Fast and Slow Conformational Fluctuations of RNA and DNA. Subnanosecond Internal Motion Correlation Times Determined by ^{31}P NMR

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Abstract: The NMR relaxation of phosphorus-31, which is strategically located in the backbone of polynucleotides, has been investigated to obtain information about the conformational fluctuations of RNA and DNA. Measurements of the spin-lattice relaxation time (T_1), $^{31}P\{^1H\}$ nuclear Overhauser effect, line width, and rotating frame spin-lattice relaxation in an off-resonance radio-frequency field ($T_{1\rho}^{off}$) were performed for poly(A), poly(I)-poly(C), and calf thymus DNA. Examination of the NMR relaxation parameters of DNA at 40.5 and 81 MHz showed that the major contribution to relaxation is dipolar and that of the chemical shift anisotropy is small. All four relaxation parameters could be fit very well by a two correlation time model involving long-range bending motions and internal motions consisting of rotational wobbling about the P-O bonds. The correlation times for double-stranded RNA and DNA are in good agreement with a model which associates the long-range bending of the molecules with isotropic reorientation of proton-phosphorus vectors at a rate of 10^5-10^7/s. The internal motion correlation times of poly(A), poly(I)-poly(C), and calf thymus DNA are found to be nearly the same, to be independent of salt and temperature in the range examined, and to be in the range of 0.3-0.5 ns. These results indicate that the local motion is not strongly coupled to the conformation of the nucleic acid in the large.

Introduction

The importance of the structural information obtained for RNA and DNA is evident in the impact these studies have had on the progress of molecular biology. The dynamical properties of the conformations of RNA and DNA are also important but have not been as amenable to investigation. The magnitude and rates of the conformational fluctuations are of interest in understanding the interaction of nucleic acids with drugs, proteins, and substances which chemically modify RNA and DNA. Determination of a preferential mode of distortion of nucleic acids may indicate a manner in which the packaging of RNA and DNA in such macrocomplexes as ribosomes, viruses, and chromatin occurs. The conformational fluctuations of nucleic acids may also be useful in obtaining some information about the conformation of RNA and DNA in macrocomplexes as the motional properties may be sensitive to conformation. Classical methods of investigating nucleic acid structure, such as X-ray diffraction, are not readily applicable to macrocomplexes owing to their size and complexity.

The conformational fluctuations of RNA and DNA are of interest in determining if there is any gross difference between the two types of nucleic acids. We have recently proposed that there is an intramolecular water bridge between the 2′-OH and 3′-phosphate of RNA which can be used to explain, at least in part, the differences in conformation and stability observed for RNA and DNA.[1] An intramolecular water bridge might be expected to restrict the mobility of RNA.

A variety of methods have been used to monitor the conformational fluctuations of bare RNA and DNA. The breathing motion of polynucleotides has been monitored by tritium exchange[2] and ^1H NMR.[3-5] These methods have been useful in examining the availability of amino and imino protons to solvent on the time scale of milliseconds to seconds (NMR) and from many seconds to minutes (tritium exchange). These two methods monitor large conformational changes resulting in exposure of the bases to solvent, which require fairly long times. Temperature-jump measurements have allowed the examination of conformational fluctuations on the time scale of milliseconds and depend on changes in the optical density of the polynucleotides which are associated with decreased stacking of the bases.[6] The temperature-jump measurements have offered information about the rate of base-pair formation, typically 1000/s. Hydrodynamic investigations have given much of the currently available information about the long-range conformational mobility of nucleic acids.[6] Such studies have shown, for example, that DNA does not have the properties expected for a rigid rod; rather, there is long-range bending such that, on the average, the angle between the base pairs which are separated from one another by about 180 base pairs, the persistence length of DNA, is 90°.[6] The rate of depolarization of the fluorescence of a drug bound to a polynucleotide allows investigation of motions on the time scale of the fluorescence lifetime of the bound drug. Only a single report of such a study has appeared, which indicated that DNA undergoes internal motion on the time scale of nanoseconds.[7] Taken together, these studies indicate that the correlation times for internal motions in nucleic acids are most likely several orders of magnitude shorter than that for overall motion of the high molecular weight polymers.

To investigate conformational fluctuations on a time scale which spans several orders of magnitude, it is necessary to have either a very versatile method or to use a combination of methods which cover the time range of interest. Since there is no known method which can be used to probe the wide range of times expected for polynucleotides, we have combined the results of four different NMR relaxation methods to obtain a model for the motion of the backbones of RNA and DNA. Phosphorus-31 was used as the magnetic probe since (a) it is strategically located in the backbone of polynucleotides; (b) it offers high sensitivity for the NMR experiments; (c) there is one phosphorus per monomer unit of the polynucleotides; (d) as phosphorus is a natural constituent of polynucleotides, it obviates any perturbation associated with a probe molecule. As we shall show, the ^{31}P NMR relaxation experiments are sensitive both to reorientation motions about internal bonds and to slower motions on the same order as those estimated for the motions responsible for the persistence length.

