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Structure of DNA in a Nucleosome Core at High Salt Concentration and at High Temperature†

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ABSTRACT: We have used hydroxyl radical cleavage of DNA to probe the organization of the nucleosome core at high salt concentration and high temperature. The rotational and translational positioning of a DNA fragment, containing part of the *Xenopus borealis* 5S RNA gene, on the histone octamer is maintained between salt concentrations of 0.0 and 0.8 M NaCl and between temperatures of 0 and 75 °C. These results provide evidence that the energy of bending DNA around the nucleosome is independent of salt concentration and temperature in this range. They illustrate the severe energetic requirements necessary to displace DNA from previously organized contacts with histones in the nucleosome core.

The most detailed analysis of protein–DNA interactions within a large nucleoprotein structure exists for the nucleosome core particle (van Holde, 1988). This fundamental unit of chromatin consists of 1.75 turns of DNA wrapped around an octamer of core histones (Richmond et al., 1984). The biophysical properties of core particles have been investigated using a variety of physical perturbants, yielding much useful information.

Nucleosome core particles alter their structure reversibly in response to an increase in ionic strength. Their sedimentation coefficient decreases from 11.7 S at very low salt concentrations (0 M NaCl) to 8.1 S at high salt concentration (1 M NaCl) (Stacks & Schumaker, 1979). The interaction of the histone octamer with DNA changes as ionic strength increases; nucleosomes slide (at 0.6 M NaCl; Lohr et al., 1977), histones H2A and H2B can be dissociated from the particle (at 0.8 M NaCl; Burton et al., 1978), and the complete histone octamer can exchange to exogenous DNA (at 0.8 M NaCl; Germond et al., 1976). This exchange of the histone octamer at high salt concentration has become a useful technique for measuring the relative affinity of the octamer for different DNA molecules (Jayasena & Behe, 1989; Shrader & Crothers, 1990; Hayes et al., 1991a,b). It is, however, possible that the organization of DNA in the nucleosome core particle at high ionic strength differs substantially from that at low ionic strength (Drew, 1991). Here we examine this possibility directly using hydroxyl radical cleavage.

The hydroxyl radical has been used to determine the helical periodicity of DNA in a nucleosome (Hayes et al., 1990, 1991a,b). The structure of free DNA (10.5 bp/turn) is altered to 10.7 bp/turn for three turns around the dyad axis and to 10.0 bp/turn in the flanking regions. This provides a reference with which to examine potential perturbations in structure. Moreover, hydroxyl radical cleavage of DNA will occur under conditions, such as high salt or temperature, that will normally inhibit enzymes. We show here that the cleavage pattern of defined sequence DNA within the nucleosome core particle at high salt (1.0 M NaCl) retains the characteristic helical periodicity observed at low salt (0.0 M NaCl).

We have also used the hydroxyl radical mediated cleavage of DNA to examine the thermal denaturation of defined sequence nucleosome core particles. Many investigators have observed a biphasic transition in hyperchromicity, proposing that the first transition (at 60 °C) corresponds to the melting of DNA (40 bp) at the ends of the core particle and the second transition (at 74 °C) corresponds to disruption of protein–nucleic acid interactions over the entire particle [see Weischet et al. (1978)]. The first transition is reversible, whereas the second transition is not. Simpson (1979) substantiated this interpretation using synthetic nucleosome core particles containing poly(dA·dT)·poly(dA·dT). These particles undergo the first transition at 45 °C. Digestion with exonuclease III under these conditions reduces DNA to a limit size of around 100 bp, consistent with the melting of approximately 40 bp of DNA at the end(s) of the particle. Nuclear magnetic resonance experiments also suggested that during the first transition two distinct conformational states exist for DNA in the nucleosome core particle (Simpson & Shindo, 1979; Shindo et al., 1980). Here we show directly for a defined sequence nucleosome that there is disruption of protein–DNA contacts at the edge of the nucleosome core particle after the first transition in hyperchromicity. Hydroxyl radical cleavage reveals that DNA conformation around the dyad axis of the nucleosome remains unchanged until after the second irreversible denaturation of the particle. These results therefore directly confirm previous suggestions of two distinct regions of protein–nucleic acid interaction in the nucleosome core particle that differ in their thermal stability.

