Whether these requirements include the use of complete cDNA copies of RNA for probes in hybridization experiments or for cloning in bacteria, the conditions described herein alleviate any previous limitations due to the length of the RNA template employed.

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Nonuniform Backbone Conformation of Deoxyribonucleic Acid Indicated by Phosphorus-31 Nuclear Magnetic Resonance Chemical Shift Anisotropy†

Heisaburo Shindo,* Jan B. Wooten, Barbara H. Pheiffer, and Steven B. Zimmerman

ABSTRACT: ^{31}P nuclear magnetic resonance of highly oriented DNA fibers has been observed for three different conformations, namely, the A, B, and C forms of DNA. At a parallel orientation of the fiber axis with respect to the magnetic field, DNA fibers in both the A and B forms exhibit a single, abnormally broad resonance; in contrast, fibers in the C form show almost the full span of the chemical shift anisotropy (170 ppm). The spectra of the fibers oriented perpendicular indicate that the DNA molecules undergo a considerable rotational motion about the helical axis, with a rate of $>2 \times 10^3$ s^{-1} for the B-form DNA. Theoretical considerations indicate that the ^{31}P chemical shift data for the B-form DNA fibers are consistent with the atomic coordinates of the phosphodiester group proposed by Langridge et al. [Langridge, R., Wilson, H. R., Hooper, C. W., Wilkins, M. H. F., & Hamilton, L. D. (1960) *J. Mol. Biol. 2*, 19-37] but not with the corresponding coordinates proposed by Arnott and Hukins [Arnott, S., & Hukins, D. W. L. (1972) *Biochem. Biophys. Res. Commun. 47*, 1504-1509], and also lead to the conclusion that the phosphodiester orientation must vary significantly along the DNA molecule. The latter result suggests that DNA has significant variations in its backbone conformation along the molecule.

DNA[1] in aqueous solution has generally been assumed to adopt the same conformation as is present in DNA fibers equilibrated at high relative humidities, namely, the B form of DNA (Langridge et al., 1960). Recently, several studies have proposed alternative secondary structures for DNA in solution based on a variety of techniques [for references, see

† From the National Institute of Arthritis, Metabolism, and Digestive Diseases (H.S., B.H.P., and S.B.Z.) and the National Institute of Child Health and Human Development (J.B.W.), National Institutes of Health, Bethesda, Maryland 20205. *Received July 24, 1979.* A preliminary account of this work was presented at the 20th Nuclear Magnetic Resonance Conference, Asilomar, CA, Feb 1979.

[1] Abbreviations used: NMR, nuclear magnetic resonance; CSA, chemical shift anisotropy; PAS, principal axis system; DNA, deoxyribonucleic acid.

Zimmerman & Pheiffer (1979)]. An extension of X-ray fiber diffraction techniques to highly concentrated DNA solutions, however, suggests that at least in these preparations the B form is maintained in solution (Zimmerman & Pheiffer, 1979). Furthermore, a careful diffraction study by Permilat & Albiser (1975) indicates that the structure of natural DNA is independent of base composition over a wide range of base ratios [cf. Bram (1971)]. Hence, we conclude that natural DNA in solution retains a conformation essentially similar to that of the B form irrespective of its base composition. However, the fiber diffraction method used in these studies will tend to average irregular changes in conformation along the DNA strands. Hence, natural DNA, with its varying sequences, may possess conformational variations undetected by the X-ray diffraction technique.

We, in fact, recently reported the observation of two well-resolved resonances of nearly equal intensity in the ^{31}P spectrum of 145 base-pair poly(dAdT)·poly(dAdT), a synthetic polydeoxyribonucleotide (Shindo et al., 1979). Analyses of the possible origins of the two resonances led to the conclusion that the phosphodiester backbone of this DNA contains two distinct phosphorus environments in an alternating array. The alternating structure of this copolymer in solution strongly suggests the possibility of local variations of the backbone conformation, which questions the concept of a uniform backbone along the DNA molecule.

In the present work, we report ^{31}P NMR studies of highly oriented DNA fibers in the A-, B-, and C-form conformations. We believe that the NMR method will be valuable for the assessment of different DNA structural models in terms of phosphodiester conformation.

Materials and Methods

Materials. Salmon sperm DNA was purchased from Calbiochem and purified as previously described (Zimmerman & Pheiffer, 1979) except for dialyzing against LiCl when the lithium salt of DNA was desired. It was then dissolved (at 1 mg/mL) in either 1 mM NaCl or LiCl and centrifuged for 16 h at 35 000 rpm in a Spinco SW 40 rotor. The pellets were used to make fibers. The vacuum-dried powder sample was used for measuring the magnitude of each principal component of the chemical shift tensor.

DNA Fibers. A drop from a pellet was suspended between two Chromel wire supports, and the DNA gel was allowed to dry at ambient temperature and humidity. It was sometimes necessary to re-form the gel into a cylindrical shape during the drying process. The fibers formed were from 100 to 200 μm in diameter and about 4 mm in length. The DNA was concentrated by ~30–100-fold upon drying so that the NaCl or LiCl concentration may be up to 6% by weight in the fibers.

X-ray Diffraction. Before NMR measurements several fibers were randomly chosen to examine their X-ray diffraction patterns. Copper Kα radiation was obtained from a Norelco fine focal spot X-ray tube. Diffraction patterns were recorded at a specimen-to-film distance of 30 mm in a Norelco microcamera at room temperature. The humidity was controlled by continuously flushing the camera with a stream of helium which had been passed through an appropriate saturated salt solution, $KClO_3$, NH_4Cl, or $MgCl_2 \cdot 6H_2O$, for 98, 79, or 33% relative humidity, respectively (O'Brien, 1948). The X-ray exposure time was 3–6 h.

NMR Measurements. From six to twenty DNA fibers were required to measure a ^{31}P NMR spectrum. They were pushed into parallel holes made in a flattened surface of a Teflon rod which can be accommodated in a NMR sample tube with an inside diameter of 5 mm. The sample mounted in the Teflon rod was allowed to stay in the presence of the appropriate saturated salt solution at room temperature for 12–16 h and then inserted into a NMR tube containing beforehand a piece of cotton saturated with the same solution. A small piece of wet cotton was pushed into the top of the tube so that the humidity was kept constant throughout the tube. The sample tube was sealed with a cap and several layers of Parafilm.

The ^{31}P NMR spectrum was measured at 24.3 MHz on a TT-14 spectrometer, equipped with a dedicated Nicolet 1080 computer and extensively modified for solid-state NMR. Proton dipolar decoupling and cross-polarization techniques were employed to gain sensitivity. The home-built probe can accommodate a 6-mm sample tube and was carefully tuned to 24.3 MHz and matched to 50 Ω before measurement. The 90° pulse was found to be ~5 μs for both ^1H and ^{31}P nuclei. The sample temperature was measured by a Doric digital thermometer, Model 400, with a thermocouple and was usually set at 24 °C unless otherwise stated. Although high decoupling power sometimes caused the sample temperature to rise, the technique of gated power level alternation allowed a minimal difference (1–3 °C) between the measured and actual temperatures. Spin–lattice relaxation times were obtained by the inversion recovery method with a 180°–τ–90° pulse sequence.