Theory

This investigation utilizes the dependence of the relaxation of the phosphorus-31 nucleus on the molecular motions of the nucleic acids. The spin-lattice relaxation time T_1, the spin-spin relaxation time T_2, the nuclear Overhauser effect NOE, and the rotating frame spin-lattice relaxation time in the presence

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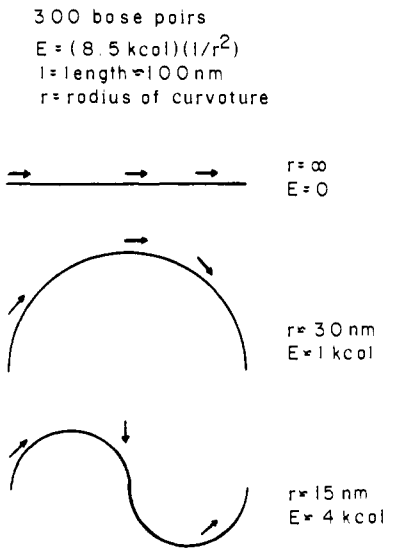

Figure 1. Schematic drawing of the reorientation of the phosphorus-hydrogen internuclear vectors, indicated by the arrows, induced by the long-range bending of RNA or DNA. The drawings indicate the orientation of the P-H vectors for different radii of curvature of a 300 base-pair segment of polynucleotide.

of an off-resonance rf field $T_{1\rho}^{\text{off}}$ are given in terms of the spectral densities $J_n(\omega)$ for dipolar coupling to a hydrogen by[8,9]

$$1/T_1 = K[J_0(\omega_H - \omega_P) + 3J_1(\omega_P) + 6J_2(\omega_H + \omega_P)] \quad (1)$$

$$1/T_2 = 1/2T_1 + K[2J_0(0) + 3J_1(\omega_H)] \quad (2)$$

$$\text{NOE} = 1 + \frac{\gamma_H}{\gamma_P} \times \frac{[6J_2(\omega_H + \omega_P) - J_0(\omega_H - \omega_P)]}{[J_0(\omega_H - \omega_P) + 3J_1(\omega_P) + 6J_2(\omega_H + \omega_P)]} \quad (3)$$

$$\frac{1}{T_{1\rho}^{\text{off}}} = K[\sin^2\theta_e\{2J_0(\omega_e)\}] + \frac{1}{T_1} = \frac{1}{T_{\text{eff}}} + \frac{1}{T_1} \quad (4)$$

$$K = \frac{\hbar^2\gamma_P^2\gamma_H^2}{20r^6} \quad (5)$$

γ_P and γ_H are the gyromagnetic ratios of phosphorus and hydrogen, ω_P and ω_H are the angular Larmor frequencies of phosphorus and hydrogen, and r is the phosphorus-proton internuclear distance. The other terms in the $T_{1\rho}^{\text{off}}$ eq 4 are[9]

$$\theta_e = \tan^{-1}(\gamma_P H_1/2\pi\nu_{\text{off}}) \quad (6)$$

$$\omega_e = 2\pi\nu_{\text{off}}/\cos\theta_e \quad (7)$$

ω_e is the angular precession frequency about the effective field vector $\overline{\mathbf{H}}_e$ created by the application of the rf field of strength H_1 at a frequency ν_{off} off-resonance:[9]

$$\overline{\mathbf{H}}_e = \frac{\overline{\omega}_e}{\gamma_P} = \frac{2\pi\nu_{\text{off}}}{\gamma_P}\overline{\mathbf{k}} + H_1\overline{\mathbf{i}} \quad (8)$$

It is noted that the expression for $T_{1\rho}^{\text{off}}$ given in eq 4 is only valid for the condition that the H_1 field is applied far off-resonance:[9]

$$\nu_{\text{off}} \gtrsim 5\gamma_P H_1/2\pi \quad (9)$$

The steady-state magnetization along the effective field $\overline{\mathbf{H}}_e$ results from the competition between T_1 and $T_{1\rho}^{\text{off}}$ relaxation:[9]

$$M_{\text{eff}} = \frac{M_0}{1 + T_1/T_{\text{eff}}} \quad (10)$$

where M_0 is the magnetization in the absence of the off-resonance field at equilibrium and M_{eff} is the magnetization in the presence of the off-resonance field at equilibrium. The ratio

$$R = M_{\text{eff}}/M_0 \quad (11)$$

can be experimentally determined as the ratio of the intensity of the phosphorus-31 resonance in the presence of an off-resonance field to that in the absence of the rf field.

The calculation of the spectral densities for the situation of random anisotropic reorientation of a spin pair about an axis which, in turn, is undergoing isotropic reorientation was developed by Woessner[10] and has been applied to macromolecules.[11,12] We use the same approach to arrive at the spectral densities:

$$J_n(\omega) = A\frac{2\tau_0}{1 + \omega^2\tau_0^2} + B\frac{2\tau_B}{1 + \omega^2\tau_B^2} + C\frac{2\tau_C}{1 + \omega^2\tau_C^2} \quad (12)$$

$$A = \frac{1}{4}(3\cos^2\phi - 1)^2 \quad B = \frac{3}{4}(\sin^2 2\phi) \quad C = \frac{3}{4}(\sin^4\phi)$$

$$\tau_B = [1/\tau_0 + 1/(6\tau_i)]^{-1} \qquad \tau_C = [1/\tau_0 + 2/(3\tau_i)]^{-1}$$

where ϕ is the angle between the phosphorus-hydrogen (P-H) internuclear vector and the axis of the internal rotation, τ_i is the correlation time for reorientation of the P-H vector about the axis of internal rotation, and τ_0 is the correlation time for isotropic reorientation of the axis of internal rotation.

