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Phosphorus-31 Nuclear Magnetic Resonance of fd Virus†

J. A. DiVerdi and S. J. Opella*

ABSTRACT: ^{31}P NMR experiments on the filamentous bacteriophage fd are used to characterize the viral DNA. Because fd is a 16.4×10^6 dalton rod-shaped particle, methods of high-resolution solid-state NMR including cross polarization, proton decoupling, and magic angle sample spinning are utilized. The ^{31}P chemical shielding tensor of solid fd is indistinguishable from that of single-stranded or double-stranded DNA in the absence of proteins; therefore the ^{31}P chemical shift does not show evidence of structural changes in DNA upon incorporation into the virus. fd in solution has a very broad ^{31}P resonance line width. The line width is due to static chemical shift anisotropy that is not motionally averaged, as shown by the generation of sidebands with magic angle sample spinning and a linear dependence of line width on magnetic field strength. These results indicate that DNA packaged inside fd is immobilized by the coat proteins.

fd is a filamentous virus that infects *Escherichia coli* (Marvin & Hohn, 1969). The virus is a protein–DNA complex with no associated membrane components. The particle weight is 16.4×10^6 daltons, 88% of which is from 2700 copies of the 5000-dalton major coat protein (Newman et al., 1977), 10% is from the 6400 nucleotides of the circle of single-stranded DNA (Beck et al., 1978), and 2% is from about 5 copies of a minor coat protein located at one end of the filament (Day & Wiseman, 1978). In solution, fd is a 900 by 9 nm rod with somewhat smaller diameter in the absence of water (Newman et al., 1977; Marvin et al., 1974). fd is similar to other single-stranded DNA bacteriophages, such as M13, Pf1, and Xf, in life cycle as well as structure (Marvin & Hohn, 1969).

There is a substantial amount of experimental evidence that filamentous bacteriophages have their DNA extended lengthwise within a tubular chamber made from the major coat-protein subunits (Marvin & Wachtel, 1975). Simple design principles are expected for biological supramolecular structures like viruses, yet there are significant problems outstanding in the description of filamentous viruses especially with regard to how the DNA is packed inside the coat-protein shell. X-ray diffraction data combined with molecular model building have shown that the coat-protein subunits are arranged in an overlapping helical array (Marvin et al., 1974). While the details of the coat-protein helix are under active investigation (Marvin, 1978; Makowski & Caspar, 1978), there is little doubt that the protein shell of these viruses is highly symmetrical.

The difficulties with understanding the architecture of fd as a nucleoprotein complex start at the most basic level since a circle of DNA is packed in a cylinder with a length to diameter ratio of around 300:1. The X-ray diffraction results that have been interpreted to give the model for the coat-protein arrangement do not have intensity recognizable as from the DNA; therefore there is no diffraction data on how the DNA is arranged in the virus interior or how the nucleotides interact with the amino acids of the coat protein (Marvin et al., 1974). There is no evidence of base pairing of the phage DNA (Beck et al., 1978). A particularly glaring piece of data about fd is the nonintegral ratio (2.3:1) of nucleotides to coat protein (Newman et al., 1977) which is difficult to reconcile with most plausible models of symmetrical DNA–protein interactions and differs from the integral ratios found for other filamentous viruses (Day & Wiseman, 1978). There are few spectroscopic means of separating the nucleotide and aromatic amino acid chromophores, although laser Raman spectroscopy does indicate that the DNA is not in the A form (Thomas & Murphy, 1975). Day and co-workers (Day & Wiseman, 1978; Day et al., 1979) have had to rely almost exclusively on physicochemical characterization of the virus particles to propose models for the packing of DNA in the filamentous viruses. Photochemical cross-linking experiments indicate that a small part of fd DNA exists in a hairpin structure, and this may fix the location of the DNA relative to one end of the particle (Shen et al., 1979); however, this approach gives no indication of overall packing arrangements of the DNA.

There are several possible explanations for the apparent lack of symmetry between the nucleotides and coat-protein subunits of fd and the absence of diffraction intensity from DNA in oriented fibers of fd. These include the DNA having significant motional freedom within the confines of the coat-protein shell or the DNA being disordered relative to the coat proteins without specific DNA–protein interactions. It is also possible that specific and rigid nucleotide–amino acid interactions exist but are complex, and the diffraction from the relatively large mass of highly ordered coat proteins simply overwhelms that from the DNA with lower symmetry.

