¹H NMR investigation of the conformational states of DNA in nucleosome core particles

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ABSTRACT

In this study ¹H NMR has been used to investigate the conformational state of DNA in nucleosome core particles. The nucleosome core particles exhibit partially resolved low field (10-15 ppm) spectra due to imino protons in Watson-Crick base pairs (one resonance per GC or AT base pair). To a first approximation, the spectrum is virtually identical with that of protein-free 140 base pair DNA, and from this observation we draw two important conclusions: (i) Since the low field spectra of DNA are known to be sensitive to conformation, the conformation of DNA in the core particles is essentially the same as that of free DNA (presumably B-form), (ii) since kinks occurring at a frequency of 1 in 10 or 1 in 20 base pairs would result in a core particle spectrum different from that of free DNA we find no NMR evidence supporting either the Crick-Klug or the Sobell models for kinking DNA around the core histones. Linewidth considerations indicate that the rotational correlation time for the core particles is approximately 1.5×10^{-7} sec, whereas the end-over-end tumbling time of the free 140 base pair DNA is 3×10^{-7} sec.

INTRODUCTION

It is now established that the basic subunit of chromatin, the nucleosome, consists of a core particle with two each of histones H2A, H2B, H3 and H4, around which 140 base pairs of DNA are wrapped and a "spacer" region of variable length DNA with which histone H1 is associated (for reviews, see 1, 2). Several different models have been proposed for the folding or wrapping of the DNA in the nucleosome particles (3-7). These models have attempted to account for the constraints imposed by the estimated size of the particle ($110 \times 110 \times 57 \text{ \AA}$) (3) and for the results of nuclease digestion studies, which indicate that nuclease susceptible sites occur with varying frequencies in multiples of 10 base pairs along the DNA in nucleosomes (8-12). The two most widely discussed models assume that most of the DNA remains in more or less standard B-form, but in one class of models the DNA is kinked every 10 or 20 base pairs (4,7) so that the

bases at the kink are unstacked, and the DNA can fold around the protein. In the other model, DNA is smoothly bent around the protein core (5,6). Theoretical calculations indicate that both models are energetically feasible.

There have been several attempts to determine the conformation of the DNA in the nucleosome core particle. At present, the resolution of both the neutron diffraction (13) and the X-ray crystallographic (3) studies is insufficient to determine the exact path of the DNA or its conformation in the nucleosome. Laser Raman scattering indicates that DNA in chromatin is in a B or slightly modified B conformation, rather than A or C form (14). Circular dichroism studies are equivocal; there is some evidence for the DNA being in a C-like conformation (15), although more recent studies indicate that the DNA is predominately in the "B-genus" (16). Neither the Raman nor the CD can distinguish between smoothly bent and kinked DNA, however. The results of ^{31}P NMR experiments on core particles are consistent with B-form DNA (17-19), but probably cannot rule out A or C forms. Although the authors (18,19) suggest that these ^{31}P NMR experiments eliminate the Sobell-type kink (1 kink per 10 base pairs), our current understanding of the various factors which affect the ^{31}P resonance shifts is such that we must question the assumptions leading to this conclusion. Since the Crick-Klug model for kinks involves little change in the O-P-O bond angles, no change in the ^{31}P chemical shifts is even expected on theoretical grounds (20,21), and therefore no conclusion could be drawn about this type of kink.

^1H NMR has also been used to investigate the conformational properties of DNA and RNA in solution, but most previous studies have been limited to low molecular weight material (generally less than 5000 and rarely over 30,000 daltons) (22-28). However, recent studies in our laboratory (Early and Kearns, submitted) indicate that DNA is sufficiently flexible that partially resolved spectra can be observed from DNA helices that are 500 base pairs long.

In the work reported in this paper, we have used resonances in the low field ^1H spectra (10 to 15 ppm downfield from DSS) of DNA to compare the properties of protein-free 140 base pair DNA helices with core particle DNA. The resonances in the low field region arise from hydrogen bonded imino protons of Watson-Crick base pairs, and their chemical shifts are known to be very sensitive to the DNA conformation (29-31). Our results show that the core particle DNA has the same conformation as free DNA in solution.

They do not support either the Crick-Klug kink (98° kink) or the Sobell κ -kink (40° kink).