MATERIALS AND METHODS

The specific DNA used in these experiments was a 152-bp *Eco*RI–*Rsa*I fragment of plasmid pXP-10, radiolabeled at the *Eco*RI site using the Klenow fragment (Wolffe et al., 1986; Hayes et al., 1991a,b). The radiolabel is 78 bp upstream from the initiation site for transcription of the *Xenopus borealis* 5S RNA gene and 75 bp from the dyad axis of the reconstituted nucleosome core particle (Rhodes, 1985; Hayes et al., 1990). Histones were reconstituted onto the radiolabeled fragment

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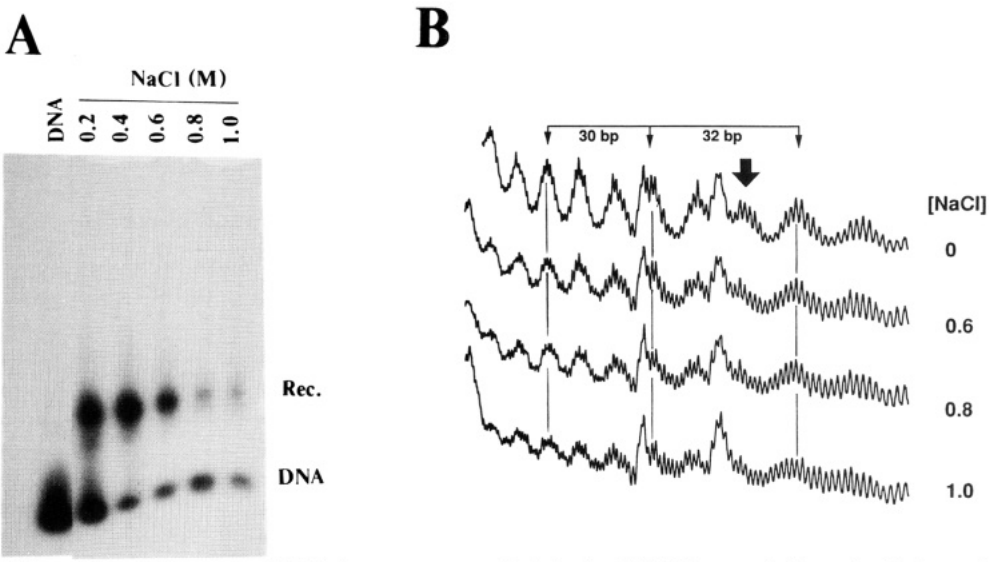

FIGURE 1: Salt stability of 5S nucleosomes. (A) Nucleosomes reconstituted onto 5S DNA were challenged with increasing concentrations of NaCl for 1 h at room temperature and then subjected to nucleoprotein electrophoresis. The indicated concentrations of salt were in addition to 10 mM Tris and 1 mM EDTA (pH 8.0) (TE) buffer. Samples were loaded directly into the wells of a running gel, and thus considerable band distortion results from salt effects. (B) Nucleosomes were probed with hydroxyl radical in the presence of the indicated concentrations of NaCl. The cleavage products were separated by denaturing electrophoresis, and the autoradiograms were analyzed by storage phosphor analysis (see Materials and Methods). The large arrow indicates the location of the nucleosomal dyad (Rhodes, 1985). The smaller arrows and brackets indicate the spacing (number of base pair steps) between peaks in cleavage and reflect the helical period in these regions (Hayes et al., 1990). The two large peaks in the center of the scans are the result of gel artifacts.

by salt/urea dialysis (Camerini-Otero et al., 1976). Chicken erythrocyte histones H2A/H2B and H3/H4 were prepared by chromatography on hydroxylapatite (Simon & Felsenfeld, 1979) and dialyzed into 10 mM Tris·HCl, pH 7.5, 1 mM Na_3EDTA, and 0.25 mM phenylmethanesulfonyl fluoride. The concentration of the histone fractions was determined by absorbance at 230 nm, using an extinction coefficient of 4.2 cm^{-2}·mg^{-1} (Stein, 1979). The integrity of the core histones was checked before and after reconstitution by denaturing polyacrylamide gel electrophoresis. The efficiency of nucleosome core particle reconstitution was monitored by nondenaturing gel electrophoresis (Wolffe & Drew, 1989). Chicken erythrocyte core particles were prepared as previously described (Ausio et al., 1989).