Computer Analysis. Model calculations were performed by using FORTRAN and the interactive MLAB system on the Digital Equipment Corp. PDP-10 at the Computer Center of the National Institutes of Health.

Analysis of Chemical Shift Anisotropy

Nuclear magnetic resonance in the solid state usually exhibits broad spectra due to the predominance of dipole–dipole interactions between nuclear spins. When these interactions are removed by, for example, spin decoupling, the resultant spectrum exhibits chemical shift anisotropy, which is directly related to the nature of the chemical bonds and thus to the structure of particular atomic groups. Consequently, the behavior of chemical shift anisotropy has been employed to investigate the structure and motions of molecules and individual atomic groups [for references see Mehring (1976)]. In the case of phosphorus, it is relatively easy to observe chemical shift anisotropy since phosphorus is relatively less abundant in biological systems and also because it has relatively weak dipole–dipole interactions with the more abundant protons.

Because of the nature of chemical shift anisotropy (CSA), the magnitude and orientation of the CSA principal axis system must be experimentally determined for individual atoms. For ^{31}P in DNA, the principal axis system (PAS) of the CSA is not known. We will test the influence of three different assumptions for the principal axis system. Principal axis system I, PAS I, is entirely based on the nature of the chemical bond and the symmetric character of the phosphodiester group (Kohler & Klein, 1976). PAS II(n) corresponds to the principal axis system which was found for a single crystal of barium diethyl phosphate by Herzfeld et al. (1978). Another alternative system, PAS II(n′), is considered equally likely based on the nearly symmetric plane made by the O(2)PO(3) bonds. These principal axis systems are illustrated in Figure 1. The phosphodiester axis system which corresponds to PAS I is chosen such that the x axis is normal to the plane of the sector PO(2)O(3), the y axis lies on the plane PO(2)O(3), and the z axis must be normal to the above two axes. The relationships among the principal axis systems are as follows. PAS II(n) results from a rotation of PAS I by $\chi = 11.1°$ about a vector N as shown in Figure 1B. PAS II(n′) is given by a rotation of PAS I by $\chi = 11.1°$ about a vector N′. Vectors N and N′ are symmetric with respect to the plane PO(2)O(3).

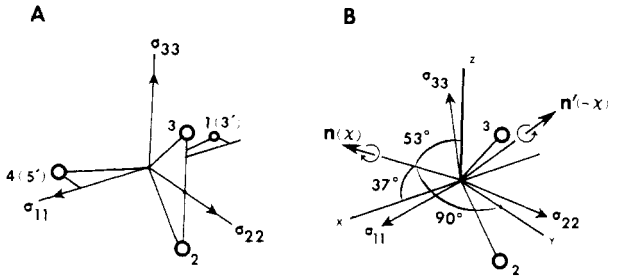

FIGURE 1: Principal axis systems (PAS) of the chemical shift tensor for phosphodiesters. The phosphorus atom is at the origin of the axis system, and the four oxygen atoms are indicated by numbers 1–4, with atoms 1 and 4 corresponding to ester linkages. (A) PAS I, the system based on the nature of the phosphodiester chemical bonds. (B) PAS II(n), the experimentally determined system for a single crystal of barium diethyl phosphate, and PAS II(n'), the system assumed from the molecular symmetry of the phosphodiester. See details in the text.

It is clear that the chemical shift will depend on the orientation of the observed nucleus with respect to the applied magnetic field and that the shift value will span the total shift anisotropy $|\sigma_{33} - \sigma_{11}|$, where σ_{11} is the smallest element and σ_{33} the largest element of the chemical shift tensor. The elements of the chemical shift tensor for ^{31}P in DNA have been reported previously (Terao et al., 1977; Shindo, 1980). Although these values vary within an experimental error of ±5 ppm, this margin does not affect our final conclusions. Therefore, we use the values of −76, −16, and 103 ppm for σ_{11}, σ_{22}, and σ_{33}, respectively, measured relative to trimethyl phosphate as an external reference (Shindo, 1980).

For calculation of the chemical shift of ^{31}P nuclei in DNA, the orientation of the phosphodiester groups must be known with respect to the DNA molecular axis system. The axis system of a phosphodiester group can be easily expressed in terms of the molecular axis system by using the atomic coordinates of the appropriate model structure. We employ the conformations proposed by Arnott & Hukins (1972), Langridge et al. (1960), and Fuller et al. (1965) as models for the A and B forms of DNA.

We now ask which component of the chemical shift tensor lies along the Z axis of the laboratory frame, i.e., the axis of the magnetic field, H_0 (Figure 2B). For the calculation of the NMR spectral pattern due to chemical shift anisotropy as a function of the angle of orientation of the DNA fiber, Φ, with respect to H_0, we consider two cases: perfect and imperfect orientations of DNA molecules along the fiber axis.

(A) Perfect Orientation. Consider first the case of perfect orientation of DNA molecules along the fiber axis. As shown in parts A and B of Figure 2, the axis system (x, y, z) is the principal axis system for chemical shift anisotropy, system (a, b, c) corresponds to the DNA molecular system where the axis c is identical with the helical axis of DNA, and system (x_g, y_g, z_g) is the goniometer system where z_g is chosen to be the rotation axis. The Z axis of the laboratory frame is taken as the axis of the magnetic field. We are interested only in the Z component of the chemical shift anisotropy (shift tensor) which is easily expressed in terms of the laboratory frame by rotational transformations of principal axis system (x, y, z) as follows: system (x, y, z) is transformed to the molecular frame by rotation matrix $\mathbf{R}_p$, the molecular frame is transformed to the goniometer system by $\mathbf{R}_m$, and the goniometer frame is transformed to the laboratory frame by $\mathbf{R}_g$. Transformation matrix $\mathbf{R}_p(\alpha, \beta, \gamma)$ is expressed in terms of Euler angles, α, β, and γ. Matrix $\mathbf{R}_m$ can be written as $\mathbf{R}_m(0°, 90°, 90°)$ when the fiber axis c lies on the axis x_g of the goniometer, and matrix $\mathbf{R}_g$ is given by $\mathbf{R}(\Phi, 90°, 90°)$ when axis Z of the laboratory frame lies on the $x_g y_g$ plane, as shown in Figure 2B.

