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Sequence Dependence of the Curvature of DNA: A Test of the Phasing Hypothesis†

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ABSTRACT: Certain DNA molecules derived from both prokaryotic and eukaryotic organisms display markedly abnormal electrophoretic behavior on polyacrylamide gels. These molecules share a common element of sequence which involves collections of A/T residues that are approximately in phase with the helix repeat. This sequence periodicity has led to the suggestion that such phasing is important in generating the abnormal behavior. We have demonstrated that such phasing is, in fact, essential, thus ruling out alternative models which invoke any form of isotropic or centrosymmetric flexibility as the source of the phenomenon. We have also shown that the abnormal behavior is not a simple consequence of marginal thermodynamic stability. The most plausible explanation for the observed behavior is that stable, local distortions of the helix axis result in macroscopic curvature when such distortions are propagated in phase with the helix repeat.

A number of duplex DNA molecules, derived as restriction fragments from both prokaryotic (Ross et al., 1982; Stellwagen, 1983; Bossi & Smith, 1984) and eukaryotic (Simpson, 1979; Challberg & Englund, 1980; Kidane et al., 1984) sources, display distinctly anomalous electrophoretic behavior. The behavior is characterized by reduced electrophoretic mobilities of the restriction fragments on polyacrylamide gels. We have invoked a hydrodynamic argument to support the notion of stable curvature of the helix axis (Hagerman, 1984); however, our approach did not exclude the possibility that a portion of the anomalous electrophoretic behavior is due to increased isotropic flexibility of the sequences of interest. This last issue is particularly germane, since (a) oligo(dA)-oligo(dT) sequences have been implicated in the generation of DNA curvature (Wu & Crothers, 1984; Kidane et al., 1984; Hagerman, 1984) and (b) it has been suggested that poly(dA)-poly(dT) sequences are more flexible than random-sequence DNA (Hogan et al., 1983). This paper represents an attempt to resolve the uncertainty of stable curvature vs. increased flexibility as the source of the anomalous electrophoretic behavior.

Before proceeding, it is important to establish what is meant by stable axial curvature. In discussing the structure of an inherently flexible molecule such as duplex DNA, stable axial curvature must be regarded as a nonlinear, minimum free energy configuration of the helix axis. Such axial curvature is expected to have an inherent phase relation to the helical repeat (Wu & Crothers, 1984); hence, if local helical distortions are propagated in phase with the helical repeat, macroscopic curvature should result (Figure 1). In contrast, increased flexibility that is dependent only on base composition would not be expected to display a phase dependence. Moreover, any form of flexibility which involves centrosymmetric deflections (i.e., deflections which do not result in a *mean*, nonzero component) will not display a sequence–helix phase dependence for the generation of macroscopic curvature. Therefore, the basic approach taken in the present study is to change the helical phasing of a particular oligo(dA)-oligo(dT) sequence in order to determine its phase dependence.

Materials and Methods

Synthesis and Purification of Oligodeoxynucleotides. All of the oligodeoxynucleotides employed in this study were synthesized by using the phosphite triester chemistry of Caruthers (Caruthers et al., 1982; Sproat & Gait, 1984). We have made a number of modifications of the basic protocols in order to reduce our cycle time to under 6 min (P. J. Hagerman, unpublished results). Final detritylation was performed

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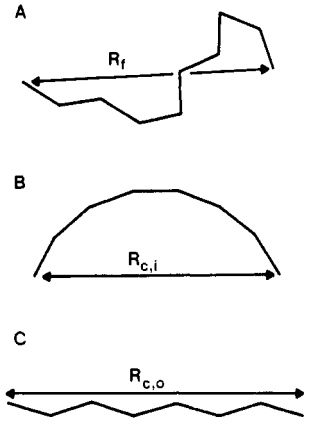

FIGURE 1: Central distinction between increased isotropic flexibility (A) and stable curvature (B, C) of the helix axis of DNA involves the absence of a phase dependence of the former. The proposal of stable curvature does not imply the absence of flexibility, only that the axial configuration of minimum free energy is not linear. In part A, R_f refers to the reduced end to end separation arising as a result of isotropic flexibility. R_f would not depend on sequence phasing with respect to the helix repeat even if axial flexure occurred in a particular direction with respect to the major and minor grooves, provided that the deflections were centrosymmetric. In parts B and C, $R_{c,i}$ and $R_{c,o}$ refer to reduced end to end separations arising from in-phase and out-of-phase axial distortions, respectively. Such distortions may either be rigid or be a result of noncentrosymmetric deflections (e.g., preferential bending into the major groove).