The correspondence between the molecular motions and the internal and overall correlation times is, of course, necessary in interpreting the experimental NMR relaxation data. The persistence length of DNA can be used to calculate the energy needed to bend a given length of DNA to a particular radius of curvature according to[6,13,14]

$$E = \left(\frac{akT}{4}\right)\left(\frac{d}{r^2}\right) \quad (13)$$

where the persistence length a is 60 nm so $(akT/4) \simeq 8.5$ kcal·nm/mol, d is the length of DNA molecule, and r is the radius of curvature. As illustrated in Figure 1, the energy needed to induce reorientation of the P-H vectors by bending of the DNA is quite small. While the drawings in Figure 1 only indicate bending of the DNA in the plane of the drawing, bending can also occur perpendicular to this plane. Although the actual long-range bending of DNA in solution will be much more complicated than that shown in Figure 1, the drawing illustrates that the bending of DNA will give rise to essentially isotropic reorientation of the P-H vectors. Calculations based on eq 13 suggest that approximately 2-4 kcal is required for the reorientation of a given P-H vector by DNA bending, which indicates that the rate of reorientation is on the order of 10^5-10^7/s. This estimate leads to a correlation time for reorientation of the P-H vectors due to the bending of the DNA of about 10^{-7}-10^{-5} s.

We note that the reorientation of the P-H vectors due to overall rotational diffusion of the DNA is much slower than that which arises from the bending motion. The rotational diffusion time for a DNA molecule with a molecular weight of a few million is on the order of 10^{-5}-10^{-4} s.[6] Thus, the overall reorientation of the P-H vectors observed in the NMR relaxation experiments will be primarily due to the bending of the DNA molecules rather than to rotational diffusion. It is presumed that the same situation occurs for double-stranded RNA.

The internal motion of the P-H vectors is illustrated in Figure 2. Rotation about the P-O bond induces a change in the orientation of the P-H vector with an angle of rotation ϕ of about 40° with respect to the rotation axis. The internuclear phosphorus-hydrogen distance for the 3′, 5′, and 5″ hydrogens is about 0.26 nm. The distances and angles were estimated

Table I. ^{31}P NMR Relaxation Parameters of Calf Thymus DNA at 40.5 and 81 MHz[a]

^{31}P frequency	40.5 MHz	81 MHz
(T_1)d[b]	2.5	3.3
$(T_1)_{CSA}$[c]	25	6
T_1 calcd[d]	2.3	2.1
T_1 obsd[e]	2.4	2.2
% CSA[f]	10%	35%
NOE calcd[g]	1.6	1.3
NOE obsd[e]	1.6	1.3

[a] The sample contained 40 mM calf thymus DNA in 2 mM EDTA, 10 mM cacodylate, pH 7.0. [b] Dipolar contribution to T_1, from Figure 3 using $\tau_{internal} = 3 \times 10^{-10}$ s, $\tau_{overall} = 10^{-6}$ s. [c] Chemical shift anisotropy contribution to T_1, from eq 14, correlation times as in footnote *a*. [d] $1/T_1$ calculated $= 1/T_{1d} + 1/T_{1\,csa}$. [e] The 40.5-MHz value is the average of measurements made at 20 and 40 °C. The 81-MHz value was obtained at 35 °C. [f] % CSA $= (1/T_{1\,csa})/(1/T_{1\,calcd}) \times 100$. [g] $NOE_{calcd} = 1 + [(NOE - 1)(T_{1d}/T_{1\,calcd})]$ with NOE from Figure 3 and eq 3 with correlation times as in footnote *a*.

from a Drieding model using the orientations determined by X-ray studies of RNA and DNA fibers.[15] The interpretation of the NMR relaxation data is not particularly sensitive to small changes in the angle of rotation (±5°) or in the proton–phosphorus internuclear distance (±0.02 nm). A recent investigation of the phosphorus relaxation of deoxyoligonucleotides has employed an effective angle of internal rotation of 60°.[16] However, effective angles of internal motion near 54.7°, the dipolar magic angle, are not consistent with our experimental results (vide infra). Rotation about the O–C bond as shown in Figure 2 will also contribute to the relaxation of the phosphorus-31 and induces changes not only in the angle of P–H vector, but the internuclear distance as well. Rotation about the C–O bond is neglected in this investigation. Since it is the correlation time of a given bond rotation that is most important in inducing relaxation of the phosphorus-31 by reorienting the P–H vectors, the details of the molecular motion are not crucial in determining the correlation time. However, it is reasonable that the main local motion contribution to reorientation of the P–H vectors is rotational wobbling about the P–O bonds.

The line width is considered to be the least reliable indicator of molecular motion due to the chemical shift inequivalence of the different phosphorus nuclei. Examination of the phosphorus-31 spectrum of low molecular weight poly(I)-poly(C) has shown that there are two peaks of equal intensity with a splitting of about 15 Hz at 40.5 MHz. For the high molecular weight samples examined in this investigation, the line widths are much larger than the chemical-shift inequivalence, which is estimated to be 15 Hz for both poly(I)-poly(C) and calf thymus DNA.

The curves in Figures 3 and 4 were calculated with complete neglect of the chemical shift anisotropy. The contribution of the chemical shift anisotropy to the relaxation of the phosphorus-31 of nucleic acids can be determined by use of[12]

$$\left(\frac{1}{T_{1\,csa}}\right) = \frac{2}{15}\omega_P^2 \Delta\sigma^2 J_1(\omega) \qquad (14)$$

where $\Delta\sigma$ is the chemical shift anisotropy, and it is assumed that the chemical shift tensor is axially symmetric. The contribution of the chemical shift anisotropy can be directly determined by observing the relaxation of the phosphorus-31 at different magnetic field strengths.

