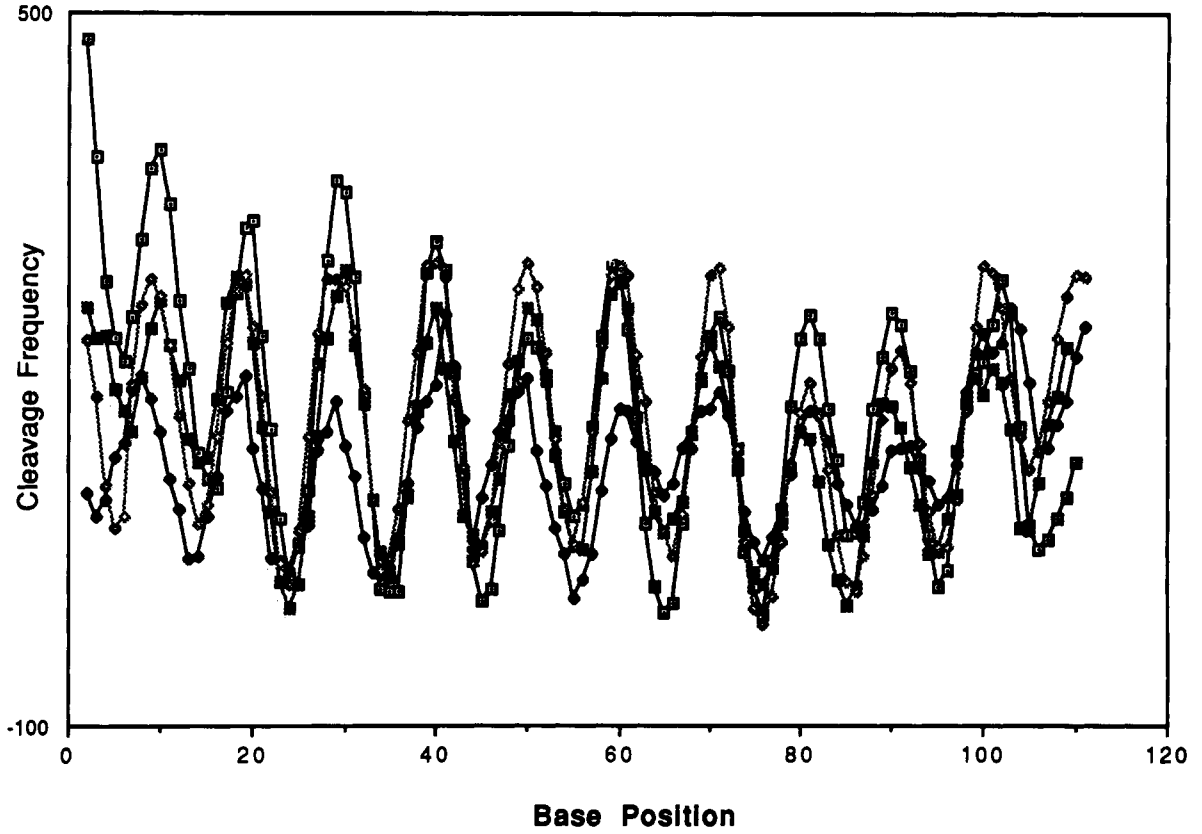

FIGURE 4: Orientation of all four DNA frgments when assembled into a nucleosome. The hydroxyl radical cleavage pattern of each of the A/T-rich DNA-containing fragments when bound on a histone surface was quantitated and plotted. Data from the DNA fragment containing oligo[d(A-T)] are plotted as open squares; curved DNA, as closed diamonds; oligo(dA)·oligo(dT), as closed squares; and oligo(dT)·oligo(dA), as open diamonds. The region containing the A/T-rich DNA insert is located from about position 50 to 100 in this figure. The individual data sets were aligned by the common sequence in regions outside of the inserts. Regions of maximal cutting indicate the places where the backbone of the DNA helix faces away from the histone surface.

DNA fragments containing unusual DNA sequences will presumably have different degrees of deformation, and consequently energy expenditure, upon association with the histone core. We therefore attempted to reconstitute each of the four 142-bp fragments of DNA into nucleosomes and to examine the degree of deformation of the region containing the unusual A/T-rich DNA sequences following incorporation into a nucleosome.

Remarkably, each of the four DNA sequences adopts the same rotational position with respect to the histone core (Figure 4). This positioning of DNA appears to be specified by the constant DNA sequence present in each fragment, i.e., the T7 RNA polymerase promoter, and the constraint of maximizing protein–nucleic acid contacts along the length of the 142-bp fragment (Wolffe & Drew, 1989). The T7 RNA polymerase promoter has alternating maxima and minima of hydroxyl radical cleavage in solution separated by 10–11 bp (Figure 2), a feature we have previously documented to be associated with known positioning elements (FitzGerald & Simpson, 1985; Hayes et al., 1990).