while the oligomer was still attached to the controlled pore glass (CPG) support (2% trichloroacetic acid in methylene chloride, 45 s). Base deprotection was carried out for at least 20 h at 56 °C using concentrated ammonium hydroxide (previously stored at −20 °C). Oligomers were subsequently lyophilized and applied in aliquots to 20% polyacrylamide/8 M urea gels (running temperature 50 °C). Oligomers were identified by UV shadowing and were eluted from macerated gel slices (>12 h) in 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl)/1 mM sodium ethylenediaminetetraacetate (NaEDTA), pH 8.1, temperature 4 °C. The eluate was then filtered and passed over a DE52 column for further purification. Purified oligomers were stored as ethanol slurries (75% v/v ethanol/0.75 M potassium acetate).

Reagents. Phosphoramidites and derivatized CPG supports were obtained from American Bionuclear. Tetrazole was Aldrich "Gold Label" and was used without further purification. Solvents were all high-pressure liquid chromatography (HPLC) grade and were used without further purification with the exception of acetonitrile, which was refluxed over calcium hydride for at least 48 h prior to the start of synthesis. Phosphoramidites and tetrazole were evacuated (<60 millitorr) for 48 h prior to synthesis.

Verification of Oligodeoxynucleotide Sequence and Integrity. Oligomer sequences were verified by employing the chemical sequencing technique of Maxam & Gilbert (1980), optimized for oligomer reactions. In addition, the T-specific cleavage reaction of Friedman & Brown (1978) was employed. The chemical approach provides us with the identities of all but the 5′ base in most cases. Sequence verification is completed by demonstrating that T4 ligase polymerized duplexes can be recut to completion with the appropriate restriction enzymes. In addition to verifying the sequence, such digestions establish the integrity of the duplexes as well as the quantitative removal of protecting groups.

To check the extent to which partial depurination occurs during purification, the decamer $G_2A_3T_3C_2$ (5′-^{32}P end labeled) was exposed to 0.25 N NaOH for 1 h at 37 °C, conditions under which we observe backbone cleavage at sites of base removal. No detectable cleavage of the decamer was observed.

Polymerization of Duplex DNA Oligomers. DNA oligomers were phosphorylated with polynucleotide kinase (Bethesda Research Labs) followed by polymerization using T4 DNA ligase (New England Biolabs) (0.5 mM ATP, 50 mM Tris-HCl, pH 8, and 10 mM $MgCl_2$; T = 12 °C). Ligase concentrations and reaction times were adjusted to give informative ligation ladders. Progress of the ligation reactions was followed for each oligomer by observing band distributions on 2.5% mini-agarose gels. Ligation reactions were terminated by the addition of NaEDTA to a final concentration of 20 mM, followed by phenol/ether extraction.

Gel Electrophoresis. Polyacrylamide gels (12% acrylamide, 37:1 monomer to bis ratio) were poured at room temperature and allowed to polymerize for at least 12 h prior to being run. The gel running buffer was 40 mM Tris–acetate, 20 mM sodium acetate, and 1 mM NaEDTA, pH 7.9. The gel spacer thickness was 0.8 mm.

*Enzyme Digests. Hpa*II digests were carried out at a ratio of 5 units of enzyme (Bethesda Research Labs) to 1 μg of DNA for 3 h at 37 °C using standard buffer conditions [no bovine serum albumin (BSA)]. S1 nuclease digests were carried out by using 500 units of enzyme (Bethesda Research Labs) to 1 μg of DNA for 3 h at 37 °C.

Results and Discussion

Sequence-Dependent, Anomalous Electrophoretic Behavior of Duplex DNA Is Strongly Dependent upon Sequence Phasing. To test the basic phasing hypothesis, a series of duplex DNA polymers of the form $[G_iA_3T_3C_i]_N$ ($N > 1$; i = 1, 2, or 3) were constructed. The oligomeric units were chosen for three reasons: (1) we had observed that synthetic polymers containing A_jT_j sequences (j > 1) were all capable of displaying abnormal electrophoretic behavior (P. J. Hagerman, unpublished results), (2) such sequences, being symmetric, could be polymerized without attendant ambiguities regarding the arrangement of oligomers within the polymer, and (3) only one of the three species (i = 2) would generate polymers having the A_3T_3 core approximately in phase with the helix repeat in solution (Peck & Wang, 1981; Rhodes & Klug, 1981; Strauss et al., 1981). Polymers of varying degrees of polymerization, N, were constructed by blunt-end ligation of the synthetic oligomers (see Materials and Methods for details).