All experiments were carried out in the presence of 10 mM Tris and 1 mM EDTA (pH 8.0). The salt concentration was adjusted by addition of concentrated NaCl solution (4 M) to attain the indicated concentrations, followed by rapid mixing and incubation at room temperature (25 °C) for 15 min before digestion and resolution on a nondenaturing agarose gel (Hayes & Wolffe, 1992). Thermal denaturation experiments were carried out in 1 mM Tris·HCl, pH 7.5, and 0.1 mM EDTA, using a temperature block and a rate of temperature increase of ~0.5 °C/min. Absorbance measurements were made every minute. Samples were equilibrated at the temperature of interest for 5 min, before digestion with hydroxyl radical for 2 min at the same temperature. Absorbance measurements did not change during the incubation. The temperature in each sample was verified by insertion of a thermocouple microprobe. Hydroxyl radical cleavage was as previously described (Tullius et al., 1987) except that the final concentrations of Fe(II) and H_2O_2 reagents in the cleavage reactions were 100 μM and 0.012%, respectively. The integrity of the reconstitute was examined by nondenaturing gel electrophoresis. Cleavage products within the nucleoprotein complex (Hayes & Wolffe, 1992) were resolved on 6% denaturing polyacrylamide gels containing 7 M urea and analyzed on a Model 400E Molecular Dynamics phosphorimager.

RESULTS AND DISCUSSION

Nucleosome Core Stability to High Salt Concentration. We examined the integrity of the nucleosome core reconstitutes containing part of the *X. borealis* 5S RNA gene after incubation salt concentration (Figure 1A). Even after incubation with 1 M NaCl added to the buffer, followed by immediate electrophoresis without dilution, over 30% of the reconstituted nucleosome core particles (Rec) remain intact. Next we examined the influence of salt concentration on the hydroxyl radical cleavage pattern of DNA in the nucleosome core particle (Figure 1B). Consistent with previous observations (Rhodes, 1985; Hayes et al., 1990) the 5S DNA is both translationally and rotationally positioned on the histone octamer. Around the dyad axis of the nucleosome core particle (bold vertical arrow) hydroxyl radical cleavage reveals a helical periodicity of ~10.7 bp/turn (32 bp per 3 turns). Outside of this region, in the flanking DNA, hydroxyl radical cleaves with DNA with a periodicity of 10 bp/turn (30 bp per 3 turns). This modulation is clearly maintained in solution with salt concentrations as high as 0.8 M NaCl and appears to be present even in the presence of 1.0 M NaCl. At these high salt concentrations (1.0 M NaCl) the modulation of hydroxyl radical cleavage is reduced, suggesting either an increased amount of free DNA or less effective rotational positioning of DNA. However, both translational and rotational positioning of the 5S DNA in contact with the histones is substantially maintained in solutions containing 0.8 M NaCl. This specific organization of the DNA in the nucleosome core is consistent with observations by van Holde and colleagues that the histone tetramer, $(H3/H4)_2$, will position on *Lytechinus* 5S DNA at high salt concentration (1.0 M NaCl) (Hansen et al., 1991).

It has been suggested that the organization of DNA in the nucleosome at physiological salt concentrations (~150 mM NaCl) might differ from that observed at 0.8 M NaCl (Drew, 1991). This might influence the comparative changes in free energy for histone octamer binding to DNA in a competitive exchange assay (Jayaseha & Behe, 1989). We have found