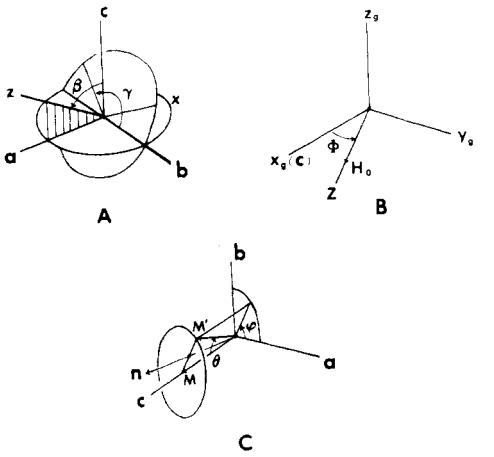

FIGURE 2: Coordinate systems. (A) System (x, y, z) represents the principal axis system of the chemical shift tensor and system (a, b, c) represents the molecular frame of the DNA fiber. (B) The long axis of the molecular frame, i.e., fiber axis c, coincides with x_g of the goniometer system (x_g, y_g, z_g), the Z axis of the laboratory frame is identical with the axis of the magnetic field, H_0, and the rotation angle of the goniometer about the z_g axis is given by Φ. (C) Dislocation of a DNA molecule from the fiber axis; vector $\mathbf{M}$ lies along fiber axis c, and $\mathbf{M}'$ represents the vector of the helical axis of disoriented DNA which makes an angle θ with respect to the fiber axis. The molecules on the surface of a cone at the angle θ have equal probability, independent of angle ψ.

If the chemical shift tensor is defined by $\mathbf{A}_p$ in the principal axis system as

$$\mathbf{A}_p = \begin{bmatrix} \sigma_{11} & 0 & 0 \\ 0 & \sigma_{22} & 0 \\ 0 & 0 & \sigma_{33} \end{bmatrix} \quad (1)$$

then the shift tensor in the goniometer frame is given by

$$\mathbf{A}_g = \mathbf{R}_m^{-1}\mathbf{R}_p^{-1}\{\mathbf{A}_p\}\mathbf{R}_p\mathbf{R}_m \quad (2)$$

Therefore, the Z component of the chemical shift in the laboratory frame is expressed by

$$\delta_{zz}(\alpha,\beta,\gamma,\Phi) = \tfrac{1}{2}[A_g(1,1) + A_g(2,2)] + \tfrac{1}{2}[A_g(1,1) - A_g(2,2)] \cos(2\Phi) + \tfrac{1}{2}[A_g(1,2) + A_g(2,1)] \sin(2\Phi) \quad (3)$$

where $A_g(i,j)$ is the matrix element of the ith row and jth column of matrix $\mathbf{A}_g$. As is seen from eq 3, the chemical shift, δ_{zz}, is a function of α, β, γ, and the rotation angle of the goniometer, Φ.

If the atomic coordinates of the phosphodiester group in DNA are known, we can determine the Euler angles, β and γ, for a given principal axis system PAS I or PAS II as shown in Figure 1. Since the atomic coordinates of the phosphodiesters are assumed to be uniform about the helical axis of DNA, the angle α must be randomly distributed about the DNA fiber axis, but the remaining angles, β and γ, are independent of the rotation of the molecule about the helical axis. Thus, the spectral intensity at an orientation Φ for a given DNA structural model is given by the derivative

$$I(\delta) = \frac{d\alpha}{d|\delta_{zz}(\alpha)|} \quad (4)$$

where α varies from 0 to 360°. When the fiber axis is parallel to the magnetic field, i.e., $\Phi = 0$, $I(\delta)$ becomes independent of the value of α since the term $A_g(1,1)$ in eq 3 does not include the variable α. In order to calculate the chemical shift for the

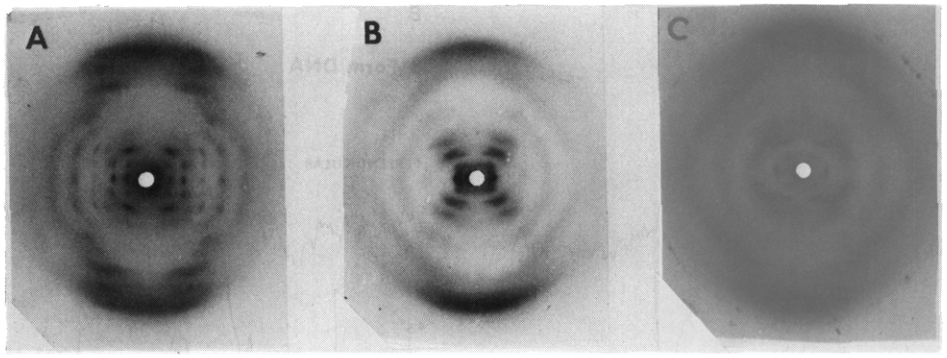

FIGURE 3: X-ray fiber diffraction patterns of different DNA conformations. (A) A-form DNA, NaDNA fiber under 79% relative humidity; (B) B-form DNA, NaDNA fiber under 98% relative humidity; (C) C-form DNA, LiDNA fiber under 33% relative humidity.

different orientations of Φ, $\delta_{zz}(\alpha)$ is calculated over the entire range of α with an increment $\Delta\alpha$, and the number of occurrences of the chemical shift in an interval δ and $\delta + \Delta\delta$ is counted. The digitized spectrum is smoothed out by applying the Gaussian convolution integral

$$H(\delta) = \int_{-\infty}^{\infty} I(\delta)G(\delta - \delta')\, d\delta' \qquad (5)$$

where $H(\delta)$ is the spectral distribution function and $G(\delta)$ is a Gaussian function. Using eq 3 and 5, we can calculate the spectrum for DNA fibers at any orientation of Φ.

(*B*) *Imperfect Orientation.* The orientation of DNA molecules within a fiber will not be perfect. It is useful to estimate the line broadening caused by imperfect orientation. As mentioned previously, we have assumed a Gaussian weighting factor so that the DNA molecules most probably align along the fiber axis with a standard deviation $\langle\theta\rangle$ from the fiber axis. For calculation of the spectrum in this case, the DNA molecules are equally distributed along the surface of a cone as shown in Figure 2C with an appropriate weighting factor. Transformation from axis M to M' can be performed by a rotation of molecular frame (a, b, c) about a vector $\mathbf{n}$ through 180°, where vector $\mathbf{n}$ is defined by two polar angles ψ and $\theta/2$. The rotation matrix for this transformation, $\mathbf{R}_d$, is expressed by

$$\mathbf{R}_d = \begin{bmatrix} 2(\cos^2\frac{\theta}{2}) - 1 & 2(\cos\frac{\theta}{2})\sin\frac{\theta}{2}\cos\psi & 2(\cos\frac{\theta}{2})\sin\frac{\theta}{2}\sin\psi \\ 2(\cos\frac{\theta}{2})\sin\frac{\theta}{2}\cos\psi & 2(\sin^2\frac{\theta}{2})\cos^2\psi - 1 & 2(\sin^2\frac{\theta}{2})\cos\psi\sin\psi \\ 2(\cos\frac{\theta}{2})\sin\frac{\theta}{2}\sin\psi & 2(\sin^2\frac{\theta}{2})\cos\psi\sin\psi & 2(\sin^2\frac{\theta}{2})\sin^2\psi - 1 \end{bmatrix} \qquad (6)$$