MATERIALS AND METHODS

Chicken blood was obtained by cardiac puncture in the presence of 10% 0.1 M sodium citrate. Preparation of nuclei, digestion of the chromatin gel, and isolation of core particles was carried out as described by W.O. Weischet, et. al. (32), except that larger (2-4x) quantities of cells and buffers were used. Briefly, the nuclei were isolated and then lysed in low ionic strength buffer. After making the solution 0.6 M in NaCl to remove H1 histone, the resulting gel was allowed to swell for 2 days with one change of buffer (with .65 M NaCl). The gel was returned to low ionic strength buffer and digested with micrococcal nuclease. The core particles were isolated on a Biogel A-5m column (33). Peak fractions from the Biogel A-5m column were pooled and concentrated by dialysis against dry Sephadex G200. Final preparation of nucleosome core particle NMR samples was done by vacuum dialysis against 1 mM sodium cacodylate (NaCac), 5 mM NaCl, pH 7.

Free DNA was prepared by isolating the DNA from nucleosome core particles prepared, as in Shaw (33), by digesting the chromatin in the nuclei with micrococcal nuclease. The nucleosomes were treated with pronase, and the DNA was extracted in 0.1 M NaCl using 0.5 volume buffer-saturated phenol (0.1 M tris, pH 7.8) and 0.5 volume isoamyl alcohol-chloroform (1:24). The residual phenol was removed with six washes of ether. The DNA was ethanol precipitated overnight twice and redissolved in 10 mM NaCl, 50 mM NaCl, pH 7. Homogeneity of the preparations was assayed by running both nucleosomes and extracted DNA on 10 cm 4% polyacrylamide tube gels. After denaturation by treatment with formaldehyde, the DNA migrated as a single peak with reduced mobility (factor of ~ 0.6) consistent with single stranded 140 base pair DNA. There was no evidence for the presence of single stranded nicks which would have produced low molecular weight fragments. Gels were scanned at 260 nm using the gel scanning attachment of the Beckman Acta CIII.

NMR spectra were obtained with a Varian Associates HR 300 field sweep spectrometer operated in the correlation mode. Spectra were averaged (typically 40 min) using a Nicolet 1180 computer to improve the signal-to-noise. Temperature was controlled to $\pm 1^\circ\text{C}$ and special Wilmad micro cells were used. Resonance positions are in parts per million (ppm) downfield relative to the standard DSS (sodium 4, 4-dimethyl-4 silapentane-1-sulfonate).

RESULTS AND DISCUSSION

The low field NMR spectra of core particles and free 140 base pair DNA are compared in Figure 1. These spectra show that it is possible to obtain partially resolved ^1H proton spectra of free $\sim 100,000$ dalton DNA and of nucleosome core particles ($\text{MW} \sim 200,000$). The effect of temperature on the core particle spectrum is shown in Fig. 2. The only noticeable changes in the spectra are a slight upfield shift of the resonances as the temperature is increased (typical for DNA (29)) and the expected increase in resolution at the higher temperature. After obtaining spectra at elevated temperatures, they were rerun at room temperature and, in the absence of thermally induced aggregation (low ionic strength buffer), identical spectra were obtained. Optically monitored thermal denaturation studies (32) indicate that the core particle should be intact up to at least 44° under our conditions, although there is CD evidence for a change in core particle conformation, beginning at 32°C when very low ionic strength buffers are used (1 mM NaCac).

Before presenting an interpretation of these spectra we first comment on the difference in buffers used for free DNA and core particles and the possible effect this might have on the spectra. A higher ionic strength

Fig. 1. Low field proton NMR spectra of nucleosome DNA (————) and free DNA (-----) at $T = 41^\circ\text{C}$. The free DNA (140 base pairs) is in 10 mM NaCac , 50 mM NaCl , pH 7 at a concentration of approximately 10 mg/ml . Nucleosomes are in 1 mM NaCac , 5 mM NaCl , pH 7, at a concentration of approximately 15 mg DNA/ml . Spectra were accumulated for 160 and 80 minutes respectively and were line broadened by 20 Hz.

