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Nuclear Magnetic Resonance Relaxation in Nucleic Acid Fragments: Models for Internal Motion[†]

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ABSTRACT: A variety of models incorporating internal motion, which can be used to extract information from nuclear magnetic resonance relaxation studies of deoxyribonucleic acid fragments, are formulated. Illustrative analyses of some recent multinuclear relaxation data are presented. Special emphasis is placed on determining whether the information extracted is unique. It is shown that the data are consistent with several physical pictures of the internal motion. However, all the models we have considered imply the existence of large-amplitude internal motions on the nanosecond time scale.

Nuclear magnetic resonance (NMR)[1] is an important technique for probing molecular motions. Recently, a large number of ^{1}H, ^{31}P, and/or ^{13}C NMR relaxation studies of DNA have appeared in the literature (Bolton & James, 1979, 1980a,b; Early & Kearns, 1979; Hogan & Jardetzky, 1979, 1980; Klevan et al., 1979; Shindo, 1980). There appears to be agreement among several groups that large-amplitude internal motions on the nanosecond time scale are present in DNA. This conclusion was extracted from the data by using highly idealized models of the internal motion (e.g., free internal rotation about an axis or two-site jumps within a plane containing the long axis of the helix). We have set out to establish whether these conclusions hold within the framework of somewhat more sophisticated models of the internal motion and whether the currently available data lead to a unique physical picture of the internal motions. In the course of this work, we found a mathematical error in the interesting work of Hogan & Jardetzky (1979, 1980) which is corrected here.

Following Hogan & Jardetzky (1979, 1980), we assume that the overall reorientation of a relatively short DNA fragment is the same as that of a freely diffusing cylinder. Motions relative to a reference frame rigidly attached to the cylinder are considered to be internal motions. Using electric dichroism, it has been shown (Hogan et al., 1978) that DNA fragments with 100–250 base pairs behave like rods to a good approximation. However, there is evidence from computer simulations (Olson, 1980) that fragments at the upper end of the above range can deviate significantly from a rodlike shape. We do not consider such bending motions although their possible influence is implicitly mimicked by our models of internal motion.

In this paper, we formulate a variety of models for internal motion. First, we consider twisting or torsional motions (using both square-well and harmonic potentials) in which the polar angle between the relevant interaction vector and the long axis of the helix remains fixed. Second, we consider wobbling motions in which the interaction vector diffuses in a cone about a director which forms a fixed angle with the long axis of the helix. Finally, we consider jump models and explicitly formulate the most general form of the two-state jump model in which the interaction vector jumps between two arbitrary and energetically inequivalent positions. We explicitly state the assumptions under which our exact treatment degenerates into

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[1] Abbreviations used: NMR, nuclear magnetic resonance; DNA, deoxyribonucleic acid; NOE, nuclear Overhauser effect.

the approximate formulation of Hogan & Jardetzky (1979, 1980) and correct a mathematical error in one of their formulas. The latter part of this paper deals with a thorough reanalysis of the data of Hogan & Jardetzky (1980) within the framework of some of the above models, placing special emphasis on whether their data can be used to eliminate certain models.

Theory

Relaxation due to dipole–dipole interaction between two nuclei can be described with the correlation function (Wallach, 1967; Wittebort & Szabo, 1978)

$$C(t) = \langle D_{qo}^{(2)*}[\Omega_{\mathrm{LF}}(0)]D_{qo}^{(2)}[\Omega_{\mathrm{LF}}(t)]\rangle \tag{1}$$

where $D_{mn}^{(2)}(\Omega)$ is a Wigner rotation matrix element (Brink & Satchler, 1961) and the Euler angles, Ω_{LF}, specify the orientation of the unit vector ($\hat{\mu}$) connecting the two nuclei in the laboratory reference frame. This correlation function also describes quadrupolar and chemical shift anisotropy relaxation in the special case where the relevant tensors are axially symmetric. The relaxation times T_1 and T_2 and, for dipolar relaxation, the NOE, are expressed in terms of the spectral density

$$J(\omega) = 2\int_0^\infty C(t)\cos\omega t\,\mathrm{d}t \tag{2}$$

in Table I of Wittebort & Szabo (1978). If several relaxation mechanisms are operative or if several dipole–dipole interactions contribute to the relaxation of a given nucleus, then

$$(T_{1,2})^{-1} = \sum_k (T_{1,2}^{(k)})^{-1} \tag{3}$$

With the exception of jump models, we explicitly consider relaxation due only to fluctuations of the orientation of the interaction vector (i.e., we assume internuclear distances remain fixed). This restriction is particularly severe for dipolar ^{31}P relaxation, where it is likely that internal motions involve changes in the phosphorus–hydrogen distances.