Results and Discussion

The experimental phosphorus-31 relaxation data can be compared with the values predicted assuming different models for the conformational fluctuations of the polynucleotides to

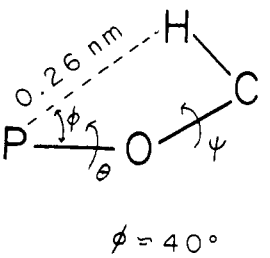

Figure 2. Schematic drawing of the angles and distances relevant to the internal motion of the phosphorus–hydrogen internuclear vector. Rotation about the P–O bond, θ, induces reorientation of the P–H vector with an effective angle of internal rotation of 40°, angle ϕ, with respect to the rotation axis. The internuclear phosphorus–proton distance is 0.26 nm. The orientation and distances are assumed to be equivalent for the 3′, 5′, and 5″ protons which assumption appears reasonable on the basis of molecular models and X-ray crystallography data.

Table II. ^{31}P NMR Relaxation Parameters of Poly(A)[a] at 40.5 MHz

temp, °C	T_1, s	NOE	$\Delta\nu_{1/2}$, Hz	R[b]	$T_{1\rho}^{off}$, s[b]
6	1.6	1.26	<10	0.7	1.1
8	1.56	1.3	<10	0.75	1.2
12	1.52	1.35	<10	0.88	1.35
20	1.54	1.29	<10	0.95	1.46
40	ND	ND	<10	0.99	ND

[a] The sample contained poly(A) at a concentration of 20–40 mM in 2 mM EDTA, 10 mM cacodylate, pH 7.0. [b] R and $T_{1\rho}^{off}$ were determined with an rf field of strength 0.47 G applied 8 kHz off-resonance.

determine which models give the most accurate description of the experimental results. Attempts to fit the data for poly(A) (see Table II) to a model first involved a description of the conformational fluctuations of poly(A) in terms of a single isotropic motion. Theoretical values of the relaxation parameters T_1, NOE, R, $T_{1\rho}^{off}$, and line width, calculated using a single correlation time model with three protons at a distance of 0.26 nm, are shown in Figure 4 as a function of the single correlation time. The experimental T_1 and NOE results alone indicate that the correlation time for the isotropic motion is about 10^{-9} s. However, consideration of only the T_1 and $T_{1\rho}^{off}$ data indicates that the correlation time is about 5×10^{-8} s. This inconsistency arises from the fact that T_1 goes through a minimum as a function of the correlation time as shown in Figure 4. The isotropic correlation time model is not adequate since the different experimental NMR relaxation results indicate different correlation times. The line width of poly(A) was less than 10 Hz in all instances and does not offer any useful information.

The apparent discrepancy between the results of the different NMR relaxation experiments can be resolved by the use of the model presented in the Theory section, which includes the bending of the molecule as well as rotation about internal bonds. The two correlation time model is consistent with all of the NMR results, permitting the determination of both correlation times. The correlation times for poly(A), summarized in Table V, indicate that the correlation time for internal motion τ_i is independent of temperature, while the correlation time τ_0 for the bending motion is strongly temperature dependent. The strong dependence of the slower motion correlation time on temperature in the range of 6–20 °C is consistent with the studies of Felsenfeld and co-workers,[17] who found that, while the optical density of poly(A) does not change appreciably over this temperature range ($T_m \sim 50$ °C), the average helix length and radius of gyration undergo dramatic changes. We note that the correlation time τ_0 for a single-stranded nucleic acid such as poly(A) may have a contri-