NMR spectroscopy of the filamentous viruses and their coat proteins and DNA can contribute to a description of their structure and dynamics (Cross et al., 1979; Cross & Opella,

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1980a,b; Opella et al., 1980b). By studying the individual protein and DNA subunits and the intact viruses, it should be possible to describe the conformational changes that occur upon assembly of the nucleoprotein structure. The only phosphorus atoms in fd are in the phosphodiester linkages of the DNA backbone; therefore ^{31}P NMR will select for resonances only from the DNA without interference from the more abundant coat proteins.

^{31}P NMR is a valuable technique for the study of nucleic acids. The chemical shift reflects electronic shielding and geometry of the phosphodiester groups (Gorenstein, 1975; Gorenstein & Kar, 1975), and additional structural information is available from ^{31}P–^{31}P and ^{31}P–^{1}H dipolar couplings. The rotational motion of the phosphates is reflected in the averaging of the chemical shift and dipolar interactions found in solid samples as well as nuclear spin relaxation induced by these interactions (Shindo, 1980).

Most ^{31}P NMR studies of nucleic acids have been carried out on oligonucleotides (Davanloo et al., 1979), low molecular weight duplex fragments (Hogan & Jardetzky, 1979; Bolton & James, 1980; Shindo, 1980), or single-stranded polymers (Akasaka et al., 1977) because these samples have rapid motions which average the chemical shift anisotropy and dipolar interactions of the phosphodiester groups sufficiently to give narrow resonances that are amenable for high-resolution solution NMR experiments and analysis. Solid-state ^{31}P NMR is necessary for the study of nucleic acids in situations where motional averaging is limited, such as for nucleic acids in the solid state (Terao et al., 1977; Shindo et al., 1980), native high molecular weight duplex DNA (Opella et al., 1981), or chromatin (J. A. DiVerdi et al., unpublished results).

Since fd is a highly organized high molecular weight particle with correspondingly slow reorientation rates in solution, solid-state NMR techniques are appropriate for its study. High-power proton decoupling removes broadening due to ^{31}P–^{1}H dipolar couplings (Sarles & Cotts, 1958; Bloch, 1958). Magic angle sample spinning is used under proton-decoupled conditions to average out the chemical shift anisotropy and give an isotropic chemical shift spectrum which corresponds to the solution spectrum of a rapidly reorienting molecule (Schaefer & Stejskal, 1976). Because of long phosphorus relaxation times in the solid state and the relatively small amounts of DNA present in virus samples, cross polarization of phosphorus magnetization is used for sensitivity enhancement (Pines et al., 1973). Other NMR experiments, such as magnetic field dependence of line shape, help describe the ^{31}P resonance properties of fd.

Materials and Methods

fd virus was prepared from 10L growths of infected *E. coli* (K3300). The bacterial cultures were grown to late log phase prior to phage infection. After several hours of postinfection growth, the bacteria were removed by low-speed centrifugation; the phage remained in the supernatant which was made 2% in poly(ethylene glycol) and 0.5 M in NaCl to precipitate the phage. Final purification was accomplished with CsCl step-gradient ultracentrifugation and dialysis against distilled water (Cross & Opella, 1980b).

Single-stranded fd DNA was isolated from fd after the virus structure was disrupted with sodium dodecyl sulfate. The DNA was separated from the coat proteins by chromatography on Sephacryl S-200 superfine in 10 mM sodium dodecyl sulfate, 40 mM borate, and 8% glycerol, pH 9.0, buffer.

The 61-MHz ^{31}P NMR experiments were performed on a home-built double-resonance spectrometer (Cross et al., 1979). The stationary spectra were obtained with a variable-temperature probe with a solenoidal coil. The magic angle spinning experiments were carried out by using a probe equipped with an Andrew-Beams type of rotor which holds about 0.3 mL of sample (Opella et al., 1980a). ^{31}P measurements at other frequencies were obtained on commercial high-resolution spectrometers.