We now consider the relaxation of a nucleus in a nucleic acid fragment. We assume that the overall motion of the fragment is the same as that of a freely diffusing cylinder. Motion relative to the reference frame which is rigidly attached to the cylinder is considered as *internal* motion. Assuming that the overall and internal motions are independent, we have (Wittebort & Szabo, 1978)

$$C(t) = \frac{1}{5}\sum_{b=-2}^{2}\exp\{-[6D_\perp + b^2(D_\parallel - D_\perp)]t\}C_b(t) \tag{4}$$

with

$$C_b(t) = \langle D_{bo}^{(2)*}[\Omega_{\mathrm{MF}}(0)]D_{bo}^{(2)}[\Omega_{\mathrm{MF}}(t)]\rangle \tag{5}$$

where Ω_{MF} specifies the orientation of $\hat{\mu}$ in the frame attached to the cylinder and $D_\parallel$ and $D_\perp$ are the diffusion constants *about* the long and short axes of the cylinder, respectively. In eq 4, terms with $b = \pm 1$ and with $b = \pm 2$ are identical.

Before modelling the correlation function which describes internal motions, $C_b(t)$, we consider the influence of such motions on the line width [$\Delta\nu_{1/2} = (\pi T_2)^{-1}$] of a nucleus in a long cylinder from a model-free point of view. For a long cylinder, the slowest motion is reorientation about the short axis ($D_\parallel \gg D_\perp$), and thus, we need to examine the term proportional to $\exp(-6D_\perp t)$ in eq 4. Assuming that internal motions are much faster than overall motions, we have

$$C(t) \simeq S^2 e^{-6D_\perp t} \tag{6}$$

where

$$\begin{aligned} S^2 &= \lim\langle D_{oo}^{(2)*}[\Omega_{\mathrm{MF}}(0)]D_{oo}^{(2)}[\Omega_{\mathrm{MF}}(t)]\rangle \\ &= \langle|D_{oo}^{(2)}(\Omega_{\mathrm{MF}})|^2\rangle \\ &= \langle P_2(\cos\beta_{\mathrm{MF}})\rangle^2 \end{aligned} \tag{7}$$

where $P_2(x)$ is the second-order Legendre polynomial. The equilibrium average is

$$\langle(\cdots)\rangle = \int(\cdots)p_{\mathrm{eq}}(\Omega)\,\mathrm{d}\Omega \tag{8}$$

where $p_{\mathrm{eq}}(\Omega)$ is the normalized equilibrium orientational distribution of $\hat{\mu}$. By use of eq 6, an estimate of the contribution to the line width due to the slow motion of the long axis is

$$\Delta\nu_{1/2} \simeq (\text{constant})S^2/D_\perp \tag{9}$$

Thus, the slow motion of the long axis is not reflected in the line width when S is small.

We note that S is the *order parameter* describing the orientation of $\hat{\mu}$ with respect to the long axis of the cylinder. The order parameter has long played an important role in the analysis of magnetic resonance studies of membranes (Bocian & Chan, 1978). The order parameter of a probe in a membrane is a measure of the degree of alignment of the probe. For a cylindrical probe whose orientation is frozen in such a way that its long axis is perpendicular to the plane of the membrane, the order parameter is unity. Deviations from unity imply that the probe can assume a variety of orientations. For the case at hand, the situation is different. If $\hat{\mu}$ forms a fixed angle β_{MF} with the long axis of the cylinder, we have