Several other interesting features emerge from a closer analysis of the conformational changes that occur following incorporation of DNA into a nucleosome. The 16-bp continuous oligo(dA)·oligo(dT) tract bends around the histone core with a helical periodicity of ~10.0 bp/turn (Figure 5C,D). This indicates that there is no absolute necessity for this type of rigid DNA structure to be at the periphery of the nucleosome where curvature is expected to be only slight (Prunell, 1982). Moreover, the change in helical twist of this sequence as it is wrapped around the core histones graphically demonstrates the dominant effect of the histone core on the conformation of DNA. In fact, the modulation of hydroxyl radical cleavage for the oligo(dA)·oligo(dT) tract is as extreme as that of the highly flexible oligo[d(A-T)] tract (Figure 5A). The dominant nucleosome positioning elements present in the constant region of DNA (the T7 promoter) and the constraints on nucleosome position due to DNA length appear to be such that they allow the curved DNA fragment (Figure 5B) to be rotationally positioned so as to place the regions of naturally narrow minor groove width in solution toward the histone core. The final configurations appear to be identical for all fragments (Figure 4), even though they each have a different helical twist in solution (Figure 3).

All of the unusual DNA sequences used in our experiment are found in vivo: oligo(dA)·oligo(dT) tracts (Valenzuela et al., 1977), oligo[d(A-T)] (Brutlag, 1980), and curved DNA (Marini et al., 1985). In agreement with others we find no experimental case in which these DNA sequences preclude nucleosome formation (Kunkel & Martinson, 1981; Losa et al., 1990). However, detailed studies have suggested that oligo(dA)·oligo(dT) tracts will have a tendency to be found at the periphery of nucleosomes (Prunell, 1982; Satchwell et al., 1986). Earlier studies, which were thought to demonstrate the failure of long (>100 bp) tracts of oligo(dA)·oligo(dT) to form nucleosomes, are not simply interpretable due to the tendency of this DNA to form stable triple-helical structures at the high salt concentrations used in these experiments (Simpson & Kunzler, 1979; Rhodes, 1979; Arnott & Selsing, 1974). We conclude that these different relatively rigid, flexible, or curved DNA sequences will adopt the same conformation in nucleosomal DNA. We have documented the various degrees of deformation of the DNA helix required for