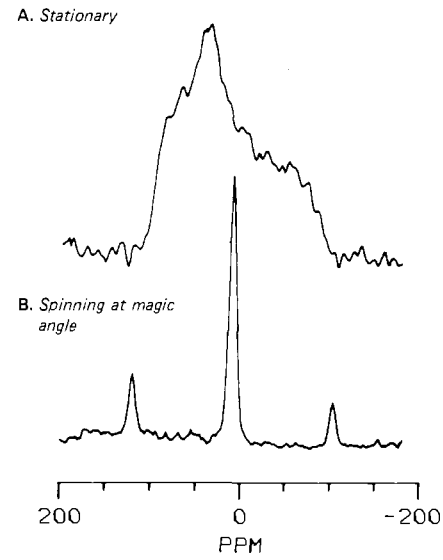

FIGURE 1: ^{31}P NMR spectra of solid fd. (A) Stationary powder (10000 scans). (B) Magic angle spinning of powder at 4.5 kHz (1000 scans). Both spectra were obtained at 61 MHz with cross polarization for 1-ms mix time, 1-s recycle delay, and 2.3-mT proton decoupling during the 19-ms data acquisition period.

Results and Discussion

The anisotropic character of nuclear spin interactions is manifested in the NMR spectra of rigid solids. Structural information is available without complications from motional averaging of the measured parameters. The ^{31}P spectra of solid fd in Figure 1 were obtained with high-power proton decoupling; therefore the spectrum of Figure 1A contains the static chemical shift powder pattern of the fd DNA phosphates broadened slightly by about 200 Hz of ^{31}P–^{31}P dipolar couplings (Opella & DiVerdi, 1981). The ^{31}P spectrum of solid fd in Figure 1A is a typical asymmetric chemical shift powder pattern of a phosphodiester (Herzfield et al., 1978). The principal values of the ^{31}P chemical shielding tensor can be measured directly at the spectral discontinuities as σ_{11} = 85 ppm, σ_{22} = 22 ppm, and σ_{33} = -109 ppm relative to external 85% phosphoric acid. The spectra of Figure 1 are from a lyophilized powder of fd; however, the spectrum of Figure 1A is indistinguishable from that obtained from a frozen solution of fd.

The spectrum of Figure 1B was obtained from a sample of fd powder spun rapidly (4.5 kHz) at the magic angle (55°) with respect to the applied magnetic field. Proton irradiation was used to decouple the ^{31}P–^{1}H interaction so the magic angle spinning averages out only the anisotropic chemical shift and the ^{31}P–^{31}P dipolar couplings to give the isotropic chemical shift spectrum of Figure 1B. Except for the presence of spinning sidebands symmetrically located about the center at the spinning frequency, this spectrum corresponds to that which would result from a conventional high-resolution experiment on a very low molecular weight sample tumbling rapidly in solution.

Since the spectra of Figure 1 are derived from powder averages of chemical shift properties, the angles of the molecular frame with respect to the principal axis system are not available and interpretation must rely on the magnitudes of

the principal values. The magic angle spinning spectrum in Figure 1B has a single center band, demonstrating the presence of a single type of phosphate group with an isotropic chemical shift of −0.9 ppm. This chemical shift is the same as that observed for many types of DNA in solution (Gorenstein et al., 1976). A single type of phosphate contributes to the chemical shift powder pattern of Figure 1A; therefore the principal values of this tensor are identical within experimental error with those observed for DNA in the absence of proteins.

These chemical shift measurements on solid fd show that the phosphates are characterized by a single chemical shielding tensor with principal elements and isotropic chemical shift indistinguishable from the constituent nucleotides. The ^{31}P chemical shift of fd DNA is not affected by packaging in the virion. There is no evidence of distortion of the backbone or altered chemical structures in the viral DNA. However, the sensitivity of ^{31}P chemical shift to phosphate conformation is not fully established. In addition, the line width of the magic angle spinning resonance of Figure 1B is about 5 ppm. This is unfortunate since that breadth is capable of masking the presence of more subtle changes or dispersion of isotropic chemical shifts. While we do not fully understand the source of this line width, it is typical of that found for other types of DNA, including samples of dinucleotides.

The presence of molecular motion has an important influence on NMR spectra. The time scale of motion that is monitored spectroscopically is a function of the nuclear spin interaction that dominates the resonance properties. With the static chemical shift parameters determined for solid fd, the influence of hydration on the ^{31}P resonance can be interpreted in terms of DNA dynamics. The ^{31}P chemical shift interaction at 61-MHz resonance frequency has a "size" of about 10^4 Hz with $\Delta\sigma = \sigma_{33} - \sigma_{11} = 194$ ppm. Motions that occur more often than about 10^4 s^{-1} will strongly influence the line shape of the chemical shift powder pattern (Mehring, 1976), with isotropic motion significantly faster than 10^4 s^{-1} averaging the powder pattern to a single line. Motions slower than about 10^{10} s^{-1} will cause efficient nuclear spin relaxation due to the fluctuating fields from the asymmetric electronic shielding.