$$S = P_2(\cos\beta_{\mathrm{MF}}) \tag{10}$$

Note that S is 0 when β_{MF} is equal to the "magic" angle (54.7°). Thus, the contribution to the line width due to the slow reorientation about the short axis of the probe can vanish even if there is no internal motion. In the presence of internal motion, S can, of course, also vanish. For future reference, we present expressions for S within two models for internal motion. First, suppose $\hat{\mu}$ jumps between sites 1 and 2 with orientations $\Omega_1 = (\theta_1,\phi_1)$ and $\Omega_2 = (\theta_2,\phi_2)$, respectively. If the equilibrium probability of finding $\hat{\mu}$ in state 1 (2) is $p_{\mathrm{eq}}(1)$ [$p_{\mathrm{eq}}(2) = 1 - p_{\mathrm{eq}}(1)$], then

$$S = p_{\mathrm{eq}}(1)P_2(\cos\theta_1) + p_{\mathrm{eq}}(2)P_2(\cos\theta_2) \tag{11}$$

In the special case where $p_{\mathrm{eq}}(1) = p_{\mathrm{eq}}(2) = {}^1/_2$, we have

$$S = \frac{1}{4}(1 + 3\cos 2\bar{\theta}\cos\Delta) \tag{12}$$

where $\bar{\theta} = (\theta_1 + \theta_2)/2$ and $\Delta = \theta_1 - \theta_2$. Second, suppose $\hat{\mu}$ diffuses in a cone of semiangle θ_0 about a director which forms a fixed angle β_{MD} with the long axis of the cylinder. Then we have

$$S = P_2(\cos\beta_{\mathrm{MD}})S_{\mathrm{cone}} \tag{13}$$

where

$$S_{\mathrm{cone}} = \frac{1}{2}\cos\theta_0(1 + \cos\theta_0) \tag{14}$$

We will now describe a variety of models for internal motion. For the sake of brevity, only the results will be presented. To make contact with some previous work, we begin by considering the case where there is no internal motion.

No Internal Motion. If $\hat{\mu}$ makes a fixed angle β_{MF} with the long axis of the cylinder, eq 5 becomes

$$C_b(t) = [d_{bo}^{(2)}(\beta_{\mathrm{MF}})]^2 \tag{15}$$

so that

$$C(t) = \frac{1}{5}\sum_{b=-2}^{2} \exp\{-[6D_\perp + b^2(D_\| - D_\perp)]t\}[d_{bo}^{(2)}(\beta_{MF})]^2 \tag{16}$$

which is equivalent to Woessner's (1962a) result for a freely diffusing ellipsoid. The angle β_{MF} can be estimated from the X-ray structure, and, for nucleic acid fragments of well-defined length, $D_\perp$ and $D_\|$ can be calculated from hydrodynamic expressions derived for an ellipsoid [see, for example, Woessner (1962a)] or for a cylinder [see, for example, Barkley & Zimm (1979)]. If the relaxation times calculated in this way agree poorly with those determined from experiment, one concludes that bending, twisting, and/or internal motions are present. One approach (Early & Kearns, 1979) to treating such situations is to vary the effective length of the fragment (i.e., vary $D_\perp$ and $D_\|$) so as to fit the data. The difference between the hydrodynamic length obtained in this way and the length calculated from the number of base pairs is taken as a reflection of the degree of internal flexibility.

Bolton & James (1979, 1980a,b) and Klevan et al. (1979) have analyzed their NMR data on very long DNA fragments, using a model (Woessner, 1962b) in which a single internal rotation is superimposed on isotropic reorientation. Specifically, $\hat{\mu}$ freely diffuses, at constant β_{MF} with diffusion constant D_i, around an axis which in turn isotropically reorients with diffusion constant D_0. The correlation function for this model is identical with the result for a freely diffusing ellipsoid (eq 16) if one identifies $D_\perp$ with D_0 and $D_\| - D_\perp$ with D_i. The assumption of free internal rotation is clearly not reasonable from the structural point of view.