Therefore, the chemical shift tensor $\boldsymbol{A}_g$ in eq 3 must be replaced by

$$\boldsymbol{A}_g = \mathbf{R}_m^{-1}\mathbf{R}_d^{-1}\mathbf{R}_p^{-1}\{\boldsymbol{A}_p\}\mathbf{R}_p\mathbf{R}_d\mathbf{R}_m \qquad (7)$$

Again, since the Euler angles β and γ are determined by the phosphodiester coordinates, for any orientation of the DNA fiber (Φ), δ_{zz} in eq 3 becomes a function only of α, ψ, and θ given by

$$\delta_{zz}(\alpha,\psi,\theta) = \tfrac{1}{2}[A_g(1,1) + A_g(2,2)] + \tfrac{1}{2}[A_g(1,1) - A_g(2,2)]\cos(2\Phi) + \tfrac{1}{2}[A_g(1,2) + A_g(2,1)]\sin(2\Phi) \qquad (8)$$

The procedure for calculating the spectrum is as follows: δ_{zz} values are calculated with an increment $\Delta\alpha$ over the range of 0–360° and ψ is incremented by $\Delta\psi$ over the range 0–360° for a given value of θ. The spectrum is calculated for a particular θ value as previously described and then weighted by the Gaussian function

$$G(\theta) = \frac{1}{(2\pi)^{1/2}\langle\theta\rangle}\exp[-\theta^2/(2\langle\theta\rangle^2)] \qquad (9)$$

where $\langle\theta\rangle$ is defined as the standard deviation of the orientation of molecules from the fiber axis. The above process is repeated for different θ values with an increment $\Delta\theta$, if necessary, up to 90°, and the spectra for the different θ values are summed up. The resultant summation represents the spectrum when there is an average deviation, $\langle\theta\rangle$, of the orientation of DNA molecules from the fiber axis. This digitized spectrum may be smoothed out by applying a convolution integral as previously described in eq 5, if desired.

Results

X-ray diffraction photographs of DNA fibers at ~25 °C are presented in Figure 3. Photograph A was obtained for NaDNA under 79% relative humidity, photograph B was obtained for NaDNA under 98% relative humidity, and photograph C was taken for LiDNA under 33% relative humidity. These photographs indicate highly oriented DNA molecules and are typical X-ray patterns of A-, B-, and C-form DNA fibers (Fuller et al., 1965; Langridge et al., 1960; Marvin et al., 1961), respectively. The proton dipolar decoupled and crosspolarized ^{31}P NMR spectra are shown in parts A and B of Figure 4 together with their respective powder patterns. It is clear that the spectra of both A- and B-form DNA fibers exhibit orientation dependence. For A-form DNA, the spectrum parallel to the magnetic field is a broad and somewhat asymmetric singlet and its chemical shift at maximum height was found to be ca. −18 ppm from external trimethyl phosphate. The spectrum perpendicular to H_0 does not exhibit a doublet as expected by theory but rather exhibits a broad asymmetric resonance with a chemical shift about 15 ppm upfield from that of the spectrum parallel to H_0. On the other hand, the B-form DNA exhibits a symmetric line shape and a generally narrower line width than that of the A-form DNA at each corresponding orientation. A similar trend is observed for their powder patterns (Figure 4, bottom). Total shift anisotropies, $|\sigma_{33} - \sigma_{11}|$, were measured to be 160 and 127 ppm for the A- and B-form powder patterns, respectively. This value, especially for the B-form DNA, is much smaller than that observed for the vacuum-dried DNA (180 ppm) in which the molecule is considered to be rigid (Terao et al., 1977; Shindo, 1980). The chemical shift of the spectrum of the B-form DNA fiber oriented at 45° to H_0 spans a wider range (−40 to 65 ppm) as compared to the other orientations tested. Chemical shift values at maximum height were measured to be −5 and 8 ppm for the parallel and perpendicular orientations, respectively. It is remarkable that the line width for the