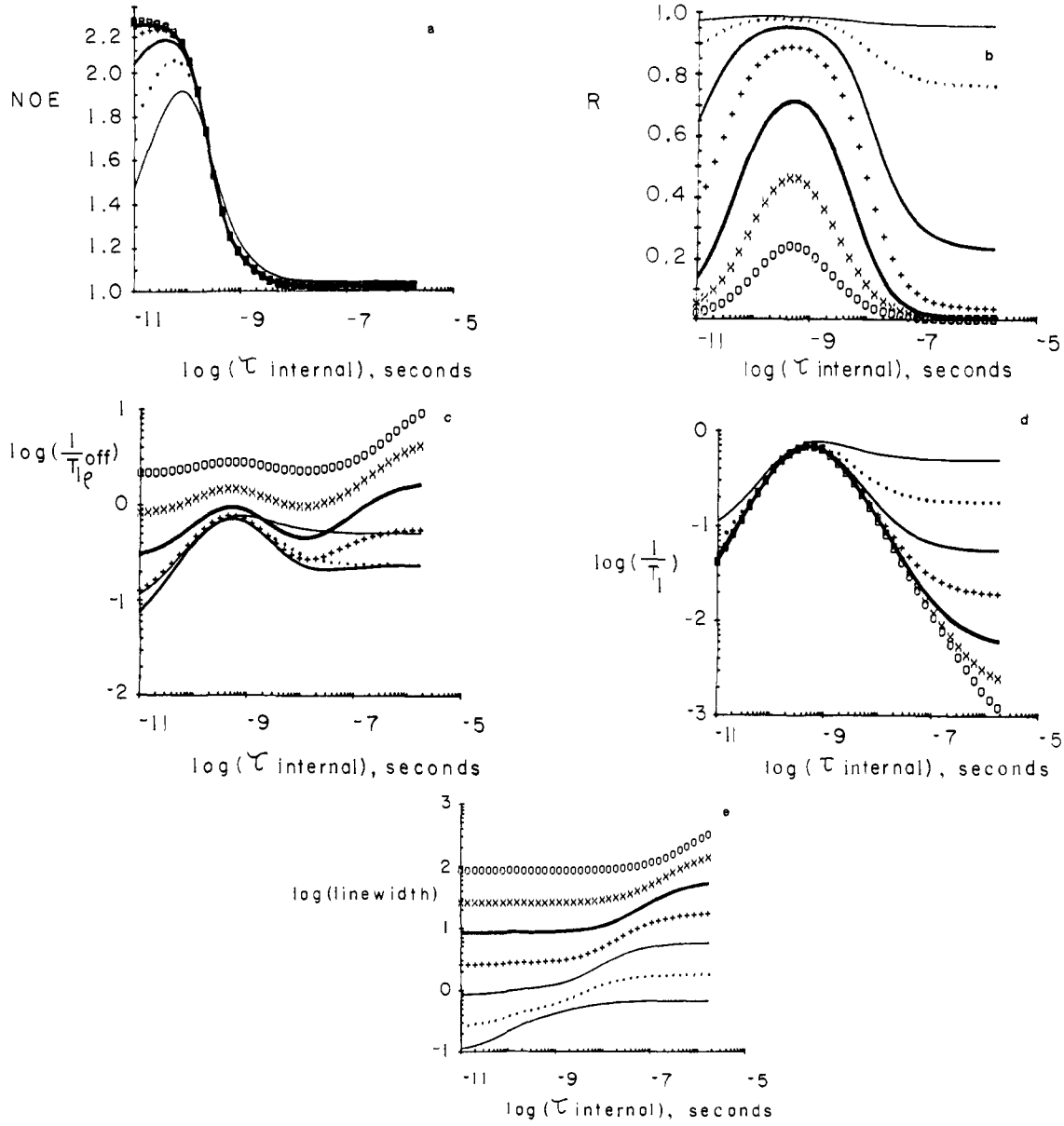

Figure 3. Theoretical values of the NMR relaxation parameters for phosphorus-31 at 40.5 MHz, calculated using the two correlation time model described in the text assuming that there are three protons 0.26 nm away from the phosphorus-31 and that the effective angle of internal rotation is 40°. The $T_{1\rho}^{off}$ and R curves were calculated assuming an off-resonance rf field of 0.47 G which is applied 8 kHz off-resonance. The curves are plotted as a function of the internal motion correlation time τ_i and are generated for bending motion correlation times τ_0 of 1×10^{-5} (○), 3×10^{-6} (×), 1×10^{-6} (■), 3×10^{-7} (+), 1×10^{-7} (▬), 3×10^{-8} (···), and 1×10^{-8} s (—).

bution from the overall rotational diffusion in addition to bending of the molecule.[17] The large decrease in the average helical length can be explained by consideration of the percent of the bases which are stacked at any temperature. At low temperature, the percent of bases stacked approaches 100%. With slight increases in temperature, the percent stacked will smoothly decrease as the melting of poly(A) is not cooperative. For example, the change in percent bases stacked from 99 to 95% decreases the average helical length from 99 to 19 bases but only changes the optical hypochromicity by 5%. Thus, we associate the large change in the slower motion correlation time of poly(A) with increasing temperature to the more rapid long-range bending of the molecule as well as to the more rapid rotational diffusion associated with the decrease in radius of gyration.

The ^{31}P NMR of poly(A) has been previously examined by Akasaka and co-workers,[18] and our T_1 and NOE results are in good agreement with theirs. On the basis of T_1 and NOE measurements, it was proposed that the mobility of poly(A) can be described in terms of a single isotropic correlation time due to local motions around the sugar phosphate backbone.[18] The additional information supplied by the $T_{1\rho}^{off}$ experiment necessitates the use of the two correlation time model discussed above. Recently Davanloo et al.[16] found that the correlation times for internal motion of oligodeoxynucleotides are on the order of nanoseconds based on ^{31}P NMR relaxation measurements. However, for such small molecules, T_1 and NOE data are not sufficient to distinguish between overall and internal motion.[16]

The NMR relaxation data for poly(I)-poly(C) were also interpreted in terms of a two correlation time model. For poly(I)-poly(C) the slower motion correlation time τ_0 is associated with the bending motion of the molecule and the internal motion correlation time τ_i is associated with rotational wobbling as presented in the Theory section. The correlation times based on the data in Table III are summarized in Table VI. It is seen that the correlation time for internal motion is nearly independent of temperature, whereas the correlation

Table III. ^{31}P NMR Relaxation Parameters of Poly(I)-Poly(C)[a] at 40.5 MHz

temp, °C	T_1, s	NOE	$\Delta\nu_{1/2}$, Hz	R^b	$T_{1\rho}^{off}$, s[b]
20	2.0	1.4	90	0.37	0.75
30	2.1	ND	65	0.45	0.95
40	2.1	1.37	50	0.52	1.04
20 + Mg^{2+} [c]	ND	1.42	135	0.2	0.4

[a] The sample contained poly(I)-poly(C) at a concentration of 20-40 mM in base pairs in 2 mM EDTA, 10 mM cacodylate, pH 7.0. [b] R and $T_{1\rho}^{off}$ were determined with an rf field of strength 0.47 G applied 8 kHz off-resonance. [c] Sample contained poly(I)-poly(C), 30 mM in base pairs, and 20 mM $MgCl_2$.