The ^{31}P NMR spectrum of solid fd in Figure 1A displays the full chemical shielding anisotropy of the phosphodiester group. Therefore, no molecular motions of significant amplitude faster than about 10^4 s^{-1} are present in the phosphodiester linkages of lyophilized or frozen solutions of fd. Figure 2 compares the proton-decoupled ^{31}P NMR spectra of fd in several situations. The chemical shift properties seem in these spectra reflect motions of DNA resulting from fd being in an aqueous environment. Figure 2B is an asymmetric powder pattern with slightly reduced magnitudes of the principal elements of the chemical shielding tensor (σ_{11} = 78 ppm, σ_{22} = 16 ppm, σ_{33} = −96 ppm) compared to those of Figure 2A which is from completely dehydrated sample. fd equilibrated in an atmosphere of 92% relative humidity corresponds to the hydration of the fibers used in the X-ray diffraction experiments (Marvin et al., 1974). The finding of the substantial $\Delta\sigma$ of 174 ppm for the ^{31}P resonance of fd rules out molecular motion as an explanation for the lack of diffraction intensity attributable to DNA in the X-ray fiber diffraction patterns of fd fibers. The DNA is not "rattling around" inside the hollow tube of coat proteins and does not have rapid local motions.

Figure 2C contains the proton-decoupled ^{31}P spectrum of fd in solution while Figure 2D has the solution spectrum of isolated single-stranded fd DNA. The chemical shift of phosphorus dominates the line shape in all the spectra of Figure

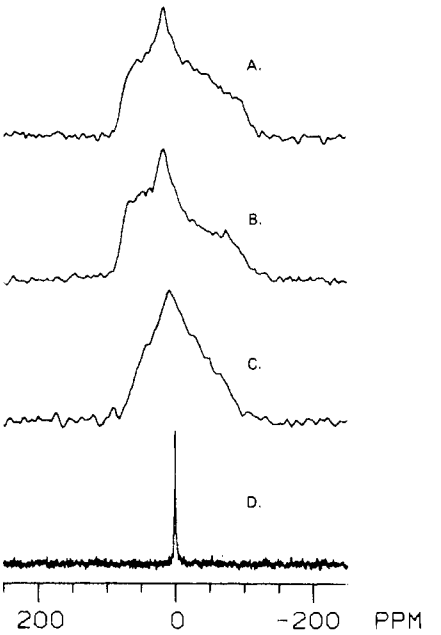

FIGURE 2: ^{31}P NMR spectra of fd and fd DNA. (A) Stationary powder of fd (0% R.H., 10000 scans). (B) Stationary powder of fd (92% R.H., 10000 scans). (C) Solution of fd (50 mg/mL, 50000 scans). (D) Isolated fd DNA in solution (2000 scans). Spectra A, B, and C were obtained with cross polarization and proton decoupling as described for Figure 1. Spectrum D resulted from pulsed free induction decays at 145 mHz by using weak (10 W) proton decoupling.

2 because of the use of proton decoupling. Single-stranded fd DNA in solution gives a ^{31}P NMR spectrum which consists of a narrow resonance near 0 ppm. The phosphodiester backbone of this DNA is undergoing very rapid rotational reorientation since the static chemical shift anisotropy is fully averaged out and the line is not broadened by very efficient relaxation processes that occur with relatively slow rotation rates. While we have not studied the NMR properties of the isolated fd DNA in detail, the line width is similar to that found for other single-stranded polynucleotides (Akasaka et al., 1977) which were found to have rotational correlation times of about 10^{-9} s for phosphate motion. The ^{31}P NMR spectrum of fd in solution is shown in Figure 2C. The fd resonance is broad with slight asymmetry and is clearly not Lorentzian in shape. The width near the base is nearly 200 ppm while the measured width at half-height is around 110 ppm (6.5 kHz at 61 MHz). The line shape of the ^{31}P resonance of fd in solution is not altered by proton decoupling or magnetic field strength; however, samples with higher concentration of virus or at lower temperatures give spectra that appear more asymmetric with partially defined discontinuities.

