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Deoxyribonucleic Acid Dynamics from Phosphorus-31 Nuclear Magnetic Resonance†

S. J. Opella,* W. B. Wise, and J. A. DiVerdi

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

† From the Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104. *Received March 28, 1980.* This work was supported by grants from the American Cancer Society (NP-225A), the National Institutes of Health (GM-24266), and the National Science Foundation (PCM-05598). The 8.5-T NMR spectra were obtained at the Middle Atlantic NMR Facility. S.J.O. is a fellow of the A. P. Sloan Foundation (1980-1982).

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here are aimed at the problem of DNA dynamics. The spectroscopic approach used for duplex DNA is also suitable for characterization of DNA in supramolecular structures, such as viruses (Cross et al., 1979; DiVerdi & Opella, 1981) or chromatin (J. A. DiVerdi et al., unpublished results).

Materials and Methods

DNA Samples. All experiments were performed on high molecular weight calf thymus DNA. The solution samples were made with DNA from U.S. Biochemicals. The fibrous material was dissolved in a buffer of 12.5 mM Tris-HCl, 50 mM NaCl, and 1 mM EDTA at pH 7.4. The samples were dialyzed exhaustively against this same buffer. The final concentration of DNA was 20 mg mL^{-1}. Samples used in the NMR experiments were handled as gently as possible with no sonication or nuclease treatments. The DNA was found to be approximately 90 kilobases in length by 1% agarose–gel electrophoresis. The solid DNA used for Figure 1a–c was Sigma Type I fibrous material directly from the bottle.

The calf thymus DNA samples used for the solution experiments of field-strength dependence, decoupling, and temperature dependence were found to be very homogeneous material by gel electrophoresis. Some samples of DNA gave high-field NMR spectra significantly different from that of Figure 1d in that a very narrow resonance shifted slightly downfield from the center was superimposed on the broad resonance. It is not clear if the sharp resonance comes from single-stranded regions of DNA or from relatively low molecular weight impurities, although the sharp resonance could not be removed by dialysis. The sharp resonance can be generated by heating the DNA above the thermal transition temperature or mechanically transferring material. This narrow resonance may have been observed by others.

NMR Spectroscopy. The spectra in a 3.5-T magnetic field (61-MHz ^{31}P resonance frequency) were obtained on a home-built double-resonance spectrometer (Cross et al., 1979). Proton-decoupling experiments were performed with a 1.5-mT coherent radiofrequency field at 150.5 MHz. The spectra of Figure 1e–f were obtained simultaneously by alternating data acquisition in different parts of the computer memory with and without decoupling in order to ensure that the change in line width was due to decoupling and not change in temperature. The ^{31}P NMR spectra of solid DNA used cross polarization of magnetization from the protons (Pines et al., 1973) with a 1-ms mix period. The mechanical sample rotation at the magic angle (θ = 55°) relative to the static magnetic field used an Andrew-Beams type rotor with a 10-mm outside diameter sample chamber holding about 300 mg of material (Opella et al., 1980).

The 1.5-T (24 MHz) spectra were taken on a modified Varian NV-14 spectrometer in the laboratory of M. Cohn. The 2.3-T (40 MHz) spectra were obtained on a JEOL PFT-100 instrument while the 8.5-T (145 MHz) work was done on a Bruker WH-360 at the Middle Atlantic NMR Facility.

Results

Analysis of ^{31}P NMR Line Width of DNA. The conclusions about DNA dynamics from this NMR study are based on the analysis of the phosphorus resonance line width from native high molecular weight DNA; this is a straightforward exercise in spin physics. Theoretical and experimental aspects of solid-state and solution NMR are required for the study of high molecular weight double-helical DNA because the polymer has dynamical characteristics between these two states. The use of ^{31}P NMR obviates any resolution or assignment problems since the phosphorus nuclei are uniformly located in the phosphodiester backbone of DNA.

The first step of the line-width analysis is to identify the nuclear spin interactions responsible for the substantially broader ^{31}P resonance of DNA in solution compared to that of a small molecule, such as a dinucleotide in solution. The measured parameter of the full width at half-maximum height, $\Delta\nu_{1/2}$, of the ^{31}P resonance as a function of applied magnetic field strength, proton decoupling, magic angle sample spinning, and temperature can then be related to molecular properties of DNA.