The central observation of this study is displayed in Figures 2 and 3, namely, that the electrophoretic behavior of the decamer series (i = 2) is distinctly abnormal, whereas the octamer (i = 1) and dodecamer (i = 3) series demonstrate essentially normal electrophoretic behavior. This observation immediately rules out any direct dependence of the abnormal electrophoretic behavior on the relative AT content since the octamer series, which is electrophoretically normal, has the highest AT content (75%) of the three A_3T_3 series. Thus, chemical interactions of AT-rich regions with the polyacrylamide gel matrix and/or increases in isotropic flexibility due to high AT content can be rigorously ruled out as explanations for the anomalous behavior. The abnormal electrophoretic behavior of the decamer series strongly supports the idea that local distortions of the helix axis, when propagated approximately in phase with the helix repeat, give rise to macroscopic curvature of the type postulated for a number of natural DNAs.

The essentially normal electrophoretic behavior of the octamer and dodecamer series does not imply the absence of local distortions. Two observations (P. J. Hagerman, unpublished results) suggest that the axial distortions are primarily de-

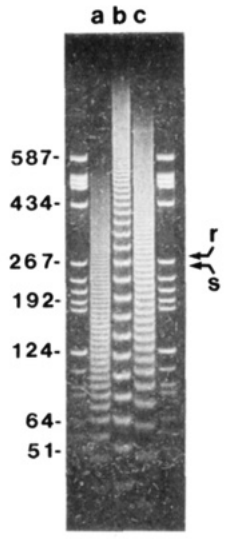

FIGURE 2: Gel electrophoretic behavior of duplex DNA polymers of the form (a) 5′-$[GA_3T_3C]_N$, (b) 5′-$[G_2A_3T_3C_2]_N$, and (c) 5′-$[G_3A_3T_3C_3]_N$. For each of the three polymer series, electrophoretic mobilities are normal for $N < 6$. Polymers of varying N were constructed by partial ligation of the basic oligomer duplexes specified within the square brackets. Lanes a–c are flanked by a *Hae*III digest of pBR322, with representative fragment sizes specified to the left of the gel pattern. To the right of the gel pattern, r indicates the position of $[G_3A_3T_3C_3]_{22}$, and s indicates the position of $[GA_3T_3C]_{33}$. The ligation patterns displayed in this figure do not represent limiting ligation patterns; continued ligation results in high molecular weight polymers. Synthetic oligomers were produced manually by using the phosphite triester approach, and sequences were verified both by chemical sequencing and by enzymatic analysis (see Materials and Methods for details). The gel temperature was approximately 22 °C.

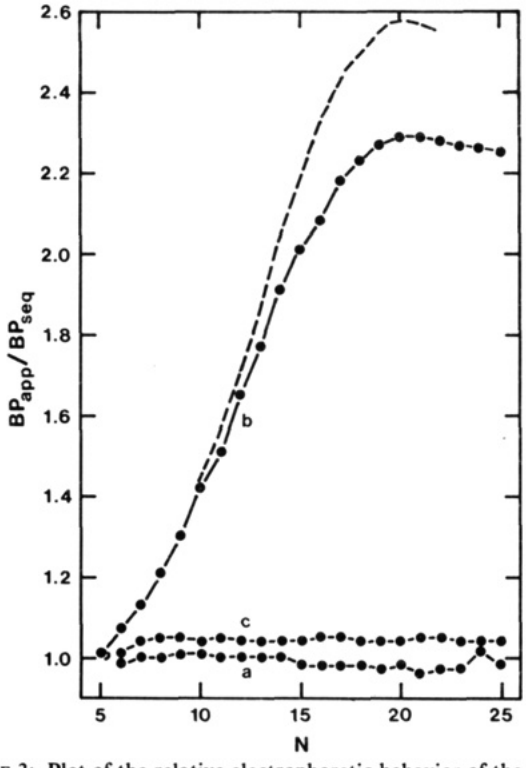

FIGURE 3: Plot of the relative electrophoretic behavior of the three oligomer ladders displayed in Figure 2. In each case, the ratio of the apparent number of base pairs [bp_{app} (BP_{app} in figure)] to the actual number of base pairs [bp_{seq} (BP_{seq} in figure)] is plotted as a function of the degree of oligomer polymerization, N. Letter designations correspond to those of Figure 2. The dashed line represents the pattern observed for $[GA_4T_4C]_N$ at 22 °C.