The ^{31}P line width of fd in solution is due to static chemical shift anisotropy that is not averaged by motion. This is shown with several NMR experiments. The line width is insensitive to high-power proton decoupling; therefore ^{31}P–^{1}H dipolar couplings do not significantly broaden the line. Figure 3 compares the ^{31}P spectra of the same sample of fd in solution for stationary and magic angle spinning experiments. When fd in solution is spun at the magic angle at a moderate rotation rate, the broad ^{31}P resonance breaks up into discrete sidebands, demonstrating that the broadening is due to the inhomogeneous chemical shift interaction. The plot of line width of the phosphorus resonance of fd vs. applied magnetic field strength in Figure 4 shows that the line width increases linearly with field strength. This is as expected for a disperison of isotropic chemical shifts or a chemical shift powder pattern but not chemical shift anisotropy relaxation. While there is substantial error in measuring broad line widths with poor signal

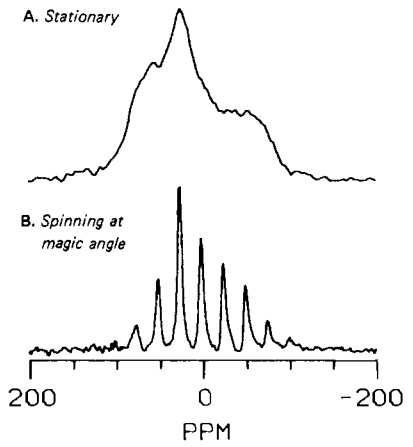

FIGURE 3: ^{31}P NMR spectra of fd in solution (200 mg/mL). (A) Stationary sample (5000 scans). (B) Magic angle sample spinning at 1.5 kHz (1000 scans). Both spectra were obtained with cross polarization and proton decoupling as described for Figure 1.

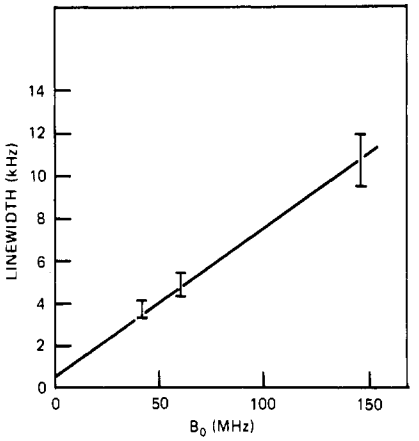

FIGURE 4: Line width of ^{31}P resonance of fd in solution (50 mg/mL) as a function of field strength. The spectra for this plot were obtained without proton decoupling.

to noise ratios that result from not being able to develop the ^{31}P magnetization from cross polarization at all field strengths, the line width clearly does not depend on the square of the field strength. The zero-field intercept is about 0.5 kHz, which is probably from unaveraged ^{31}P–^{1}H dipolar couplings and is sufficiently small as to not be apparent in the spectra compared to the several kilohertz broadening due to chemical shift. This conclusion is reinforced by the more asymmetric line shapes seen for samples with higher virus concentration (Figure 3A) as well as by ^{31}P spin–echo experiments that indicate that the magnetization can be refocussed after relatively long time intervals.

fd in solution is well approximated as a 900 by 9 nm cylinder (Newman et al., 1977). The diffusion coefficients for rotation parallel ($D_{\parallel}$) and perpendicular ($D_{\perp}$) to the long axis of fd can be calculated for such a cylinder (Edsall, 1953). $D_{\parallel}$ = 20 s^{-1} is calculated for fd in solution, and a value of $D_{\parallel}$ = 21 s^{-1} has been measured by Newman et al. (1977). This reorientation rate is too slow to affect the NMR spectra. However, $D_{\perp} = 10^4$ s^{-1} by these calculations. This is the time scale of the ^{31}P chemical shift anisotropy. The rounding of the chemical shift powder pattern seen for fd in solution is consistent with such a rotational diffusion constant (Mehring, 1976; Speiss, 1978; Campbell et al., 1979).

The spectra of Figure 2C,D demonstrate the drastic influence of virus assembly on the dynamics of the single-stranded fd DNA. Since the data on solid fd show no evidence of structural change in the phosphodiester backbone induced by the coat-protein shell, the spectral changes in Figure 2 can be only due to motional effects. While single-stranded fd DNA in solution has substantial motions in the nanosecond time range, the DNA in the virion has a limited amount of motion faster than $\sim 10^{-4}$ s, which indicates the DNA is immobilized by being packaged inside the virus particle.