The width of nuclear resonance signals arises from interactions of the nuclear spin with the applied magnetic field and nearby spins. ^{31}P has a nuclear spin of $^1/_2$; therefore, dipole–dipole couplings and chemical shift anisotropy are the interactions which are most likely to affect the line width. Dipole–dipole interactions arise from the mutual magnetic coupling through space of two or more nuclei with nonzero spin. In a rigid lattice, the dipolar interactions split the energy levels, resulting in broadening due to the sum of splittings from the various distances, angles, and neighbors that are involved. The phosphorus atoms of DNA are relatively distant from other phosphate groups and protons because of the phosphodiester linkage; therefore, the broadening due to ^{31}P–^{31}P and ^{31}P–^{1}H interactions are less than those typically seen in ^{13}C or ^{1}H NMR of solids. Chemical shift anisotropy (CSA) arises from the nonspherical distribution of electrons in the phosphate group screening the applied magnetic field to different extents, depending on the orientation of the group in the magnetic field. Static CSA results in a characteristic chemical shift powder pattern for the line shape of a polycrystalline sample (Mehring, 1976). Phosphodiester groups have large asymmetric chemical shift anisotropy that is emphasized by the relatively large phosphorus gyromagnetic ratio.

The line broadening from static interactions can be reduced in magnitude by motion. Rapid isotropic motion as in a liquid removes the influence of static nuclear interactions, and, to a first approximation, an infinitely sharp line results. In order to completely remove static line broadening, the effective motions must be fast compared to the strength of the interaction. Line broadening can also occur when the static broadening mechanisms are averaged by motion through nuclear spin relaxation which manifests itself in several relaxation parameters including T_2, the spin–spin or transverse relaxation time. The observed line width at half-height is described by $\Delta\nu_{1/2} = 1/(\pi T_2)$ when the line width is determined by relaxation. Motion has the dual function of removing static line-broadening effects and inducing fluctuations of local magnetic fields that result in relaxation broadening.

Dipolar couplings and chemical shift anisotropy can induce nuclear relaxation. Both of these interactions involve asymmetric local fields at the nuclear site, and molecular motion causes these fields to become time dependent. The relaxation parameters can be calculated by taking into account the physical basis of the interaction and the relevant spectral density functions, J_m, which describe the effectiveness of rotational reorientation rates, τ_i, for inducing relaxation. The expression for calculation of line width due to chemical shift anisotropy relaxation is given by eq 1 (Abragam, 1961; Hull & Sykes, 1975; Shindo, 1980)

$$\Delta\nu_{1/2} = \frac{1}{\pi T_2} = \frac{1}{40\pi}\gamma^2 B_0^2 \delta_z^2 \sum_{i=0}^{2} C_i[3J_1(\omega_0) + 4J_0(0)] \quad (1)$$

where γ is the gyromagnetic ratio, B_0 is the strength of the applied magnetic field, δ_z is the z component of the traceless

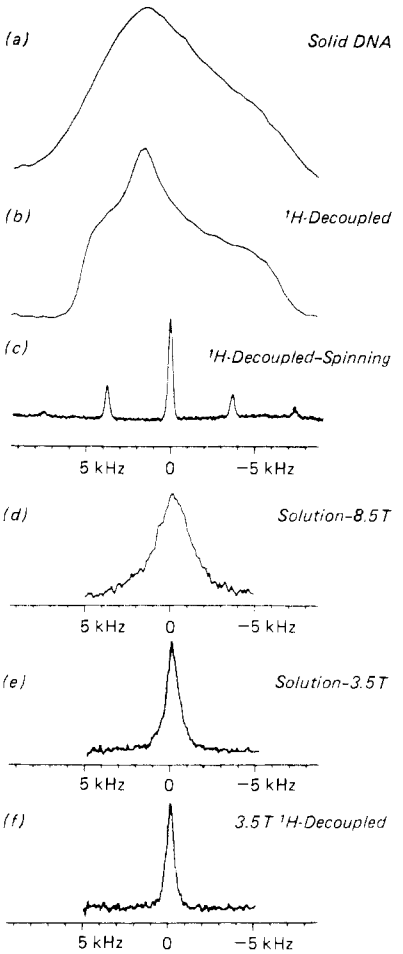

FIGURE 1: ^{31}P NMR spectra of calf thymus DNA. (a) Solid DNA, cross polarized but undecoupled spectrum at 61 MHz. (b) Solid DNA, proton decoupled at 61 MHz. (c) Solid DNA, magic angle spinning and proton decoupled at 61 MHz. (d) DNA in solution at 30 °C, 145 MHz. (e) DNA in solution at 30 °C, 61 MHz. (f) DNA in solution at 30 °C, proton decoupled at 61 MHz.