Internal Motions at Constant β_{MF} (Twisting). We now turn to the class of internal motions in which the angle between $\hat{\mu}$ and the long axis of the cylinder remains constant but the azimuthal angle, ϕ, fluctuates. In this case, eq 5 becomes

$$C_b(t) = [d_{bo}^{(2)}(\beta_{MF})]^2\langle e^{ib\varphi(0)}e^{-ib\varphi(t)}\rangle \tag{17}$$

For free diffusion (with diffusion constant D), the azimuthal correlation function is (Wallach, 1967)

$$\langle e^{ib\varphi(0)}e^{-ib\varphi(t)}\rangle = e^{-b^2Dt} \tag{18}$$

With this result, the total correlation function (eq 4) is identical with eq 16 when $D_\|$ is replaced by $D_\| + D$, as to be expected from physical considerations. The azimuthal correlation function can be obtained for a variety of models involving restricted diffusive motion. For motion in a square well, i.e.

$$p_{eq}(\varphi) = (2\varphi_0)^{-1} \qquad -\varphi_0 \le \varphi \le \varphi_0$$
$$= 0 \text{ otherwise}$$

the azimuthal correlation function has been given by Wittebort & Szabo (1978) and by London & Avitabile (1978). For motion in a harmonic well, i.e.

$$p_{eq}(\varphi) = [\omega^2/(2\pi)]^{1/2}e^{-(1/2)\omega^2\varphi^2}$$

it can be shown that

$$\langle e^{ib\varphi(0)}e^{-ib\varphi(t)}\rangle = \exp\left[-\frac{b^2}{\omega^2}(1 - e^{-D\omega^2t})\right] \tag{19}$$

We note that when $\omega \rightarrow 0$ (i.e., the angular force constant vanishes), eq 19 reduces to the free diffusion result in eq 18.

Wobbling in a Cone. We consider the model shown in Figure 1. In this model, $\hat{\mu}$ diffuses, with diffusion constant D_w, in a cone of semiangle θ_0 about a director, $\vec{d}$, which forms a fixed angle β_{MD} with the long axis of the cylinder. The motion of $\hat{\mu}$ is azimuthally unrestricted about $\vec{d}$. The correlation function for internal motions is well approximated by (Lipari & Szabo, 1981)

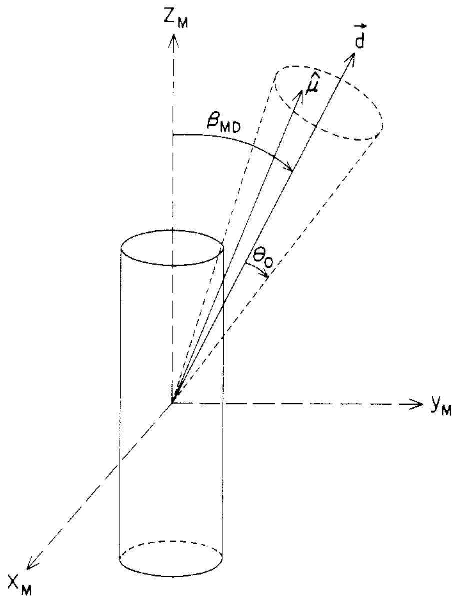

FIGURE 1: Diffusion in a cone model.

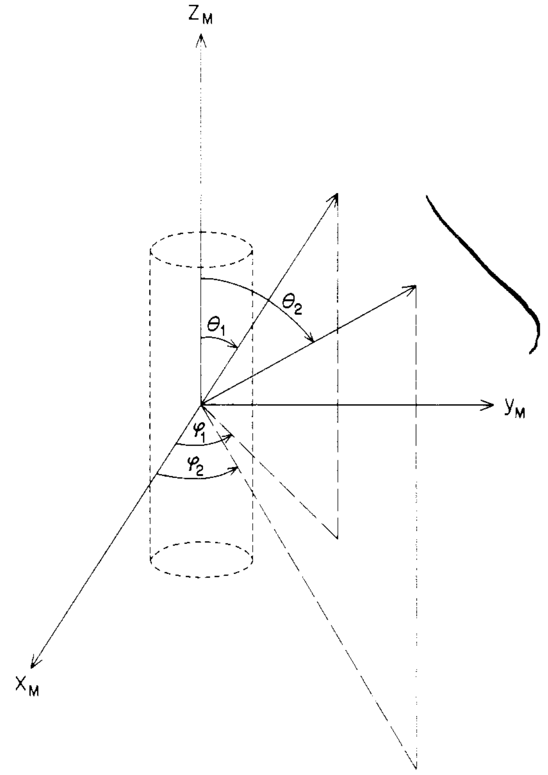

FIGURE 2: Two-state jump model.

$$C_b(t) = \sum_{c=-2}^{2}[d_{bc}^{(2)}(\beta_{MD})]^2\{G_c(\infty) + [G_c(0) - G_c(\infty)]e^{-t/\tau_{eff}^{(c)}}\} \tag{20}$$

Simple closed-form expressions of $G_c(\infty)$, $G_c(0)$, and $\tau_{eff}^{(c)}$ in terms of θ_0 and D_w are given by Lipari & Szabo (1981).