Fig. 2. The temperature dependence of the low field NMR spectra of nucleosome core particle DNA. See caption for Fig. 1 for experimental details. Spectra were accumulated for 40 to 80 minutes.

buffer was used for the free DNA for two reasons. First, it prevents early melting of the free DNA. Second, the electrostatic environment of the DNA in the nucleohistone complex is probably more similar to DNA in high salt (1 M), although it is by no means clear just what ionic strength is required to mimic the histone environment. Thus, while we used ~ 60 mM ionic strength buffer solutions for the DNA, we could probably have used 1-2 M solutions. Judging from previous studies on synthetic DNA duplexes, we

anticipate very little change in the free DNA spectra (less than 0.1 ppm shifts) over this range of salt concentration (22,29). Thus, we believe that the spectra of the free DNA in 60 mM buffer can be directly compared with spectra of nucleosomal DNA obtained using a buffer of one-tenth the ionic strength. A low ionic strength buffer was used for the nucleosome core particle NMR samples to avoid aggregation problems which occurred as the sample temperature was raised in higher ionic strength buffers. However, we note that the spectra taken initially in 10 mM cacodylate and 50 mM NaCl were identical to those at lower ionic strength (1 mM NaCac and 5 mM NaCl), until aggregation occurred. When aggregation occurred the spectra were broadened.

The two partially resolved peaks observed in the low field spectrum at 13.5 and 12.6 ppm of free DNA and core particle DNA arise from a collection of resonances due to the hydrogen bonded imino proton of T and G respectively. The wide range of chemical shifts is due to the variety of environments experienced by the protons in different base pairs (31). The primary effect is due to ring current shifts induced by nearest neighbor base pairs (16 different possibilities), and these range from 0.1-1.5 ppm. A secondary, much smaller, effect (0.0-0.3 ppm) is due to second neighbor ring current shifts. A detailed analysis of the low field spectra of calf thymus and salmon sperm DNA helices (which are virtually identical with the spectra presented here) indicates that the intrinsic linewidths of the individual resonances are probably less than 100 Hz (Early and Kearns, submitted) and it is these individual resonances which overlap in DNA to give rise to a characteristic AT envelope extending from about 14.5 to 13.0 ppm (max @ 13.5) and a GC envelope, with resonances from about 13.3 to 11.8 ppm (max @ 12.6).

The low field NMR spectra of the free DNA and the nucleosome core particle DNA (Fig. 1) are, to a first approximation, identical, and from this we conclude that the conformational states are nearly identical. There do, however, appear to be slight differences between the two spectra in the region near 13.0 ppm and around 12 ppm which might be due to a slight conformational change in the DNA. The protons receiving the largest nearest neighbor ring current shifts are those which would be most sensitive to small conformational changes, and these are located around 13 ppm for highly shifted AT resonances or around 12 ppm for highly shifted GC resonances. Some indication of the magnitude of the conformational changes which might be involved is provided by comparison of RNA and DNA spectra. The low field resonance from the RNA poly (A)·poly (U) is at 13.5 ppm, whereas the corres-

ponding resonance from poly (dA)·poly (dT) is at 14.1 ppm; similarly, the resonances from poly (G)·poly (C) and poly (dG)·poly (dC) are at 12.5 and 13.0 ppm, respectively. Thus, chemical shift differences on the order of 0.5 to 0.8 ppm are observed in going from an RNA A-like conformation to a DNA B-like conformation, and this provides some indication of the spectral changes which would attend a similarly large structural perturbation. According to Levitt's calculations for smooth bending of DNA around a nucleosome core (6), the base tilt would be distorted by about 5 degrees from linear B-form DNA. Derivatives of calculated ring current shifts on the ring nitrogen proton of thymine (29,31) indicate that the expected chemical shift for a 5° change in tilt would be about 0.09 ppm, consistent with the magnitudes of the observed spectral changes.

Our NMR data provide no evidence for kinks in the DNA. In contrast to smoothly bent DNA, the structural perturbations introduced in DNA by kinks are expected to give rise to observable changes in the low field resonances of such DNA. In both the Sobell and in the Crick and Klug models for kinked DNA two base pairs become unstacked at each kink, but their hydrogen bonds remain intact. For the Crick-Klug model this would result in the loss of, on the average, half of the ring current shift for each of the two base pairs located at each kink. If there is one kink every 20 base pairs, then 10% of the base pairs (and hence 10% of the intensity in the low field spectra) will be affected. For example, a resonance from a typical A·T base pair occurs at 13.5 ppm, as a result of a net upfield shift of about 1.0 ppm caused by neighboring bases (30). If this base pair were located at a kink, then the nearest neighbor shifts would, on the average, be reduced by a factor of 2, and the resonance would appear at 13.9 ppm, instead of 13.5 ppm. Similar considerations apply to a resonance from a typical G·C base pair, which we would expect to be shifted from 12.6 to 13.0 ppm when present at a kink site. Assuming that any base pair located at a kink site loses half of its nearest neighbor ring current shifts, we can compute the changes in the DNA spectra to be expected if kinks were present, and these results are presented in Fig. 3d. It is clear that we could easily have detected this type of kink. In the Sobell model (1 kink per 10 base pairs) the increase in the angle between the planes of the base pairs at the kink (from 0° to about 40°) and the increased separation between base pairs centers causes an estimated 70% reduction in shift from the neighboring base pair at the kink. The spectrum calculated for this model (Fig. 3c) predicts a downfield shift (0.15 to 0.2 ppm) of both the AT and GC peaks and a change in