chemical shift tensor, and the C_i are geometric parameters which relate the principal axes systems of the diffusion and chemical shielding tensors.

The static dipolar and CSA interactions of DNA are illustrated with ^{31}P NMR spectra of solid fibrous DNA in Figure 1a–c. These spectra were obtained with cross polarization of the phosphorus nuclei from the protons for increased sensitivity. Figure 1a is a broad, nearly featureless resonance that represents the natural ^{31}P line shape of solid DNA; the approximately 10-kHz line width is the sum of all ^{31}P–^{31}P and ^{31}P–^{1}H dipolar splittings and the ^{31}P chemical shift anisotropy. The application of strong radiofrequency irradiation at the proton resonance frequency decouples the ^{31}P–^{1}H dipolar interaction, giving the characteristic asymmetric chemical shift powder pattern of a phosphodiester in Figure 1b. About 5 kHz of Gaussian line broadening is removed with proton decoupling, reflecting the magnitude of the ^{31}P–^{1}H dipolar interactions.

The ^{31}P chemical shift powder pattern is slightly rounded or broadened compared to the theoretical line shape with abrupt discontinuities. This is due to ^{31}P–^{31}P dipolar couplings which represent about 400 Hz of broadening, which is small because of the large distances between phosphate groups. The principal values of the DNA phosphate chemical shielding tensor can be measured from the discontinuities of the powder spectrum of Figure 1b as σ_{11} = 85 ppm, σ_{22} = 25 ppm, and σ_{33} = −109 ppm relative to external phosphoric acid; these values are within experimental error of those reported by Terao et al. (1977) and Shindo et al. (1980).

Rapid rotation of a powder sample at the magic angle (θ = 55°) with respect to the applied magnetic field averages the chemical shift anisotropy to its isotropic value ($\sigma_{iso} = {}^1/_3(\sigma_{11} + \sigma_{22} + \sigma_{33})$) and reduces the ^{31}P–^{31}P dipolar coupling (Andrew et al., 1958). The effect of this procedure on the proton-decoupled ^{31}P NMR spectrum of solid DNA is shown in Figure 1c. A relatively sharp, single-line spectrum results with the intense center band at the isotropic chemical shift position flanked by spinning sidebands separated by the rotation rate of 3.5 kHz. The line width of the central line is about 5 ppm, significantly larger than expected although it is similar to that observed for dinucleotides and other small polycrystalline molecules. Only about 0.5 ppm of the breadth would be expected to be from chemical shift dispersion among the various nucleotide neighbors (Patel, 1979a,b). A variety of NMR experiments have ruled out inadequate ^{1}H decoupling or T_2 of the phosphate group as sources of the line width of Figure 2c.

High molecular weight DNA in solution gives the ^{31}P NMR spectra shown in Figure 1c–e under various experimental conditions. Figure 1e is the solution spectrum in a 3.5-T field with a line width of about 800 Hz at 30 °C. The reduction of line width due to all nuclear spin interactions from Figure 1a (10 kHz) to Figure 1e (0.8 kHz) is a reflection of the motional averaging that occurs in solution compared to the rigid solid. A comparison of Figure 1b to Figure 1f also reflects motional averaging, but, because proton decoupling is utilized, only a single interaction, chemical shift, is influencing the spectra.

The moderate line width of the ^{31}P resonance of DNA in solution implies that no single interaction is extremely effective in inducing nuclear spin relaxation and the analysis of the line width might be complex. The solution spectra of Figure 1d–f illustrate some of the experiments performed to sort out the contributions to the line width. The ^{31}P resonance line width measured at 145 MHz (Figure 1d) is much larger than that measured at 61 MHz (Figure 1e); therefore, the line-broadening mechanism has a strong field strength dependence. The application of proton irradiation at levels suitable for decoupling ^{31}P–^{1}H dipolar interactions in solids narrows the ^{31}P line by about 400 Hz; therefore, there is some static ^{31}P–^{1}H dipolar coupling that is not removed by motion. Magic angle sample spinning has little effect on the proton-decoupled ^{31}P spectrum of DNA in solution at 61 MHz.