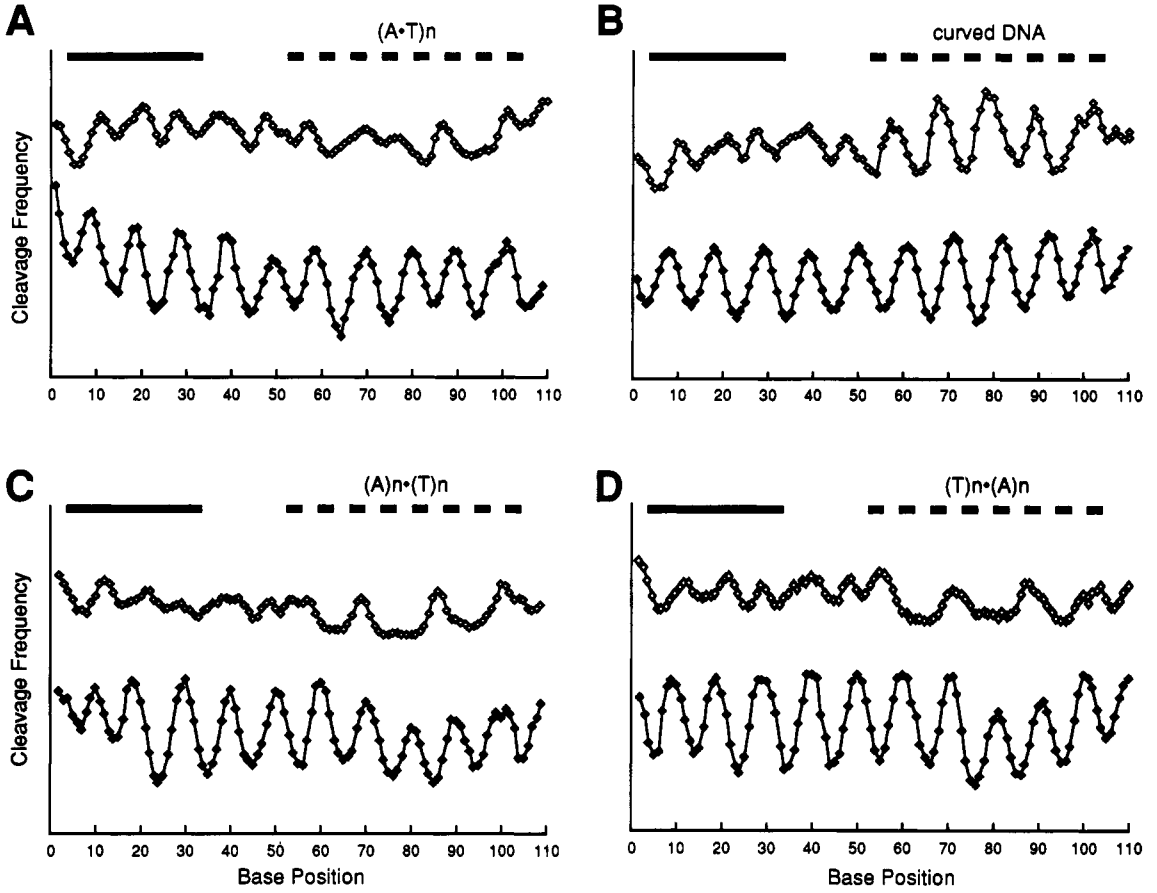

FIGURE 5: Comparison of the hydroxyl radical cleavage patterns of nucleosomal and free DNA for the different DNA frgments. The quantitated frequency of cleavage is plotted for free DNA (◇) and for nucleosomal DNA (◆). Panels A, B, C, and D show the cleavage data from the oligo[d(A-T)], curved DNA, oligo(dA)·oligo(dT)a, and oligo(dA)·oligo(dT)b insert-containing fragments, respectively. The regions containing the T7 promoter or the A/T-rich DNA insert are indicated by a solid or a dashed line, respectively. Base position 1 in this figure corresponds to the eighth base from the *Dde*I end of the fragments (see Figure 1). A/T-rich DNA inserts are located in the 55–105 region.

this conformation to be achieved in each of these cases. We now wished to determine if there exists a correlation between these various degrees of structural deformation and the energetics of nucleosome formation.

Relative Affinity of the Histone Core for Unusual DNA sequences. Several workers (Jayasena & Behe, 1989; Schrader & Crothers, 1989, 1990) have used competitive exchange of histone octamers to measure the preference of the octamer for particular DNA fragments. Their results suggest that there is little preference for particular DNA fragments relative to the mixture of DNA molecules present in chicken erythrocyte nucleosome core particles. The conformation of DNA in solution and in the nucleosome was not, however, comprehensively defined. Having established that major conformational changes occur in DNA following incorporation into a nucleosome (Figures 2, 3, and 4) and that the final DNA structure is almost identical for widely differing DNA sequences (Figure 4), we next compared the relative affinity of the histone core for DNA fragments containing these unusual DNA sequences. Results from a competitive reconstitution experiment are compared by assigning the relative energy difference of nucleosome formation for a particular DNA with respect to random sequence core DNA. We find that there is little difference in the free energy of formation ($\Delta\Delta G$) between these various DNA fragments when incorporated into nucleosomes. However, the trend in $\Delta\Delta G$ reflects the relative amount of change in DNA structure that occurs upon nucleosome formation. The differences in ΔG of nucleosome formation with respect to random sequence core DNA (0 ± 0.04 kcal/mol) are 1.40 ± 0.30 kcal/mol for the curved-DNA-containing fragment; −0.33 ± 0.23 kcal/mol for the oligo[d(A-T)] fragment; −1.15 ± 0.01 kcal/mol for oligo(dA)·oligo(dT); and −1.20 ± .03 for the oligo(dT)·oligo(dA) fragment. Two additional curved DNA fragments, curve B and curve D from Wolffe and Drew (1989), were also found to have $\Delta\Delta G$ values of 0.85 ± 0.33 and 0.86 ± 0.18 kcal/mol, respectively. The largest difference occurs between fragments containing curves (Wolffe & Drew, 1990) that are favored by <3 kcal/mol over those containing oligo(dA)·oligo(dT) tracts. These results agree with the conclusions of Schrader and Crothers (1990) and Jayasena and Behe (1989) and demonstrate the conformational plasticity of DNA on nucleosome formation.

It should be emphasized that the equilibrium measurement of the affinity of a histone octamer for a DNA fragment reflects the affinity relative to mixed sequence DNA in chicken erythrocyte core particles at 0.5 M salt or higher, since high salt concentrations are required to facilitate exchange. It is possible that differences of binding free energy among different DNA sequences toward the histone octamer might be much less at high salt than at low salt because the DNA is not wrapped so firmly about the protein at high salt than at low salt (Spadafora et al., 1979; H. R. Drew, personal communication).

Conclusion

The major conclusion of this paper is that the histone core exerts a dominant constraint on the structure of DNA in the nucleosome. Relatively rigid, flexible, and curved DNA sequences adopt the same conformation in nucleosomal DNA.

The deformation of the DNA helix demonstrated in these experiments has relatively little effect on the free energy of binding of a DNA fragment to the histone octamer. Practically all naturally occurring DNA sequences will therefore have the capacity to be incorporated into a nucleosome. Therefore, nucleosome positioning might be expected to be more strongly influenced in vivo by factors other than structural features within the DNA helix.

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