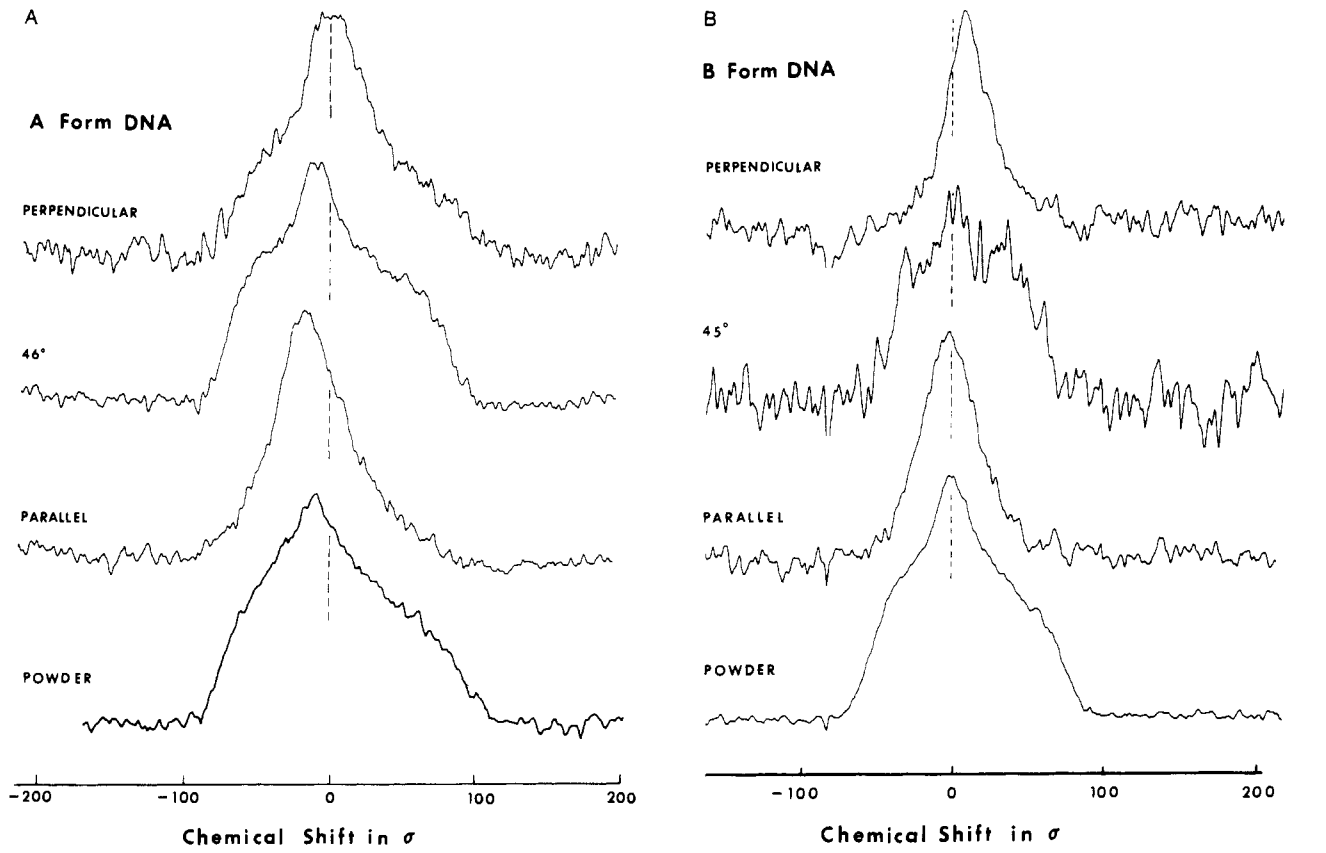

FIGURE 4: (A) Proton dipolar decoupled ^{31}P NMR spectra of the A-form NaDNA at different orientations of the fiber axis to the magnetic field as indicated, together with powder patterns; all at 79% relative humidity at 25 °C. Spectrometer conditions: spectral window, ±20 KHz; data points, 1024 real; cross-polarization time, 1.5 ms; delay time, 2.0 s; line broadening, 50 Hz; scan numbers ranged from 40 000 to 65 000 for DNA fibers and were 1024 for powder samples. (B) Proton dipolar decoupled ^{31}P NMR spectra of the B-form NaDNA at different orientations of the fiber axis as indicated, under 98% relative humidity at 25 °C. Spectrometer conditions are the same as those described in (A).

perpendicular orientation is narrower by 10 ppm than for the parallel orientation. The B form was also obtained from the lithium salt of DNA; its spectra at 25 °C are very similar to those of the B form obtained from fibers of the sodium salt of DNA (see Table I). This NMR result is consistent with that of X-ray diffraction studies (Langridge et al., 1960). None of the spectra of DNA fibers at any orientation exhibit resolved features. One possible reason for this is that rotational motion about the long axis of DNA takes place. In fact, the spin–lattice relaxation time (T_1) of ^{31}P in the B-form fiber was found to be twofold shorter for the perpendicular orientation than for the parallel (see Table I). This anisotropic effect can only be interpreted in terms of the rotational motion about the helical axis of DNA, which essentially corresponds to the fiber axis, since the orientation of the long axis of DNA is virtually fixed along the fiber axis. Because of this motion, the chemical shifts and line widths of the broad spectra for perpendicularly oriented fibers may be considered to be averaged by the rotational motion.

For confirmation of the existence of this rotational motion, the spectra were measured for the B-form DNA at 2 and −13 °C as shown in parts A and B of Figure 5. The relative humidity at 2 °C was kept at 95% by using a saturated K_2SO_4 solution. The spectrum corresponding to each orientation broadened as the temperature was decreased (see Figures 4B and 5). The effect of temperature on the line width confirms the presence of rapid molecular motions at 25 °C. DNA may alter its structure from the normal B form at −13 °C where water, of course, is frozen and relative humidity becomes meaningless. It seems, however, judging from the spectra in Figure 5 (right), that the orientation of DNA along the fiber axis is still highly ordered at −13 °C; this is emphasized in the comparison of the spectra at rotation angles Φ = 90 and 45°.

Table I: ^{31}P NMR Data of DNA Fibers at Different Orientations Relative to Magnetic Field

	rel humidity (%)	rotation angle[a]	chemical shift[b] (δ)	line width[c] (ppm)	T_1 (s)
A-form NaDNA	79	0	−18	53.5	
		45	−69 to 79		
		90	−3 to 8		
B-form NaDNA	98	0	−5	41.2	1.8 ± 0.2
		45	−38 to 61		
		90	8	31.7	0.8 ± 0.2
C-form LiDNA	33	0	−68 to 93		
B-form LiDNA	98	0	−6	40.2	
		90	7	31.8	

[a] The rotation angle of goniometer, Φ = 0°, represents the parallel orientation to H_0, and an angle Φ = 90° corresponds to the perpendicular orientation. [b] Chemical shift, δ, was measured in ppm from trimethyl phosphate in H_2O as an external reference (upfield is positive). [c] Line width is defined as the width at half-height of the nearly symmetrical resonance.

Figure 6 shows a comparison of spectra from the C form of LiDNA at 25 °C with those of NaDNA fibers at −80 °C. (The NaDNA fibers exhibited a B-form X-ray diffraction pattern at 25 °C.) It is clear from this comparison that the spectral patterns are very similar to each other. This may indicate that the NaDNA fiber is converted to the C conformation at such a low temperature, suggesting that the DNA fiber is dehydrated as the temperature is lowered below zero.

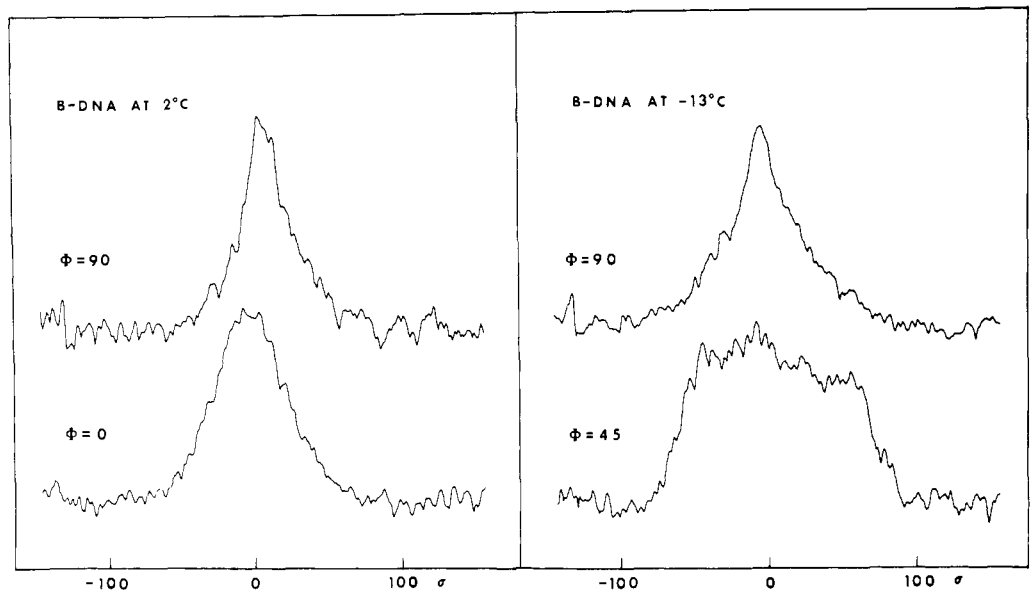

FIGURE 5: Proton dipolar decoupled ^{31}P NMR spectra for the B-form NaDNA fibers oriented to the magnetic field as indicated. (Left) The spectra obtained under 95% relative humidity at 2 °C (K_2SO_4-saturated solution); (right) the spectra of the same fibers at −13 °C but the relative humidity is unknown. Spectrometer conditions are the same as those described in Figure 4A.