The effect of field strength on the line width of the DNA phosphorus resonance is shown in Figure 2. The line width is a linear function of the magnetic field strength squared, B_0^2. This type of dependence is completely diagnostic for CSA relaxation as a line-broadening mechanism, as can be seen from eq 1. Other likely contributions to the phosphorus line width have quite different dependencies on field strength, since residual static chemical shift anisotropy or dispersion of chemical shifts have a linear dependence on B_0, dipolar relaxation does not have a strong B_0 dependence, and static dipolar couplings do not depend on B_0. If the entire line width is due to CSA relaxation, then the plot of line width vs. B_0^2 should pass through the origin of 0 Hz width at B_0 = 0. Instead, the intercept for no applied field is about 400 Hz; this 400 Hz of line width has to be from some spin interaction other than CSA relaxation.

Proton decoupling significantly narrows the phosphorus resonance of DNA in solution, as can be seen in the comparison of Figure 1e–1f. The line width at 61 MHz and 30 °C goes from about 800 Hz to about 400 Hz. Proton de-

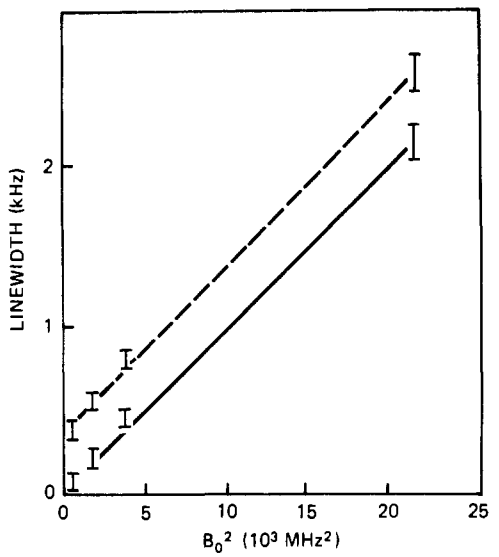

FIGURE 2: Magnetic field dependence of line width at half-height for phosphorus resonance from a solution of DNA. The dashed line represents widths measured directly. The solid line was plotted after subtraction of 400 Hz of static dipolar width.

coupling removes the line broadening due to ^{31}P–^{1}H dipole–dipole couplings; therefore, the 400-Hz difference in line width corresponds to the strength of the dipolar interactions that are not averaged by motion. The strength of static dipolar couplings are independent of applied magnetic field; thus, the phosphorus line width of DNA in solution has a 400-Hz component from ^{31}P–^{1}H dipolar couplings at all field strengths, including zero field.

There are two spin interactions that dominate the ^{31}P resonance line width of DNA in solution, CSA relaxation and residual ^{31}P–^{1}H static dipolar couplings. The combination of magnetic field strength dependence and decoupling experiments separates these two effects. There are probably additional interactions that make small contributions to the phosphate line width that are not apparent in this analysis because of errors in measurement of line width and temperature as well as their influence being simply overwhelmed by the two large effects; the most likely candidates are dispersion of the isotropic chemical shift among the various phosphates (0.5 ppm) and heteronuclear dipolar relaxation.

The 400-Hz dipolar contribution is present in the line widths at all values of B_0 because of the field independence of static dipolar splittings. When the dipolar part is subtracted from the observed line width, the solid line plot of Figure 2 is generated. This line has the squared-field dependence and passes through the 0 Hz origin at $B_0 = 0$. This line represents the CSA relaxation behavior of the phosphorus of DNA and can be used to determine the rotational correlation time of the phosphate group.

Effective Rotational Correlation Time of Phosphate Motion. Rates of rotational diffusion can be determined from relaxation parameters, such as line width, when the relaxation mechanism is known. The evaluation of chemical shift anisotropy relaxation depends on knowing the appropriate chemical shielding tensor.