Table IV. ^{31}P NMR Relaxation Parameters of Calf Thymus DNA[a] at 40.5 MHz

temp, °C	T_1, s	NOE	$\Delta\nu_{1/2}$	R^b	$T_{1\rho}^{off}$, s[b]
20	2.5	1.56	95	0.37	0.9
40	2.3	1.6	45	0.53	1.2

[a] The sample contained sonicated calf thymus DNA (mol wt ~few million) at a concentration of 20-40 mM in 2 mM EDTA, 10 mM cacodylate, pH 7.0. [b] R and $T_{1\rho}^{off}$ were determined with an rf field of strength 0.47 G applied 8 kHz off-resonance.

Table V. Internal and Bending Motion Correlation Times of Poly(A) Determined from ^{31}P NMR Relaxation Data[a]

temp, °C	τ_0, ns	τ_i, ns
6	300	0.5
8	200	0.5
12	100	0.5
20	10	0.5
40	≤10	0.5

[a] The correlation times were determined using the mathematical model comprised of eq 1-12 with the relaxation data of Table II.

Table VI. Internal and Bending Motion Correlation Times of Poly(I)-Poly(C) Determined from ^{31}P NMR Relaxation Data[a]

temp, °C	τ_0, ns	τ_i, ns
20	1000	0.5
30	700	0.5
40	500	0.5
20, Mg^{2+}	3000	0.5

[a] The correlation times were calculated using the mathematical model comprised of eq 1-12 with the relaxation data of Table III. [b] The sample contained poly(I)-poly(C), 30 mM in base pairs, and 20 mM $MgCl_2$.

Table VII. Internal and Bending Motion Correlation Times of Calf Thymus DNA Determined from ^{31}P NMR Relaxation Data[a]

temp, °C	τ_0, ns	τ_i, ns
20	1000	0.3
40	500	0.3

[a] The correlation times were calculated using the mathematical model comprised of eq 1-12 with the relaxation data of Table IV.

time for bending motion changes by about an order of magnitude over the range of experimental conditions investigated. The temperature dependence of the bending motion correlation time can be associated with a decrease in the persistence length of poly(I)-poly(C) with temperature according to eq 13 as has been observed for DNA.[14] The temperature independence of the internal motion correlation time, the observation that the

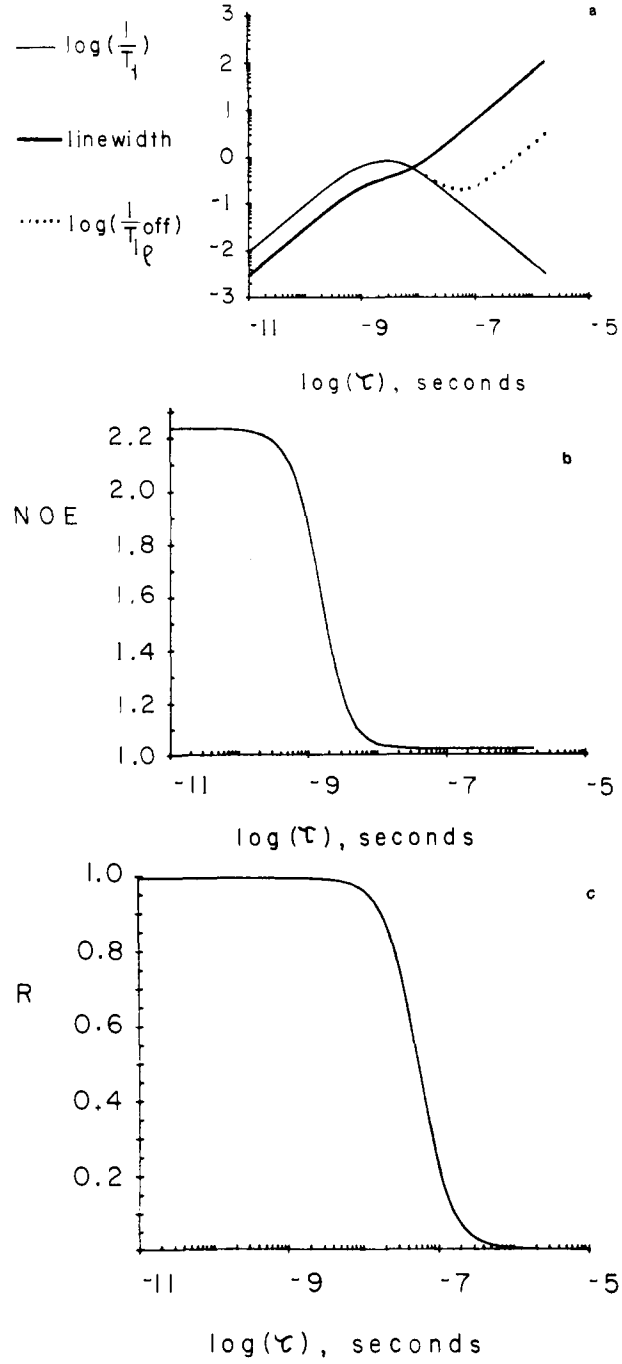

Figure 4. Theoretical values of the NMR relaxation parameters for phosphorus-31 at 40.5 MHz, calculated using the single correlation time model described in the text. The model assumes that there are three protons which are 0.26 nm away from the phosphorus. The $T_{1\rho}^{off}$ and R curves were calculated assuming an off-resonance field of 0.47 G applied 8 kHz off-resonance.

internal motion correlation time is about the same for poly(A) and poly(I)-poly(C), and the fact that the internal motion correlation time is less than 1 ns indicate that the internal motion is not strongly coupled to the conformation of the molecule in the large.