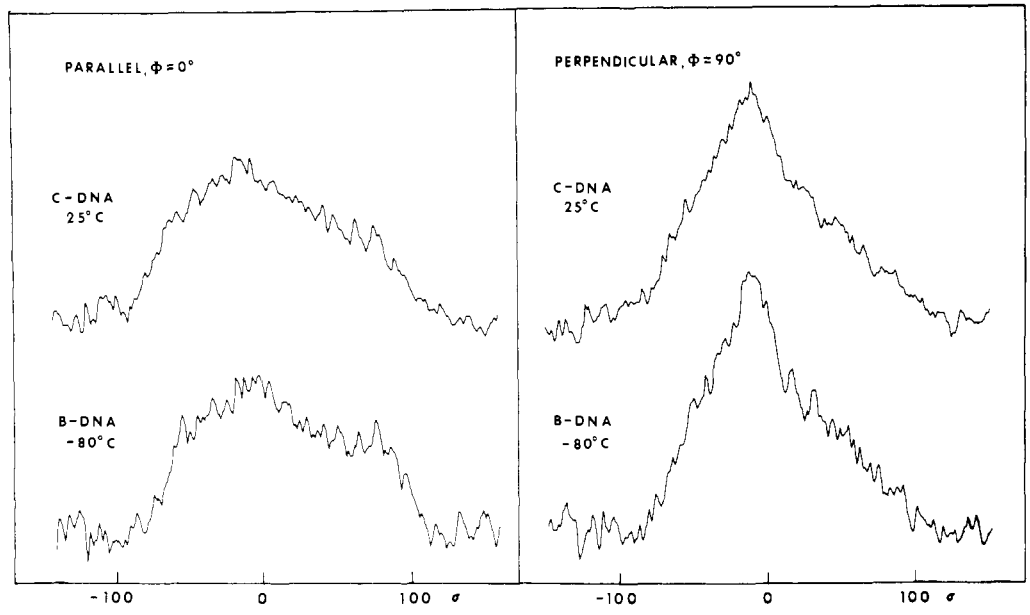

FIGURE 6: Comparison of ^{31}P NMR spectra of the C-form LiDNA at 25 °C and those of NaDNA fibers at −80 °C. (Left) The spectra of the fibers oriented parallel to H_0; (right) the spectra of the fibers oriented perpendicular.

Unfortunately, conformational data are not available at such a low temperature.

Discussion

Molecular Motion and Conformation of Phosphodiester in DNA. If the atomic coordinates of the phosphodiester groups in DNA were known exactly, the NMR spectrum of a DNA fiber at any orientation relative to the magnetic field could be calculated. Structural parameters related to the orientation of phosphodiester groups can be obtained by comparison of simulated and observed spectra. The number of observable data is reduced, however, due to the rotational motion about the helical axis of the DNA, since the spectral pattern for the perpendicular orientation of the fibers is affected by this motion. The shift value for the parallel orientation should not be affected, however, at least in principle, assuming a uniform backbone conformation of DNA, since the chemical shift δ_{zz} is not a function of α when the orientation angle $\Phi = 0°$ (eq 3). Thus, the chemical shift is considered to be a much more critical quantity for the parallel orientation than for the perpendicular orientation.

Assuming perfect orientation of DNA molecules along the fiber axis, chemical shifts and spectra were calculated for the A- and B-form DNA by using eq 3 and 5. Two examples are shown for the A- and B-form conformations in parts A and B of Figure 7, in which the phosphodiester coordinates used for the A form were proposed by Arnott & Hukins (1972) and for the B form were proposed by Langridge et al. (1960). Two common features can be recognized from these spectra: (1) a singlet for the parallel orientation and a doublet for the perpendicular orientation as expected from the helical symmetry of the DNA structure; (2) a triplet for the orientation $\Phi = 45°$ (a theoretical quartet in which the central doublet consists of two overlapping peaks). In contrast, the chemical shift and its span are different for the A- and B-form conformations.

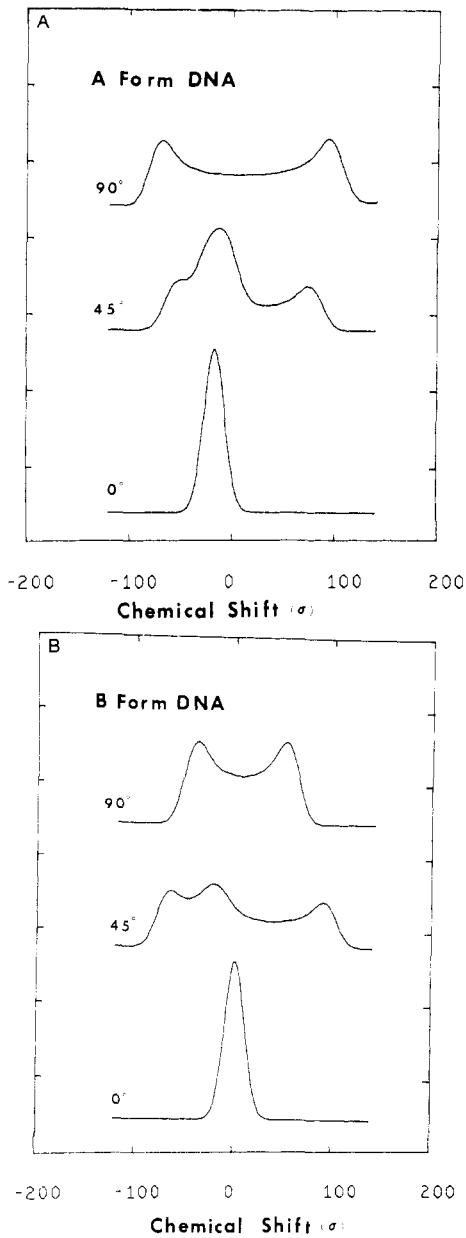

FIGURE 7: (A) Calculated spectra for the A-form DNA at different orientations. Phosphodiester atomic coordinates used were for the A-form DNA model determined by Arnott & Hukins (1972). A line broadening of 10 ppm was applied to the digitized spectrum by Gaussian convolution. (B) Calculated spectra for the B-form DNA at different orientations. Phosphodiester atomic coordinates used were for the B-form DNA model determined by Langridge et al. (1960).

It is interesting to compare these calculated spectra to the observed spectra. The observed spectra for perpendicular orientation do not exhibit resolved peaks at 25 °C but rather exhibit a singlet, which is particularly obvious for the B-form DNA fiber. This discrepancy between the calculated and observed spectra can be interpreted in terms of rotational motion of the molecules about their helical axis. Furthermore, the fact that the line width is narrower for the B-form DNA than for the A-form DNA can be attributed to a difference in hydration of the molecules and/or intermolecular interaction. From the difference of the maximum span of the chemical shift (180 ppm at 24.3 MHz) for the vacuum-dried DNA species and the observed total shift anisotropy (160 and 127 ppm for the A- and B-form DNA, respectively), the minimum rate of rotational motion may be estimated to be 5×10^2 s^{-1} for the A-form DNA and 1.5×10^3 s^{-1} for the B-form DNA, respectively. The azimuthal angle of the rotational motion may be small for the A-form DNA, although it is very difficult to estimate this angle from the broad spectrum of the A-form DNA fibers oriented perpendicular to H_0. In contrast, a comparison of the observed single spectrum and the calculated doublet for the B-form DNA perpendicular to H_0 indicates that the azimuthal angle of the rotational motion must exceed 90° and that the rate of the motion is over 2.2×10^3 s^{-1}.