In principle, a thorough relaxation analysis including longitudinal and transverse relaxation processes that utilizes the (as yet undetermined) complete chemical shielding tensor from an oligonucleotide could characterize the directions and rates of phosphate motion of DNA in great detail. The more limited data set of the established principal values, σ_{ii}, of the chemical shielding tensor of DNA combined with the CSA relaxation contribution to the resonance line width can give

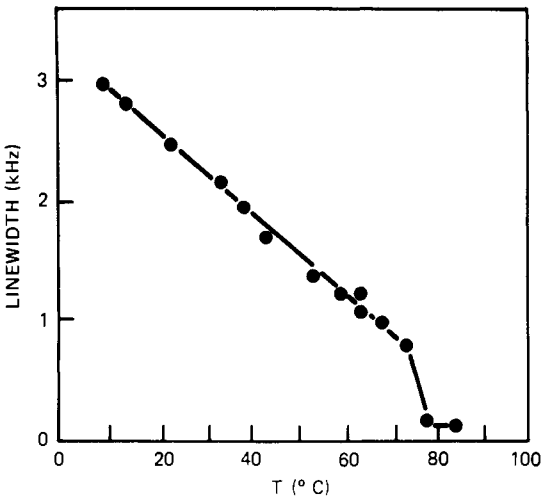

FIGURE 3: Plot of ^{31}P line width vs. temperature. It was obtained at 145 MHz.

a rotational diffusion constant for the phosphate group that is explicitly restricted to isotropic reorientation. The line width at half-height, $\Delta\nu_{1/2}$, can be directly calculated for a given correlation time, τ_c, by eq 2.

$$\Delta\nu_{1/2} = \frac{1}{40\pi}\omega_0^2\delta_z^2(1 + \eta/3)\left[\frac{3\tau_c}{1 + \omega_0^2\tau_c^2} + 4\tau_c\right] \quad (2)$$

This was derived from eq 1 for the case of isotropic reorientation where $J(\omega_0) = 2\tau_c/(1 + \omega_0^2\tau_c^2)$, $\sum C_i = 1 + \eta/3$, $\eta = (\delta_x - \delta_y)/\delta_z$, and $\delta_j = \sigma_{ii} - \sigma_{iso}$. At 30 °C the observed line width for $B_0 = 8.5$ T is 2.15 kHz, of which 400 Hz is dipolar, so the relevant line width for eq 1 is 1.75 kHz, which corresponds to an isotropic rotational correlation time of about 2×10^{-6} s.

Activation Energy of Phosphate Motion. There is a substantial temperature dependence of the ^{31}P resonance line width of DNA as shown in Figure 3. The change in this parameter over a wide range of temperatures is by itself strong evidence for motion of the DNA structure at temperatures well below the thermal melting transition. The data of Figure 3 are for a relatively high magnetic field, so the line width is predominantly determined by the efficiency of the CSA relaxation which is directly related to the rate of phosphate reorientation. Because the plot is linear from approximately 5 to 70 °C, only a single dynamical process is effective in inducing relaxation. A relatively small but abrupt change in line width is seen at the melting temperature of the DNA, which emphasizes the important role of fluctuations in the helical structure relative to denaturation.

The rotational correlation time calculated from the NMR data at 30 °C is a reliable and valuable number because the resonance properties due to a single spin interaction are evaluated. The data of Figure 3 can be used to determine the activation energy for the phosphate motions in DNA by calculating the rotational correlation time for different temperatures; however, to do this rigorously, the relative dipolar and CSA contributions have to be determined for the entire temperature range. There are technical difficulties in doing variable temperature work on aqueous samples when applying large decoupling fields because of dielectric heating that would make this part of the experiment unreliable with the present equipment. It is probable that at higher temperatures where phosphate motion is faster the ^{31}P–^{1}H dipolar couplings will be reduced from the 400-Hz contribution measured at 30 °C, and the correspondingly smaller amount should be subtracted from the experimental line width of Figure 3. Although these

values are not known at the present time, the activation energy for backbone motion of DNA can be determined with eq 3 within relatively narrow limits.