Fig. 3. A comparison of the observed low field NMR spectrum of protein free 140 base pair DNA (a) with the spectra computed assuming one kink every 10 (c) or 20 (d) base pairs. See text for details. Curve (b) is a computer simulation of the observed free DNA spectrum. The dashed (— — —) curves in c and d are the same as b and are placed in the figure for reference. The simulated spectra were obtained as follows. First the observed DNA spectrum (a) was fit using two 80% gaussian, 20% lorentzian lines (half widths of 288 Hz and 274 Hz respectively) centered at 13.5 and 12.6 ppm, respectively (b). For 1 kink in 10 base pairs, (c), 20% of the intensity in each peak was removed and replaced by peaks with the same integrated intensity, but .65 the original line width, centered at 13.9 or 12.9 ppm, respectively. In this way the reduced chemical ring current shift on base pairs at kinks was taken into account. In the case of 1 kink per 20 base pairs (d) 10% of the intensity in each peak was shifted to peaks centered @ 14.0 and 13.0 ppm with .50 the original line widths.

relative intensity of the two peaks to make them more nearly equal. Experimentally, there is practically no change in the AT and GC peak positions (Fig. 1) and the observed intensity changes are in the opposite direction

from that predicted. Our data thus do not support the Sobell model.

Although the proposed kink models require, for energetic considerations, that the bases at kink sites remain base paired, it could be argued that the base pairing at a kink would be weakened, allowing rapid exchange of the imino protons with water. If this were to occur, the resonances from base pairs at the kinks would be lost. While this possibility cannot be eliminated at present, we believe it is unlikely for the following reason. While it is true that the terminal base pairs of helices exhibit early melting (34), this is not the case when the two strands of the helix are linked together as in a loop or (as they would be in a kink) by contiguous base pairs (35). We therefore interpret the NMR results to indicate that kinks are not present in core particle DNA with the frequencies proposed.

It is interesting to note that the spectra of the free DNA and the core particle DNA, shown in Fig. 1, are virtually identical despite the fact that the core particle has a molecular weight approximately twice that of the free DNA. Analysis of the free DNA spectra (Early and Kearns, submitted) reveals that the proton-proton dipolar contribution to broadening of resonances in the low field spectra of 140 BP DNA is about 110 ± 20 Hz, corresponding to a rotational correlation time (end-over-end tumbling) of about 3×10^{-7} sec. In the core particle spectra, the linewidths are slightly larger (by about 20-30 Hz). Assuming a spherical shape for the core particle, this corresponds to a rotational correlation time of 1.2×10^{-7} sec, which compares closely with the value of 1.5×10^{-7} sec calculated using the approximate expression $\tau = 6 \times 10^{-13} M$ sec for a protein of molecular weight M in a solvent of viscosity of 2 cp (36). The rotational correlation times derived from the NMR experiments are somewhat shorter than the values of 3×10^{-6} sec and $6-8 \times 10^{-7}$ sec for 140 base pair DNA and nucleosome core particles, respectively, deduced from electric field dichroism measurements (37,38). It should be noted, however, that the electric dichroism measurements were limited to 1 μ sec or longer (37).

CONCLUSIONS

In this paper we have shown that it is possible to obtain useful low field proton NMR spectra of intact nucleosome core particles. Analysis of these NMR spectra supports nucleosome models in which the DNA is smoothly wrapped around the protein core without significantly altering its solution state conformation. Two models in which DNA is kinked around the nucleosome

are not supported by the ^1H NMR data. Future ^1H NMR studies should provide additional information about nucleosome structure and fluctuations in structure.

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