Calculated chemical shift values are listed in Table II. Column 1 gives the structural models employed for the calculation, column 3 shows the principal axis system (PAS) as described in the analysis section (Figure 1), columns 4 and 5 list Euler angles for the rotational transformation of the PAS to the molecular frame, column 6 lists the chemical shift values for the parallel orientation of the fiber, i.e., $\Phi = 0°$, and column 7 gives chemical shift values for the perpendicular orientation ($\Phi = 90°$) when rotational motion is rapid enough to collapse the doublet to a singlet. The uncertainty of the calculated chemical shifts may reach about ±14 ppm, because of the experimental error in the chemical shift anisotropy (±5 ppm) and also because of the assumption of the orientation of the principal axis system; a choice of the principal axis system (PAS) may change the chemical shift values for the parallel orientation by 18 ppm at most as in Table II. Furthermore, it should be recalled that the chemical shift value for $\Phi = 0°$ is more reliable than for $\Phi = 90°$ because of lack of a rapid motion particularly for the A-form DNA. The last column indicates an evaluation of the agreement between the calculated and observed chemical shifts, taking into consideration the uncertainties mentioned above. The following conclusions can be drawn from Table II: (1) the NMR data for B-form DNA can be described in terms of phosphodiester coordinates given by Langridge et al. (1960) but not by Arnott & Hukins (1972) and (2) the NMR data for the A-form DNA can be reasonably interpreted in terms of two different sets of coordinate systems given by Fuller et al. (1965) or by Arnott & Hukins (1972) but cannot differentiate between them. It should be emphasized that these conclusions only apply to the phosphodiester coordinates. We have initially aimed to define the exact orientation of phosphodiesters in the molecular frame from NMR data alone and to compare with the above-mentioned DNA models but it was found to be a very difficult task because of the lack of resolved features of spectral patterns. It seems likely, however, that there is a basic reason for precluding such a resolved spectrum: that is, the lack of a uniform phosphodiester conformation in DNA.

Nonuniform Backbone Conformation of DNA. We face two questions about the line width of the spectra for the B-form DNA: (1) why is the parallel spectrum so broad (41 ppm at 24.3 MHz) and (2) why is the perpendicular spectrum narrower than the parallel by 10 ppm? With regard to the first question, one might imagine that the orientation of DNA may be decreased either due to lack of truly parallel orientation among the fibers or due to lack of orientation of DNA molecules relative to the fiber axis or due to both effects. Both types of disorder could artifactually broaden the line width of the spectra. The former, disorientation between fibers, should be neglible, since machine tolerances to make a set of parallel holes (400 μm in diameter and 3 mm in length) are accurate enough for our purposes. The latter problem was avoided as much as possible by selection of fibers with excellent extinction when examined with a polarizing microscope. The molecular orientation within the fibers was evaluated from X-ray diffraction patterns of representative fibers. The in-

Table II: Calculated Chemical Shift Values of Different Conformations of DNA Fibers for Parallel and Perpendicular Orientations of Fiber to H_0

coordinates used	DNA form	PAS[a]	Euler angle		chemical shift (δ)		agreement[c] with obsd
			β	γ	0°	90°[b]	
Arnott & Hukins (1972)	A	I	103.1	−79.1	−11.9	12	fair
		II(n)	94.5	−72.6	−20.6	15	good
		II(n′)	94.3	−85.7	−15.7	14	good
Fuller et al. (1965)	A	I	115.4	−60.1	−6.3	15	poor
		II(n)	108.2	−54.2	−22.9	17	fair
		II(n′)	107.7	−66.2	−13.9	12	good
Arnott & Hukins (1972)	B	I	76.9	−42.2	−41.1	26	none
		II(n)	71.7	−36.3	−39.4		none
Langridge et al. (1960)	B	I	119.8	−130.5	−5.8	9	good
		II(n)	125.8	−125.7	11.2		none
		II(n′)	124.9	−135.0	2.6	4	good

[a] See the principal axis system (PAS) of the chemical shift tensor in the text. [b] Chemical shift values for the perpendicular orientation are the averaged values of two peaks. [c] Agreement between the observed and calculated chemical shifts.

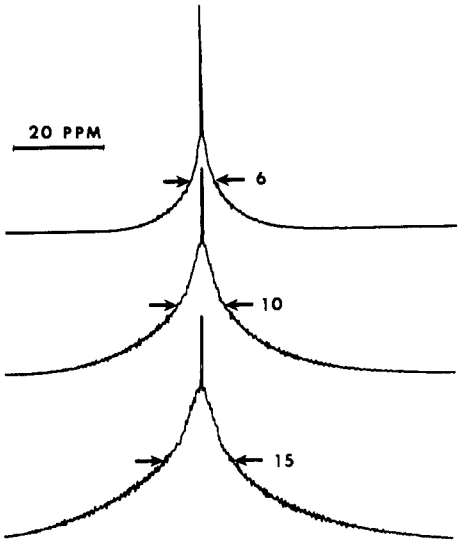

FIGURE 8: Line broadening caused by disorientation of rigid DNA molecules about the fiber axis. The average deviation angle from the fiber axis, $\langle\theta\rangle$, is 4, 8, and 12° from the top spectrum.

fluence of the disorientation of DNA within the fiber on the line broadening will be calculated as follows. Using eq 6–9 and taking an interval from 0 to 360° in steps of 5° for α and ψ in the case of imperfect orientation, we calculated theoretical spectra for parallel orientation of the B-form DNA fiber. These spectra are shown in Figure 8 for standard deviations of $\langle\theta\rangle$ = 4, 8, and 12°. As is seen, there is a sharp peak at the center of the spectrum and the intensity smoothly decreases as the chemical shift moves out from the center. If a convolution integral were applied by using eq 5, the sharp central peak in the spectra would be smoothed out. It is reasonable to define a line width due to imperfect orientation as the line width at half-height, neglecting the sharp peak at the center, whose line width is indicated by the number in parts per million in Figure 8. Even for a standard deviation $\langle\theta\rangle$ of 12°, the spectrum is broadened by only 15 ppm, which is much less than the observed line width (41 ppm). The assumed deviation of 12° is unreasonably large in view of the observed X-ray diffraction patterns for the fibers studied here. Recalling that a line width of 15 ppm was calculated for a rigid molecule, the spectrum should become even narrower in the presence of molecular rotational motion. Thus, it can be concluded that imperfect orientation of molecules along the fiber axis is not sufficient to rationalize the line width observed for the B-form DNA. Residual ^{31}P–^{1}H coupling, ^{31}P–^{31}P dipolar interaction, and susceptibility changes near the surfaces of fibers may cause some line broadening, but these should be smaller by an order of magnitude than that observed.