The activation energy, E_A, calculated by using eq 3 and the data of Figure 3 is between 5 and 8 kcal/mol.

$$\tau_c = \tau_0 \exp(E_A/kT) \quad (3)$$

Discussion

The phosphodiester linkage of DNA in solution has substantial mobility. The backbone motions of duplex DNA are the reason for the drastic reduction of the phosphate resonance line width of DNA in solution compared to the solid state. This is true when all nuclear spin interactions are taken into account (Figure 1a–e) as well as for just the chemical shift interaction (Figure 1b–f). The primary question we want to address in the interpretation of the motional averaging of the ^{31}P resonance properties is whether the results can be explained by the relatively long-range flexibility of the polymer as expressed in the hydrodynamic persistence length or if the existence of large amplitude fast local motions is required.

The experimental results show that the motions of DNA influence the ^{31}P resonance line width through three different effects on three different time scales. The static ^{31}P chemical shift anisotropy and ^{31}P–^{1}H dipolar interactions are significantly reduced by the motions of DNA in solution. Efficient ^{31}P nuclear spin relaxation is induced by the chemical shift anisotropy due to the motion of the phosphates.

Rotational motions in solids or gels are generally restricted in amplitude, angular dispersion, or rate compared to those in liquids. However, these motions are sufficient to reduce or average static nuclear spin interactions. A typical example is that of methyl carbon chemical shielding tensors of single crystals which are axially symmetric due to rapid rotation of the methyl group about its C_3 axis (Mehring, 1976). Substantial reductions of dipolar couplings and chemical shift anisotropy are found in liquid crystals and membranes (Urbina & Waugh, 1974). The motions of the DNA phosphates are such that the static ^{31}P–^{1}H dipolar couplings are reduced in magnitude to a fraction of their static value but are not completely eliminated. This finding is somewhat unusual but not unprecedented. This situation is found for the well-studied plastic crystal adamantane where reorientation of the molecules in the solid state is rapid enough to average out ^{13}C chemical shift anisotropy and intramolecular dipolar couplings but not intermolecular dipolar couplings (Pines et al., 1973). The extended aliphatic tail of cholesterol in model membranes behaves similarly, since the C_{26} resonance narrows with proton decoupling to a very sharp line (Opella et al., 1976). Averaging of all of the CSA but not the full magnitude of the dipolar interactions has been seen for some of the amino acid side-chain carbon resonances in collagen (Jelinski & Torchia, 1980).

The motions of the DNA backbone completely average the 10^4-Hz static CSA but only partially average the 5×10^3 Hz static heteronuclear dipolar couplings of the phosphates. This discrepancy in frequency scales is not severe and can be explained in several ways. First of all, the chemical shift powder pattern is a "tentlike" shape with abrupt discontinuities at the frequency limits of σ_{11} and σ_{33}, while the dipolar ^{31}P–^{1}H broadening has the form of a Gaussian function with some intensity significantly beyond the nominal 5×10^3 Hz at half-height of the static interaction. Therefore, much more rapid motions are required to completely remove the dipolar coupling than an equivalent chemical shift anisotropy. Exactly the same argument is used in describing the formation of spinning sidebands separate from the center band at rotation rates on the order of or less than the magnitude of the chemical shift anisotropy (Waugh et al., 1978), and this is illustrated in Figure 1c where rotation at 3.5×10^3 Hz is sufficient to narrow the 10-kHz chemical shift powder pattern to a recognizable isotropic chemical shift with small sidebands. A second reason may be that the effective motion is not isotropic and the chemical shielding tensor or dipolar coupling tensors respond differently to the rotations. Additional possibilities include the CSA reflecting only phosphate side motions, while the ^{31}P–^{1}H dipolar couplings must take into account the changes in any distances between protons and phosphorus atoms as well as angular dispersions.