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Deoxyribonucleic Acid Dynamics from Phosphorus-31 Nuclear Magnetic Resonance†

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ABSTRACT: ^{31}P NMR of native high molecular weight DNA shows that the phosphodiester backbone undergoes reorientation with a rotational correlation time of about 2×10^{-6} s at 30 °C. This rate is consistent with the flexibility of the polymer and does not require the presence of internal phosphate motions. The line width of the phosphorus resonance of DNA is due to incompletely averaged ^{31}P-^{1}H dipolar couplings and ^{31}P chemical shift anisotropy relaxation; high-power proton decoupling and magnetic field dependence experiments separate the two effects and allow the use of the line width for determination of the rotational correlation time. The line width is dependent on temperature, and an activation energy of 5-8 kcal/mol is calculated for the motion of DNA.

While the main structural features of DNA are generally regarded as established from diffraction studies of fibers (Watson & Crick, 1953; Arnott, 1970) as well as oligonucleotide crystals (Kallenbach & Berman, 1977), much less is known about the dynamical properties of DNA. The structure of DNA as it is significantly affected by its environment of proteins, drugs, ions, etc., is not well-characterized; in these situations, more than structural details are of interest since the motions of DNA are an important influence on conformational flexibility (Crick & Klug, 1975; Sobell et al., 1976). In general, the dynamics of native double-stranded DNA have not been described, although processes with rates varying over at least 10 orders of magnitude have been detected experimentally (Wahl et al., 1970; Teitelbaum & Englander, 1975). Theoretical studies also suggest that a wide range of motions are present in DNA (Barkley & Zimm, 1979).

NMR spectroscopy can, in principle, provide a detailed description of the microscopic dynamics of all atoms in a molecule. However, NMR studies of DNA are problematical because of the very broad line widths of nucleotide resonances that are a consequence of the motions of the polymer being too slow to effectively average out static nuclear spin line-broadening mechanisms (McDonald et al., 1964). Most previous NMR studies have been performed on low molecular weight or single-stranded materials because these samples have narrow resonances that can be characterized by using conventional high-resolution spectrometers (Yamane, 1971). ^{31}P NMR of nucleic acids has been successfully employed to monitor the conformation of phosphodiester groups (Gorenstein et al., 1976) and their environment in oligonucleotides (Patel, 1979a; Davanloo et al., 1979), polynucleotides (Akasaka, 1974; Akasaka et al., 1975, 1977; Patel & Canuel, 1976), and drug-nucleic acid complexes (Patel, 1979b). Some motional information has come from ^{31}P relaxation studies of oligonucleotides and single-stranded polynucleotides (Davanloo, et al., 1979; Akasaka, 1974; Akasaka et al., 1975).

Recently, a number of NMR studies of small fragments of double-helical DNA have been reported, generally with an analysis of DNA dynamics. The ^{31}P NMR experiments of Mariam & Wilson (1979) relied on the phosphate chemical shifts to describe the helix to coil transition. Three groups have analyzed the ^{31}P relaxation properties of double-helical DNA fragments. Both Hogan & Jardetzky (1979) and Bolton & James (1979, 1980a,b) combine the phosphorus relaxation data with ^{1}H or ^{13}C data to derive a picture of a double helix having rapid internal motions, especially in the phosphodiester backbone. Shindo (1980) interprets his ^{31}P NMR results as showing some flexibility in 140 base-pair pieces of duplex DNA. The ^{1}H NMR results of Early & Kearns 1979) and the ^{13}C NMR spectra of Rill et al. (1980) of similar DNA fragments are consistent with the presence of rapid segmental motions. Parallel ^{31}P NMR studies of the nucleosome core particles containing 140 base-pair segments of DNA (Cotter & Lilley, 1977; Kallenbach et al., 1978; Klevan et al., 1979; Shindo et al., 1980) and the ^{1}H NMR study of Feigon & Kearns (1979) indicate that apparently the DNA motions are affected only slightly, if at all, by the presence of chromosomal proteins.

While there have been two reports of ^{31}P NMR spectra of high molecular weight native DNA (Hanlon et al., 1976; Yamada et al., 1978), a combination of high-resolution solid-state and solution NMR techniques is needed to obtain reliable and interpretable data. The NMR studies described

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