Magnesium ion is known to stabilize double-stranded polynucleotides to thermal denaturation, so we investigated the effect of magnesium ion on the NMR relaxation parameters of poly(I)-poly(C). The data (Table III) indicate that magnesium decreases the rate of the slower motion and has little or no effect on the rate of internal motion (see Table VI). This suggests that magnesium increases the effective persistence length of the poly(I)-poly(C) and, as proposed above,

the internal motions are not strongly coupled to the long-range conformational motion.

The correlation times observed for calf thymus DNA are very similar to those observed for poly(I)-poly(C), as indicated by the comparison of the correlation times listed in Tables VI and VII. The observation that the double-stranded RNA and DNA samples have about the same bending motion correlation times over the temperature range studied indicates that the persistence lengths and their temperature dependence are comparable for the two molecules. The similarity of the internal motion correlation times for poly(A), poly(I)-poly(C), and the calf thymus DNA is consistent with the notion that the internal motion is not influenced strongly by the conformation of the polynucleotide. It should be noted that the ^{31}P NMR data indicate that the internal motions of the polynucleotides investigated here are all about the same regardless of the method of interpreting the NMR data.

The ^{31}P NMR of calf thymus DNA was also examined at 81 MHz to investigate the contribution of the chemical shift anisotropy to the relaxation since the chemical shift anisotropy contribution to the relaxation is dependent on the square of the magnetic field strength, but the dipolar contribution is not (eq 1-4 and 14). The experimental and calculated T_1 and NOE parameters obtained at 40.5 and 81 MHz are compared in Table I. The close correspondence between the calculated and observed parameters indicates that the chemical shift anisotropy has been properly taken into account. It is noted that the contribution of the chemical shift anisotropy to the relaxation of the phosphorus-31 of DNA is about 35% at 81 MHz. This implies that future studies may take advantage of the high sensitivity of the wide-bore 4.7 T multinuclear spectrometers now available without serious loss of resolution or sensitivity due to chemical shift anisotropy contributions to the relaxation.

The NMR results can also be used to obtain some information about the kinetics of the water molecules which are hydrogen bonded to the phosphate groups of nucleic acids. Comparison of the ^{31}P NMR relaxation data obtained in 95% ^2H_2O with those obtained in 90% H_2O-10% ^2H_2O showed no significant difference in any of the relaxation parameters at 40.5 MHz (data not shown). This indicates that the water molecules do not contribute significantly to the relaxation of the phosphorus-31 even though there are at least four water molecules which are hydrogen bonded to the phosphate group. As the protons of the water molecules hydrogen bonded to the phosphate group are only about 0.28 nm away from the phosphorus nucleus, the lack of effect of the water protons on the phosphorus-31 relaxation needs to be explained. Exchange of the hydrogen-bonded water molecules with bulk solvent, $\tau \simeq 10^{-6}$ s, coupled with rapid reorientation of the water molecules, $\tau \simeq 10^{-10}$ s, would make the contribution of the water molecules to the relaxation of the phosphorus negligible when compared to that of the ribose protons. Thus, the NMR experiments can be used to obtain limits on the motion of the water molecules which are hydrogen bonded to the phosphate groups even though the water protons are not observed directly.

The correlation time for the overall motion of the double-stranded RNA and DNA molecules is approximately that expected from a consideration of the long-range bending of these molecules. Similarly, the overall motion of the single-stranded poly(A) is in good agreement with that predicted on the basis of its known hydrodynamic properties. However, the correlation time for the internal motion of the polynucleotides may, at first, appear to be unreasonably short.[22,23] The only other data which we know to bear directly on the rate of internal motion on the time scale of nanoseconds is the investigation of the depolarization of the dye ethidium intercalated between the base pairs of DNA. The data indicated that DNA possesses considerable internal motion on the time scale of nanoseconds.[7] The correlation time for reorientation in the backbone of a polynucleotide might be expected to be shorter than that for the reorientation of a dye intercalated between base pairs.

One of the objectives of this study was to determine if there are gross differences in the local mobility of RNA and DNA which can be attributed to the intramolecular water bridge between the 2′-OH and 3′-phosphate of RNA. The correlation times deduced from the NMR data, see Tables VI and VII, suggest that the internal motion correlation time for RNA is slightly longer than that for the DNA, which is consistent with some restriction of the local motion of RNA relative to DNA. However, the difference is so small that it cannot be interpreted in a meaningful way. It may be possible that examination of the motion of the ribose moiety RNA and DNA, via ^{13}C NMR relaxation experiments analogous to those described here, will allow the determination of the effect of the intramolecular water bridge, if any, on the internal motion of RNA.