pendent upon the A_3T_3 core sequence: (1) various decamer-based block copolymers comprising primarily GC are normal,

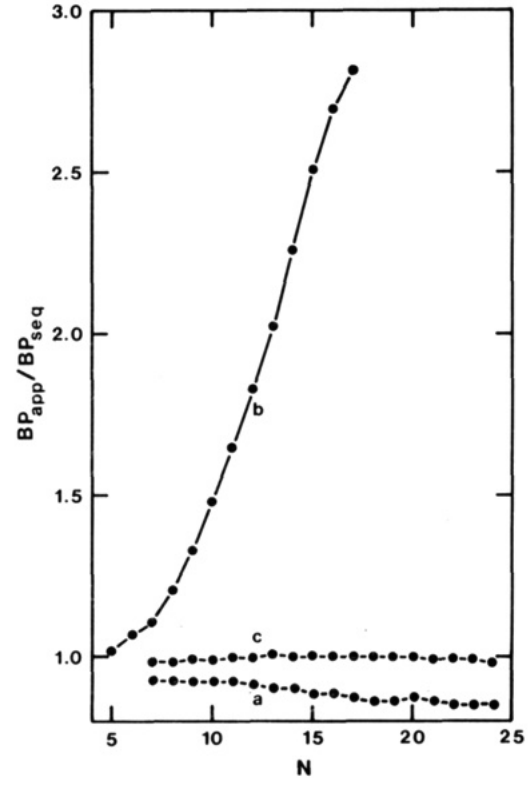

FIGURE 4: Plot similar to that displayed in Figure 3: (a) 5′-$[A_4T_4]_N$; (b) 5′-$[GA_4T_4C]_N$; (c) 5′-$[G_2A_4T_4C_2]_N$. The gel temperature was approximately 6 °C.

or nearly so, and (2) replacement of flanking 5′G with a C and of the flanking 3′C with a G does not reduce the degree of electrophoretic anomaly of the block copolymers. The most likely explanation for the observed behavior of the various series is that sequences containing oligo(dA)-oligo(dT) runs form secondary helices of various cross section, depending upon the phasing of the AT blocks. Secondary helices with narrow cross sections, which would occur if axial distortions were not in phase with the helical repeat, may not be significantly retarded in polyacrylamide gels.

It should be noted (Figure 2) that the behavior of the dodecamer series $[G_3A_3T_3C_3]_N$ is not precisely normal, as evidenced by the slightly different mobilities of the 264 base pair (bp) molecules $[GA_3T_3C]_{33}$ and $[G_3A_3T_3C_3]_{22}$. The former runs almost exactly as expected for a 264 bp fragment, whereas the latter runs at approximately 280 bp. This difference may reflect the fact that the helical repeat is slightly greater than 10.0 bp/turn, thus producing a somewhat larger secondary helical cross section for the dodecamer series than for its octameric counterpart. This last suggestion is consistent with the essentially normal behavior of the $[G_2A_4T_4C_2]_N$ series (Figure 4), which should have a helix repeat closer to 10.0 bp/turn than its $G_3A_3T_3C_3$ counterpart.

Electrophoretic Behavior of the Decamer Series $[G_2A_3T_3C_2]_N$ Is Not a Consequence of Cruciform or Hairpin Formation. The decamer series displayed in Figures 2 and 3 has been examined for the presence of cruciform and/or hairpin structures. Such structures might conceivably arise during the various manipulations involved in preparing the polymers and running the polyacrylamide gels. The first test of the duplex character of the polymer was made by noting that the polymeric material contains potential *Hpa*II sites (CCGG) every 10 bp. *Hpa*II digestion of a separate aliquot of the ligated material displayed in lane b of Figure 2 yielded a single decamer band which contained greater than 95% of

the DNA (limit of detection). This observation militates against the presence of significant loop-back structures, since such species would yield primarily 5, 15, 25, etc. base-pair-equivalent molecules. A second observation involves the absence of any increased susceptibility of the decamer ladder to the action of S1 nuclease (relative to normal duplex DNA). This last observation is consistent with the results of similar studies with natural kinetoplast DNA (Marini et al., 1984). It is also clear that the various N-mers are not being capped by hairpins, since continued ligation results in the conversion of the ladder to very high molecular weight species (>1 kbp). Finally, under certain conditions (high temperatures or high ethanol concentrations), we do observe partial conversion of certain N-mers (usually for $N < 10$) to various hairpin forms as expected. These altered species run with mobilities distinct from those of the normal ligation ladder.