Two-State Jump Model. Suppose $\hat{\mu}$ jumps among N nonequivalent sites. The orientation of $\hat{\mu}$ in state i is specified by $\Omega_i = (\theta_i,\phi_i)$, and the probability that at equilibrium this state is realized is $p_{eq}(i)$. Wittebort & Szabo (1978) showed how the appropriate correlation function can be constructed from the eigenvalues and eigenvectors of an $N \times N$ *symmetric* matrix formed from the rate matrix. The case $N = 2$ is shown in Figure 2. If the rates of interconversion of the two states are defined as

$$(1) \underset{k_r}{\overset{k_f}{\rightleftharpoons}} (2)$$

then

$$p_{eq}(1) = \frac{k_r}{k_f + k_r} \qquad p_{eq}(2) = \frac{k_f}{k_r + k_f}$$

and the correlation function for internal motions which is exact within the model, $C_b(t)$, is given by

$$C_b(t) = C_b(\infty) + [C_b(0) - C_b(\infty)]e^{-t/\tau_{\mathrm{int}}} \tag{21}$$

where

$$\tau_{\mathrm{int}} = (k_r + k_f)^{-1} \tag{22}$$

and

$$C_b(\infty) = |\langle D_{bo}^{(2)}(\Omega_{\mathrm{MF}})\rangle|^2 = \{p_{eq}^2(1)[d_{bo}^{(2)}(\theta_1)]^2 + p_{eq}^2(2) \times [d_{bo}^{(2)}(\theta_2)]^2 + 2p_{eq}(1)p_{eq}(2)d_{bo}^{(2)}(\theta_1)d_{bo}^{(2)}(\theta_2) \cos b(\varphi_1 - \varphi_2)\} \tag{23}$$

and

$$C_b(0) = \langle|D_{bo}^{(2)}(\Omega_{\mathrm{MF}})|^2\rangle = p_{eq}(1)[d_{bo}^{(2)}(\theta_1)]^2 + p_{eq}(2)[d_{bo}^{(2)}(\theta_2)]^2 \tag{24}$$

We note that $C_0(\infty)$ is simply the square of the *order parameter* of $\hat{\mu}$, i.e.

$$C_0(\infty) = S^2 \tag{25}$$

where S is given by eq 11 and, in the special case that $p_{eq}(1) = p_{eq}(2) = {}^1/_2$, by eq 12.

In contrast to diffusive models, the jump formalism easily handles the contribution to relaxation due to fluctuations in the *magnitude* of the interaction vector (i.e., the internuclear distance in state i is r_i). For example, eq 23 and 24 can be generalized by simply replacing $d_{bo}^{(2)}(\theta_i)$ by $d_{bo}^{(2)}(\theta_i)/r_i^3$, $i = 1, 2$.

Connection with Hogan & Jardetzky (1979, 1980). Recently, Hogan & Jardetzky (1979, 1980) considered the two-state jump model in the special case that $p_{eq}(1) = p_{eq}(2)$ and $\phi_1 = \phi_2$. Hogan & Jardetzky (1979) present approximations for T_1^{-1} and T_2^{-1} which supposedly hold when $\tau_{\mathrm{int}}^{-1} > D_{\|} \gg D_{\perp}$ and when the measured line widths are too small to be consistent with overall motion alone. Here, we examine the approximations inherent in their expressions in more detail and show that their expression for a parameter they call α (which turns out to be S^2 in our terminology) is *incorrect*. If we assume (1) that $\tau_{\mathrm{int}}^{-1} > D_{\|} \gg D_{\perp}$ and (2) that