The results of this study have offered some new information about the conformational mobility of RNA and DNA in solution and have also set the stage for the investigation of the conformational fluctuations of nucleic acids in such macrocomplexes as viruses, ribosomes, and chromosomes. The method is particularly well suited to large complexes with molecular weights of several million since for these cases contributions of the overall and internal motions to the relaxation of the phosphorus-31 are easily separable. Nucleosomes, for example, may be too small to allow separation of the overall and internal motion correlation times by the method used here. A preliminary report on the phosphorus-31 relaxation of ribosomes[19] has appeared, and the results appear to be consistent with the ribosomal RNA exhibiting an internal motion correlation time comparable to that observed for free RNA.[24]

Experimental Section

Poly(A), poly(I), and poly(C) were obtained from P-L Biochemicals. The NMR samples of poly(A) were prepared by dialysis against 0.1 M NaCl, 10 mM cacodylate, and 2 mm EDTA at pH 7.0. The sample was then lyophilized and reconstituted with either 99.8% ^2H_2O or with 10% ^2H_2O-90% H_2O. Samples of poly(I)-poly(C) were prepared by mixing stoichiometric amounts of the two homopolymers in the dialysis buffer used for poly(A) followed by dialysis against the same buffer. Calf thymus DNA was sonicated in high-salt-high-buffer solutions as described elsewhere[20] to minimize the formation of loose ends during sonication. After sonication the DNA was dialyzed against the same buffer as was used for poly(A). The molecular weight of the ribohomopolymers was greater than 100 000 and had a minimum $s_{20,w}$ of 7.2. The molecular weight of the sonicated calf thymus DNA is estimated to be a few million.[20] The NMR samples were 20-40 mM in base pairs poly(I)-poly(C) and calf thymus DNA, or in nucleotide units. No concentration dependence of the NMR relaxation parameters was observed for this range of concentration.

To establish that all phosphorus-31 nuclei in the polynucleotide samples contribute to the NMR signal, two tests were conducted. The intensity of the polynucleotide phosphorus-31 signal was compared with that of added phosphate buffer at 40.5 and 81 MHz. Also, the intensities of the polynucleotide signals at temperatures above and below the melting temperature were compared. Both tests indicated that all of the polynucleotide phosphorus-31 nuclei were contributing to the NMR peak.

The phosphorus-31 T_1 values were measured using the inversion recovery technique. The gated method was utilized for measuring the NOE using a waiting time of at least four T_1s.[21] The $T_{1\rho}^{off}$ experiments were performed as described previously.[9] Line widths were measured directly from plots of the spectra or by use of a Lorentzian line-fitting program. The ^{31}P NMR experiments were performed with 12-mm tubes at 40.5 MHz using an XL-100-15 spectrometer coupled to a Nicolet TT-100 computer system and with 20-mm tubes at 81 MHz using a Nicolet 200 wide-bore NMR spectrometer.

Calculations of the relaxation parameters were performed using the PROPHET computer system with associated graphics. The figures

illustrating the dependence of the relaxation parameters on the correlation times utilize curves generated by the computer system.

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Solution Characterization of "Intermediate-Spin" Iron(III) Porphyrins by NMR Spectroscopy

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Abstract: ^1H and ^{13}C NMR spectra of iron(III) porphyrin perchlorate derivatives in noncoordinating solvents are anomalous with respect to the isotropic shift patterns observed for other iron(III) porphyrin species. Both synthetic and natural-derivative iron(III) porphyrins have been examined. Variation of solution conditions rule out chemical equilibria as an explanation for the unusual behavior. The perchlorate ion remains coordinated or tightly ion paired in solution. The measurements support earlier solid-state work which suggests a quantum mechanically admixed $S = 3/2$, $S = 5/2$ spin state formulation. The unperturbed quartet state is of lower energy. Magnetic anisotropy for the $S = 3/2$ state is significant with $\chi_\perp > \chi_\parallel$. The $d_{x^2-y^2}$ orbital is vacant, and the most likely formal ground state for the unperturbed $S = 3/2$ species is $(d_{xy})^2 (d_{z^2})^1 (d_{xz}, d_{yz})^2$. Changes in isotropic shift patterns and magnetic moments with temperature presumably reflect thermal population of Kramer's doublets with varying amounts of $S = 3/2$ and $S = 5/2$ character. Solvent-dependence studies show that aromatic solvents increase the $S = 5/2$ character as compared with chlorinated solvents. Solvent perturbation results may be relevant to the chemistry of cytochrome *c'*, which is also believed to exhibit quantum mechanical spin admixture.

Introduction

Preparation and physical characterization of metalloporphyrins in all possible spin and oxidation states constitutes a major effort among bioinorganic chemists. Biologically relevant iron(II) and iron(III) porphyrin complexes in both high- and low-spin states have received the most attention in this regard.[1] Experimental work[2-4] has also confirmed the theoretical prediction[5] of intermediate-spin $S = 1$ iron(II) porphyrins. Generation of this spin state demands a square-planar environment (no axial ligands) in which a vacant $d_{x^2-y^2}$ orbital is raised in energy and an occupied d_{z^2} orbital is stabilized. Intermediate-spin iron(II) phthalocyanines[6] and macrocyclic complexes[7] are also recognized.

Intermediate-spin $S = 3/2$ character in ferric cytochrome *c'* has been invoked by Maltempo to explain unusual ESR properties and a decrease in magnetic moment at low temperature for this bacterial hemoprotein.[8,9] A *quantum-mechanical* admixture of $S = 3/2$ and $S = 5/2$ states is proposed. This formulation is to be distinguished from a *thermal mixture* of magnetically distinct $S = 3/2$ and $S = 5/2$ states. Quantum-mechanical admixing is presumably the result of a small energy separation between pure spin states. A spin–orbit coupling constant of comparable energy ($\sim$300 cm^{-1}) provides a direct mechanism for interaction of the two spin systems. Suggestion of intermediate-spin involvement is not unreasonable in view of literature descriptions of $S = 3/2$ bis(*N*,*N*-dialkyldithiocar-

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