Anomalous Electrophoretic Behavior of the Decamer Repeat Is Not a Simple Consequence of Any Relative Thermodynamic Instability of the Duplex. In order to examine the possibility that the abnormal electrophoretic behavior is a simple consequence of local melting, we have constructed octamer, decamer, and dodecamer series, with each oligomer containing 2 additional AT base pairs (e.g., $[G_iA_4T_4C_i]_N$, where $i = 0$, 1, or 2). As can be seen by inspection of Figure 4, the same qualitative behavior is evident for the three A_4T_4-containing series. When the $[GA_4T_4C]_N$ decamer series was run at 22 °C (Figure 3), that series displayed a reduced degree of maximum retardation compared to the same sequence ladder run at 6 °C (Figure 4), a result which is opposite to that predicted for models based on relative thermodynamic stabilities. This obervation is consistent with the temperature-dependent electrophoretic anomalies observed with natural kinetoplast fragments (Marini et al., 1984).

It is also interesting to note (Figure 4) that the octamer series $[A_4T_4]_N$, which is 100% AT, migrates *faster* than expected on the basis of size ($bp_{app}/bp_{seq} < 1$ where bp_{app} is the apparent number of base pairs and bp_{seq} is the actual number of base pairs). This behavior may reflect a greater intrinsic flexibility associated with pure AT sequences; however, the effect is opposite to that observed with stable axial curvature. Moreover, its $[A_5T_5]_N$ counterpart (also 100% AT) displays reductions in electrophoretic mobility similar to its $G_2A_3T_3C_2$ and GA_4T_4C counterparts (P. J. Hagerman, unpublished results). One possible explanation of the above observations is that intrinsic flexibility attenuates the effect of stable curvature on electrophoretic mobility and that decreasing temperature renders the effect of curvature more dramatic by reducing such flexibility. It is also possible that temperature-dependent effects are, in part, due to changes in helical repeat. These proposals are currently being investigated.

CONCLUSIONS

The central conclusion of this study is that the reduced electrophoretic mobilities of certain AT-containing DNA molecules are a direct consequence of a phase coherence between sequence periodicity and the helical repeat. This conclusion immediately eliminates any model for the observed electrophoretic behavior which depends solely on AT content. Therefore, the phenomenon is not dependent on any specific chemical interaction between AT-rich regions and the polyacrylamide gel matrix, nor is it a simple consequence of any increased isotropic flexibility (or other centrosymmetric deflections) of the helix axis, as might occur within the AT regions. Furthermore, the electrophoretic behavior is not a consequence of marginal thermodynamic stability; rather, the phenomenon must be related to an intrinsic alteration in the mean helix direction, most probably originating within the AT blocks.

The observations presented above do not distinguish between *static* curvature and anisotropic flexibility with an equilibrium-average (mean) curvature (Ulyanov & Zhurkin, 1984). In fact, stable axial curvature must still take into account the intrinsic flexibility of the helix axis.

Finally, the absence of macroscopic curvature (normal electrophoretic or hydrodynamic behavior) does not imply the absence of regions of significant microscopic curvature, as evidenced by the essentially normal macroscopic behavior of the octamer and dodecamer series, all of which contain the A_iT_i core sequences. Regions of local curvature may exert a profound influence on the interactions of sequence-specific proteins with DNA. Such a contribution to the energetics and specificity of a particular protein–DNA interaction should always be considered. Highly localized distortions will probably turn out to be much more significant biochemically than macroscopic, delocalized curves, due to the higher free energy densities associated with the former.

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Registry No. 5′-$[GA_3T_3C]_N$, 98678-52-5; 5′-$[G_2A_3T_3C_2]_N$, 98687-69-5; 5′-$[G_3A_3T_3C_3]_N$, 98687-71-9; 5′-$[A_4T_4]_N$, 98678-54-7; 5′-$[GA_4T_4C]_N$, 98687-73-1; 5′-$[G_2A_4T_4C_2]_N$, 98687-75-3.