The phosphate motion of DNA is characterized by the rotational correlation time of 2×10^{-6} s at 30 °C derived from the chemical shift anisotropy relaxation contribution to the line width. This rotational correltion time can only be considered an order of magnitude estimate because of the relatively large errors in measuring broad line widths and the explicit assumption used in eq 2 that the motion effective in inducing relaxation is isotropic. However, all of the ^{31}P NMR results on DNA in solution are consistent with the presence of effectively isotropic phosphate motion. If rotations of the phosphate groups in one or a few directions are rapid while other motions were slow, then an axially symmetric ^{31}P chemical shift powder pattern reduced in magnitude would be observed. This is the case for the phosphodiester head groups of lipids in membranes (Griffin, 1976). There is no evidence in the line shapes of Figure 1, the frequency dependence of line width in Figure 2, or the magic angle spinning results on DNA in solution that the ^{31}P resonance has any residual powder pattern from CSA. This means motions in all directions are fast compared to 10^4 Hz. The effective rotational diffusion constant of the phosphate groups is readily derived from the calculated isotropic rotational correlation time as $D_{rot} = {}^1/_6\tau_c = 8 \times 10^4$ s^{-1} at 30 °C. This value is larger than the static CSA (10^4 Hz) and ^{31}P–^{1}H dipolar couplings (5×10^3 Hz) but not so much larger that it is unreasonable for a relatively small part of the Gaussian dipolar broadening to be unaveraged.

High molecular weight native DNA is a flexible polymer rather than a rigid rod. Hydrodynamic results show that DNA has a persistence length of about 180 base pairs that corresponds to the distance between independently oriented parts of the polymer (Bloomfield et al., 1974). This independence of phosphate groups is both spatial and angular and is a consequence of bending motions in the backbone of the polymer. Bending motions occur in all directions, giving rise to apparently isotropic motion at a given position in the chain. A correlation time for this long-range bending has been calculated to be around 10^{-7}–10^{-5} s (Bloomfield et al., 1974; Barkley & Zimm, 1979; Bolton & James, 1980a,b). This general description of DNA as a flexible polymer is in excellent accord with the ^{31}P NMR results, in particular, the finding of an effectively isotropic rotational correlation times of 2×10^{-6} s at 30 °C. The slow fluctuations in structure detected by hydrogen-exchange experiments or formaldehyde reactions with bases are invisible on the time scales of the ^{31}P NMR experiments (Teitelbaum & Englander, 1975; McGhee & von Hippel, 1975).

There are several sources of evidence for the existence of rapid local motions in DNA with correlation times near 10^{-9} s, which are quite distinct from those with correlation times near 10^{-6} s associated with bending as well as the very slow breathing modes of the double helix. Fluorescence studies of

ethidium bromide intercalated into DNA bases indicate that fast fluctuations of the bases are occurring (Wahl et al., 1970; Genest & Wahl, 1978). Robinson et al. (1980) have intercalated acridines with attached stable free radicals into DNA and obtained electron spin resonance spectra consistent with rapid internal motions of DNA. Both of these experiments suffer from the limitations associated with the use of probe molecules which can have independent motions or perturb the system being monitored.

Recent high-resolution NMR studies on duplex DNA do not have to contend with probe molecules; however, they do have to use relatively low molecular weight DNA to obtain narrow lines with conventional high-resolution spectrometers. These studies strongly suggest the presence of large amplitude rapid motions in the backbone of DNA. ^{31}P NMR studies of 140 base-pair fragments of DNA obtained by nuclease digestion by Hogan & Jardetzky (1979) are interpreted in terms of internal phosphate motions with rates near 10^{-9} s while those of Shindo (1980) are interpreted as indicating some flexibility of the DNA rod through bending or twisting motions. The ^{31}P and ^{13}C NMR experiments of Bolton & James (1979, 1980a,b) on somewhat larger sized fragments of DNA formed by sonication indicate that both a long correlation time process (10^{-6} s) and short correlation time processes (10^{-9} s) are occurring in duplex DNA. ^{1}H NMR studies of Early & Kearns (1979) and the ^{13}C NMR spectra of Rill et al. (1980) also suggest that fragments of DNA have some rapid internal motions. This group of NMR experiments indicates that the phosphodiester linkage of DNA has motions with correlation times around 10^{-9} s.

There is about 3 orders of magnitude difference in rates of phosphate motion between our ^{31}P NMR results in high molecular weight double-helical DNA and those of the work cited above on fragments of double-helical DNA since we measure an effective rotational correlation time of 10^{-6} s. The qualitative conclusions about DNA dynamics are very different since with phosphate motion on the microsecond time scale there is no need to invoke the presence of large amplitude local phosphate motions.