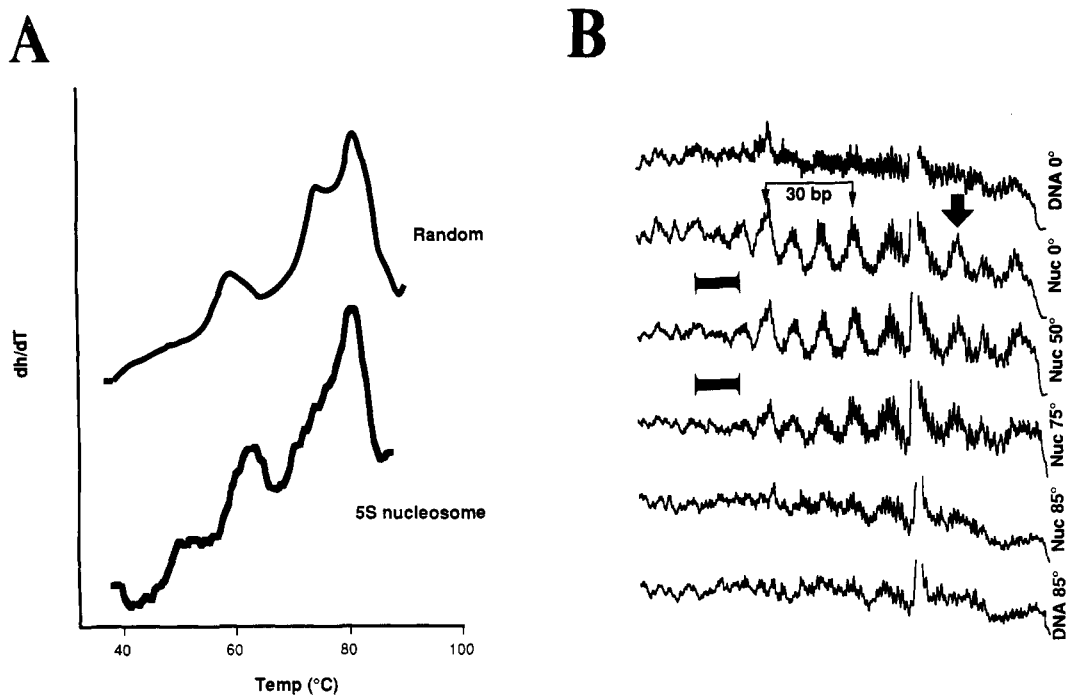

FIGURE 2: Thermal stability of 5S nucleosomes. (A) Comparison of the thermal melting profile of 5S nucleosomes with that of random sequence core particles. Data are plotted as the derivative of the absorbance (A_{260}) as a function of temperature (dh/dT). (B) Effect of temperature on the hydroxyl radical footprint of the 5S nucleosome. 5S nucleosomes were probed at various temperatures as indicated. The cleavage products were separated by denaturing electrophoresis, and the autoradiograms were analyzed by densitometry (see Materials and Methods). The large arrow and smaller arrows and brackets are as in Figure 1. The thick horizontal line indicates the location of the region of the core DNA where the changes in the cleavage pattern are most evident between 0 and 50 or 75 °C. The large peak due to gel artifact (Figure 1B) appears in a slightly different position here because of a slightly different radiolabeling scheme.

that the helical periodicity of DNA at the dyad axis of the nucleosome core and immediately flanking this region is not significantly different between salt concentrations at which histone octamers do exchange (0.8 M NaCl) and those at which they do not (0.6 M NaCl). Furthermore, this organization is similar to that at physiological ionic strength. Thus we conclude that the comparative free energies obtained for histone octamer association with DNA at 0.8 M NaCl are likely to compare favorably with those at lower ionic strengths (Jayasena & Behe, 1989; Shrader & Crothers, 1990; Hayes et al., 1991a,b). Other evidence that is consistent with this conclusion includes the observation that the energy required to deform DNA does not change significantly from 0.1 to 1.0 M NaCl (Hagerman, 1988). Therefore, the energy required to wrap DNA around the histone core and to alter the number of base pairs per turn of the double helix might be expected not to be influenced substantially by comparable changes in salt concentration.

Nucleosome core particle conformation does change from a sedimentation coefficient of 11.7 S at very low salt (0 M NaCl) to one of 9.9 S at 0.25 M NaCl and of 8.9 S at 0.8 M NaCl (Stacks & Schumaker, 1979). The small alteration in sedimentation coefficient between 0.25 and 0.8 M NaCl might be explained by dissociation of the core histone tails from contact with DNA in this salt range (Walker, 1984). This would not be expected to influence either nucleosome core positioning (Dong et al., 1990; Hayes et al., 1991a,b) or the helical periodicity of DNA in the nucleosome core (Hayes et al., 1991a,b).