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Proton NMR Investigation of the Nucleosome Core Particle: Evidence for Regions of Altered Hydrogen Bonding†

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ABSTRACT: Novel ^{1}H nuclear magnetic resonance (NMR) resonances, arising from exchangeable protons and centered at ~11.2 and 10.1 parts per million (ppm), have been observed in the low-field spectrum (10–15 ppm) of the chicken erythrocyte core particle [145 ± 2 base pairs (bp)]. These peaks are located upfield from the normal adenine-thymine (A-T) and guanine-cytosine (G-C) imino peaks characteristic of B-form deoxyribonucleic acid (DNA) and are not observed in free DNA under identical conditions. The appearance of the new peaks is ionic strength dependent and temperature-reversible below 75 °C. At 25 °C, the upfield peak area represents 5% of the DNA base pairs (7 bp), while between 45 and 55 °C, the area increases to 18%, affecting approximately 25 bp. Area increases in the upfield resonances result in a complementary decrease in the A-T and G-C imino peaks found between 12 and 14 ppm. We believe these novel proton signals represent a histone-induced DNA conformational change which involves localized alteration of base pairing in the core particle.

Because the packing ratio within the nucleosomal structure has been determined to be 7, i.e., seven lengths of deoxyribonucleic acid (DNA)[1] contained per length of nucleosome core, conformational states have been proposed for the DNA in the nucleosome which would allow for a tightly coiled structure and would agree with nuclease digestion patterns. Early considerations led to the suggestion that the phosphodiester backbone in the core particle was primarily B form but might contain bends (Crick & Klug, 1975) or kinks (Sobell et al., 1976) occurring at regular intervals. Crick & Klug (1975) proposed a helix which was primarily B form but contained a 98° bend every 20 bp. Sobell (1976) predicted a more drastic alteration of the phosphodiester backbone which resulted in a 40° kink every 10 bp. The 10 bp kink, in this model, was conjectured to be the molecular basis for the 10 bp cutting frequency pattern characteristic of DNase I. Both models predicted base unstacking to occur at the bend. Until recently, no data have supported the presence of any conformationally distinct region (i.e., bend) in the backbone of nucleosomal DNA. Measurements made by ^{31}P NMR (Cotter & Lilley, 1977; Kallenbach et al., 1978; Klevan et al., 1979; Shindo et al., 1980), a technique which is quite sensitive to conformational changes in the DNA helix, suggested only one average nucleosomal conformation, which was not significantly different from the conformation in free DNA. ^{1}H NMR (Feigon & Kearns, 1979) of the imino protons of the A-T and G-C base pairs similarly displayed little difference between the spectra of the core particle and free DNA. Consistent with these results, energy calculations (Levitt, 1978; Sussman & Trifonov, 1978) showed that whereas kinked and abruptly bent DNAs were energetically possible, smooth isotropic bending of DNA changed the local conformation of the phosphodiester backbone only slightly and the resulting increase in energy was small.

Recently, Richmond et al. (1984) have determined the crystal structure of the core particle to 7-Å resolution. Unexpectedly, the nucleosome crystal structure revealed the presence of bent regions located at about 30, 60, 80, and 110 bp which displayed "departure from good base stacking" (Richmond et al., 1984). The lack of detection of such altered regions in solution studies of nucleosomes again raises the question as to whether any such regions of altered structure exist under physiological conditions. The presence of bends

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[1] Abbreviations: NMR, nuclear magnetic resonance; ppm, parts per million; bp, base pair(s) of DNA; A-T, adenine-thymine base pair; G-C, guanine-cytosine base pair; H1, H2, H3, H4, and H5, histone proteins 1, 2, 3, 4, and 5, respectively; DNA, deoxyribonucleic acid; EDTA, (ethylenedinitrilo)tetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; PMSF, phenylmethanesulfonyl fluoride; SDS, sodium dodecyl sulfate; CD, circular dichroism; UV, ultraviolet light; U, uridine; D_2O, deuterium oxide; Hz, hertz; MHz, megahertz; Tris, tris(hydroxymethyl)aminomethane; TSP, 3-(trimethylsilyl)propionic acid; Pipes, piperazine-*N,N′*-bis(2-ethanesulfonic acid).

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