There are several possibilities for this discrepancy in rates of phosphate motion in DNA. First of all, the DNA samples are substantially different in length with our experiments performed on material with 9×10^4 base pairs compared to 140 base pairs for most of the other studies. Fragments of DNA may have internal modes of motion not present in high molecular weight DNA. It is not unreasonable that rotational motions are significantly damped for a group in the middle of a long polymer compared to those of a short rod, especially since a phosphate group in the middle of a 140 base-pair fragment of DNA is much less than a persistence length from either end. A second possibility concerns the fragile nature of the DNA double helix since even in the best quality high molecular weight samples of DNA rapid backbone motions can be easily induced experimentally by handling the sample. The fragments of DNA found to have rapid motions were generated by harsh nuclease or sonication procedures which may have irreversibly altered the DNA. In addition to these reasons associated with the DNA samples being of different sizes, the NMR experiments and interpretation are quite different. The rapid overall and internal motions of DNA fragments result in narrow resonances that were studied with spectrometers and relaxation theory suitable for liquids, while the high molecular weight DNA line widths are broad from motional averaging with both solid-like and liquid-like character which required both the solid-state and solution NMR experiments. Further work on well-defined high molecular weight DNA samples, such as plasmids, using solid-state NMR and relaxation measurements at multiple field strengths can more completely describe DNA dynamics for the native double helix.

Acknowledgments

We thank N. Kallenbach for helpful discussion, H. Nick for help with the experiments, and M. Cohn and J. Engle for use of the spectrometer for the 1.5-T field strength measurements.

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Hydrogen-1 and Carbon-13 Nuclear Magnetic Resonance of the Aromatic Residues of fd Coat Protein†

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ABSTRACT: The aromatic residues of fd coat protein in sodium dodecyl sulfate micelles are characterized by ^1H and ^{13}C NMR. Resonances from both types of nuclei show structure-induced chemical shift dispersion and line widths indicative of a folded native structure for the protein. The two tyrosines were found to have pK_as of 12.3 and 12.5 by ^1H NMR and spectrophotometric titrations. ^{13}C relaxation measurements show that two of the three Phe rings have significant internal mobility, the two Tyr rings have moderate internal mobility, and the Trp side chain is completely immobilized. Qualitative comparisons are made between the intact virus and the isolated coat protein.

Studies of protein structure and dynamics have been greatly facilitated by the spectroscopic accessibility of the aromatic amino acid residues; this is particularly true for ^1H and ^{13}C NMR.[1] The relatively few aromatic amino acids present in a protein are usually distributed throughout the sequence; therefore they are selective monitors of various protein domains. Resonance frequencies of aromatic carbons and protons do not overlap with those from aliphatic sites, allowing ready discrimination. The aromatic groups are of substantial biochemical interest because of their hydrophobic interactions, acid–base chemistry, hydrogen bonding, and ability to intercalate between other planar groups.

The filamentous bacteriophages are an attractive system for study because of their small size, experimental tractability, and interesting properties. In particular, the major coat protein of these viruses is extraordinary in that it exists as a cytoplasmic protein, as a structural element of a nucleoprotein complex, and as an integral membrane protein at different stages of its life cycle (Denhart et al., 1978; Kornberg, 1974). When *Escherichia coli* is infected with fd, the cells produce very large amounts of the viral coat protein. This protein is synthesized as the water-soluble procoat protein which has a 23 amino acid amino-terminal leader sequence (Chang et al., 1978). After insertion into the cell membrane, the leader sequence is cleaved to form the mature coat protein which is stored in the membrane bilayer (Smilowitz et al., 1972). Virus assembly occurs at the cell membrane; as the DNA is extruded

† From the Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104. *Received March 28, 1980.* This work was supported by grants from the National Institutes of Health (GM-24266) and the American Cancer Society (NP-225A). The ^{13}C NMR experiments were performed on a spectrometer obtained with a National Science Foundation grant. ^1H NMR spectra were obtained at the Middle Atlantic NMR Facility. T.A.C. is supported by a Cell and Molecular Biology Training Grant. S.J.O. is a fellow of the A. P. Sloan Foundation (1980–1982).

[1] Abbreviations used: NMR, nuclear magnetic resonance; NOE, nuclear Overhauser enhancement; PFU, plaque-forming units; $NaDodSO_4$, sodium dodecyl sulfate; Me_4Si, tetramethylsilane; DSS, 4,4-dimethyl-4-silapentane-1-sulfonic acid.

0006-2960/81/0420-0290$01.00/0