Nucleosome Core Stability to High Temperature. We next compared the derivative melting curve of the defined sequence nucleosome containing part of the *X. borealis* 5S RNA gene with that of random sequence nucleosome core particles (Figure 2A). Consistent with established results for mixed sequence nucleosome core particles (Weischet et al., 1978; Shindo et al., 1980; Walker & Wolffe, 1984), we observed a biphasic hyperchromicity profile, with the first transition centered at 59 °C and the second at 75 °C. The hyperchromicity profile of the nucleosome core containing 5S DNA is similar; however, both hyperchromicity transitions are shifted to higher temperatures: 62 °C for the first transition and 79 °C for the second. The appearance of secondary peaks in the profile is probably due to the multiphased melting of a unique sequence DNA fragment. The differences in nucleosome core-specific transitions, however, probably reflect differences in stability between the mixed sequence nucleosome cores and the nucleosome cores containing 5S DNA. The 5S DNA is relatively rich in G·C base pairs, and the association of a histone octamer with 5S DNA is favored over that with mixed sequence DNA (Shrader & Crothers, 1989).

The organization of DNA in the nucleosome cr as probed by hydroxyl radical cleavage shows several changes as the temperature of the reaction increases (Figure 2B). Between 0 and 50 °C, the start of the first hyperchromicity transition, there is little change in hydroxyl radical cleavage pattern. After the first transition, the helical periodicity of DNA revealed by the cleavage pattern around the dyad axis of the nucleosome core (large vertical arrow) does not change as far as six helical turns of DNA away (30 bp indicated by brackets). However, the degree of modulation of hydroxyl radical cleavage decreases a little over this region, suggesting that DNA is less constained and that the rotational positioning of the double helix on the histone octamer has decreased. This central region of DNA in the nucleosome core is predominantly organized by a tetramer of histone H3/H4 (Hayes et al., 1991a,b; Dong & van Holde, 1991), and our results therefore indicate that histone–DNA contacts in this region remain intact during the first transition. Three detectable peaks in the 0 °C scan outside of this central region of contact are highlighted by the bar in Figure 2B, one at either end of the

bar and one exactly in the center. These peaks, although clearly evident, are slightly diminished in the 50 °C scan and completely undetectable in the 75 °C scan at the beginning of the second transition in hyperchromicity, suggesting that DNA is free from constraints imposed by the histones. After the second transition, the hydroxyl radical cleavage pattern is like that of free denatured 5S DNA at this temperature. Thus, our results suggest that the first transition involves the complete release of DNA constraints outside the central 120 bp of the 5S nucleosome, together with some release of constraint within this central region. The second transition involves the complete loss of any detectable specific organization of DNA through contact with the histones. This interpretation is consistent with earlier work suggesting a loss of stable histone–DNA contacts at the periphery of the nucleosome core particle during the first transition, followed by a complete loss of organized structure in the second transition (Weischet et al., 1978; Simpson, 1979; Walker & Wolffe, 1984).

CONCLUSIONS

The organization of 5S DNA on the histone octamer in a nucleosome core particle is remarkably stable. Rotational and translational positioning of DNA on the histone octamer is apparent even at a salt concentration of 0.8 M NaCl. Likewise, positioning of the histone octamer is evident even at a temperature of 75 °C. Disruption or rearrangement of histone–DNA contacts in the nucleosome core particle will therefore require a substantial energetic input. It is noteworthy that two of the proven means of displacing histones from DNA that might have physiological significance require either processive enzymes (Lorch et al., 1987; Clark & Felsenfeld, 1992) or the binding of the transition proteins during spermiogenesis (Singh & Rao, 1987). These mechanisms have in common the melting of the DNA duplex, which appears necessary to disrupt histone–DNA interactions in the nucleosome. Histones do not appear able to efficiently organize single-stranded DNA into nucleosomal structures (Almouzni et al., 1990). Disruption of histone–DNA contacts can also be achieved by the addition of high concentrations of cationic compounds such as protamines (Oliva & Dixon, 1991). This would apparently mimic the effect of high salt concentrations (greater than 0.8 M) and remove histones from duplex DNA competitively. In vivo the protamines, which can contain extensive tracts of polyarginine, accumulate to a large excess during spermatogenesis to achieve this displacement of histones. The energetic considerations involved in the binding of single transcription factor to a specific sequence are likely to be insufficient to disrupt all of the histone–DNA interactions in a complete nucleosome core particle; other mechanisms must mediate nucleosome disruption (Pina et al., 1990; Archer et al., 1991; Perlmann, 1992; Hayes & Wolffe, 1992).

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