We must consider possible contamination of one form of DNA with another. The highly cooperative nature of the transition between A- and B-NaDNAs induced by hydration of DNA (Cooper & Hamilton, 1966; Arnott & Selsing, 1974) makes this possibility less likely. Occasionally, A-form fibers (at 79% relative humidity) were observed to have traces (<5%) of the B form by X-ray diffraction. However, the B form was never observed to be contaminated with the A form in samples held at 98% relative humidity. Also, the lithium salt of DNA has not been observed to give the A form of DNA (Langridge et al., 1960); the very similar spectra for the B form of DNA from the lithium and sodium salts are therefore further evidence for the absence of contamination by the A form.

Gorenstein et al. (1976) have suggested that the torsion angle of the O–P–O bonds is correlated with the isotropic chemical shifts of the phosphodiester groups. We found that the isotropic chemical shift has a variation of only 0.5 ppm for the phosphodiesters of DNA in solution (Shindo, 1980). Therefore, we can assume that the magnitude and orientation of the chemical shift tensor with respect to the atomic coordiantes of the phosphodiester group are virtually identical for all conformers of DNA in the fiber, especially for the B-form DNA.

We suggest that the variation in the orientation of phosphodiester groups of DNA in the fiber is the predominant factor in the line broadening. In fact, it is easy to show that rotation of a phosphodiester group about an arbitrary axis by 20° can cause a change in the chemical shift for the oriented fibers by as much as 30 ppm. This hypothesis may help to explain why the line width of the perpendicularly aligned B-form DNA fiber is narrower than that of the parallel fiber. It is reasonable to expect the doublet of the perpendicular spectrum to collapse into a singlet due to rapid motion about the helical axis of DNA. The largest element of the chemical shift tensor, σ_{33}, can be considered alone without adversely affecting the qualitative conclusions. According to the B-form coordinates of Langridge et al. (1960), one principal axis, z, of the chemical shift tensor in PAS I can be projected onto the molecular frame where a choice of the principal axis system is not essential. The angle β = 119.75° between z and c, the helical axis of DNA, is taken from Table II. The z axis is nearly perpendicular to the axis c. Therefore, the component of the shift tensor element σ_{33} along the axis c is much smaller than the component along the axis perpendicular to the axis

c. Consider changes in chemical shifts for axes c and the axis perpendicular to axis c associated with variations of angle β by ±7°. Chemical shift variation induced by a change in β by ±7° is estimated to be $(\sigma_{33} - \sigma_i)[\cos(\beta - 7) - \cos(\beta + 7)] = 21$ ppm for the component along the axis perpendicular to the axis c and $(\sigma_{33} - \sigma_i)[\sin(\beta - 7) - \sin(\beta + 7)] = 12$ ppm for the component along the c axis. Here σ_i is the isotropic chemical shift, $(\sigma_{11} + \sigma_{22} + \sigma_{33})/3$. In other words, the variation of angle β emphasizes the change in the chemical shift for the parallel component more than for the perpendicular. A difference of 9 ppm observed between the line widths for the parallel and perpendicular spectra can be quantitatively interpreted in terms of a variation of angle β by ±7°.

The hypothesis mentioned above may also be supported by the facts that the spectra of the perpendicularly oriented fibers do not exhibit doublet patterns even at low temperatures and that the spectra of the parallel-oriented fibers exhibit a wide range of chemical shift dispersion for the C-form LiDNA at 25 °C and for NaDNA at -80 °C at which temperatures both DNA molecules are essentially rigid. Thus, it can be concluded that all of our ^{31}P NMR spectra from DNA fibers are interpreted consistently in terms of the presence of significant variations in the backbone conformation of DNA; the magnitude of the variation in phosphodiester orientations may reach as much as ±20° from an average conformation. Yet it remains to be established to what extent variation in the backbone conformation can be allowed in interpreting X-ray diffraction data from DNA fibers.

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Characterization of the Inhibitory (ϵ) Subunit of the Proton-Translocating Adenosine Triphosphatase from *Escherichia coli*†

Paul C. Sternweis‡ and Jeffrey B. Smith*

ABSTRACT: The inhibitory subunit (ϵ) of the F_1 adenosine triphosphatase (ATPase) was purified to homogeneity from the ML 308-225 and K12(λ) strains of *Escherichia coli*. No tryptophan or cysteine was detected in the subunit from either strain. The highly active ϵ from both strains was found to be a globular protein with a Stokes' radius of 18-19 Å. Circular dichroism spectra suggested an α-helix content of ~40%. The molecular weight of ϵ was ~15 000-16 000 by sedimentation equilibrium centrifugation in the presence and absence of guanidinium hydrochloride, molecular sieve chromatography, and gel electrophoresis in the presence of sodium dodecyl sulfate and 8 M urea. The $s_{20,w}$ of ϵ was ~1.6 s^{-1}. Inhibition of the purified F_1 ATPase by ϵ displayed noncompetitive kinetics with a K_i of ~10 nM. The inhibition of the ATPase was rapidly reversed by diluting the enzyme-ϵ mixture. [^{125}I]ϵ which was incorporated into ECF_1 was readily displaced by unlabeled ϵ. ϵ had no significant effect on the ATPase activity of "native" or reconstituted everted membrane vesicles under a variety of assay conditions. Combining the ϵ-inhibited F_1 ATPase with its hydrophobic portion in everted membrane vesicles reconstituted the reversible proton-translocating ATPase and restored nearly full ATPase activity. These results suggest that ϵ inhibits the enzyme only when the F_1 ATPase becomes detached from its hydrophobic subunits.

Subunit ϵ[1] is the smallest or fifth subunit of the F_1 ATPase from mitochondria, chloroplasts, and bacteria (Senior, 1973; Pedersen, 1975; Wilson & Smith, 1978). Homogeneous ϵ from *Escherichia coli* (Smith & Sternweis, 1977) and chloroplasts

† From the Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14853. *Received July 9, 1979.* This work was supported by grants from the National Science Foundation and the National Institutes of Health to Dr. Leon A. Heppel and a National Research Award (IF 32 GM 02419) to J.B.S.

‡ Present address: Department of Pharmacology, University of Virginia School of Medicine, Charlottesville, VA 22903.

[1] Abbreviations used: F_1, that portion of the proton-translocating ATPase which is peripheral to the membrane; F_0, that portion of the enzyme which is an integral component of the membrane; ECF_1, F_1 from *E. coli*; MF_1, F_1 from mitochondria; α, β, γ, δ, and ϵ, individual subunits of F_1 in order of decreasing size; ATPase, adenosine triphosphatase; DTT, dithiothreitol; EDTA, (ethylenedinitrilo)tetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate.

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