$$\sum_{\substack{b=-2\\ b\neq 0}}^{2} C_b(\infty)e^{-b^2D\|t} = e^{-6D\|t}\sum_{\substack{b=-2\\ b\neq 0}}^{2} C_b(\infty) \tag{26}$$

our expression for $C(t)$ reduces to

$$C(t) = \frac{1}{5}[S^2e^{-t/\tau_{\mathrm{L}}} + (1 - S^2 - \beta)e^{-t/\tau_{\mathrm{S}}} + \beta e^{-t/\tau_{\mathrm{int}}}] \tag{27}$$

where we have defined

$$\tau_{\mathrm{L}} = (6D_{\perp})^{-1} \tag{28a}$$

$$\tau_{\mathrm{S}} = (6D_{\|})^{-1} \tag{28b}$$

and where we have shown that

$$\beta = \frac{3}{4}\sin^2\Delta \tag{29}$$

with $\Delta = \theta_1 - \theta_2$ and S given by eq 12. The "decoupling" approximation shown in eq 26 is not expected to be accurate (e.g., the factor 6 multiplying $D_{\|}$ on the right-hand side is clearly not optimal).

Using eq 27, we make our final approximation (3) by neglecting the term involving τ_{int} in the calculation of T_2 and by neglecting the term involving τ_{L} in the calculation of T_1. In this way we find

$$\frac{1}{T_1} = \frac{\beta}{T_1(\tau_{\mathrm{int}})} + \frac{(1 - \beta - S^2)}{T_1(\tau_{\mathrm{S}})} \tag{30}$$

$$\frac{1}{T_2} = \frac{S_2}{T_2(\tau_{\mathrm{L}})} + \frac{(1 - \beta - S^2)}{T_2(\tau_{\mathrm{S}})} \tag{31}$$

where $T_{1,2}(\tau)$ is the relaxation time calculated by using a single exponential correlation function of the form $5^{-1}\exp(-t/\tau)$.

Equations 30 and 31 are formally identical with the results of Hogan and Jardetzky provided we identify our β with their β and our S^2 with their α

$$\alpha = S^2 = \frac{1}{16}(1 + 3\cos 2\bar{\theta}\cos\Delta)^2 \tag{32}$$

where $\bar{\theta} = (\theta_1 - \theta_2)/2$ and $\Delta = \theta_1 - \theta_2$ (see eq 12). Our expression for β given in eq 29 agrees with their result. Unfortunately, this is not the case for α. Hogan & Jardetzky (1979, 1980) give

$$\alpha_{\mathrm{HJ}} = \frac{1}{16}(1 + 3\cos^2\bar{\theta}\cos\Delta) \tag{33}$$

which is clearly different from our result in eq 32. We thought that the difference between the two expressions was a result of misprints in their two papers, but this is not the case (O. Jardetzky, private communication).

Their incorrect expression for α does not negate their analysis of data *within the framework of the approximate* eq 30 and 31 because they treated α and β directly as adjustable parameters. It only prevented them from giving an interpretation of the fitted α and β within the jump model. For example, Hogan & Jardetzky (1980) fitted data with $\alpha < 0.05$ and even $\alpha = 0$ although for $\Delta < 90°$ the minimum value of α_{HJ} is 0.0625.

However, their data analysis within the framework of the two-state jump model clearly depends on the validity of the approximations 1–3 used to obtain eq 30 and 31. As we shall see below, eq 30 and 31 sometimes work (i.e., they give the same result as those obtained by using the exact formulation of the model), and sometimes they do not [e.g., when α (or S^2) is close to zero, the term involving τ_{S} in eq 31 makes the dominant contribution to T_2, and the consequences of the decoupling approximation in eq 26 are particularly severe]. Since T_1 and T_2 within the two-state jump model can be evaluated exactly almost as easily as they can be calculated approximately, it does not appear worthwhile to try to improve upon eq 30 and 31 or use them to fit data. Finally, we comment on the statement made by these authors in their second paper (Hogan & Jardetzky, 1980), namely, that eq 30 and 31, with α (S^2) and β as adjustable parameters, are valid for all models of the internal motion as long as the motion is described by a single correlation time. Since eq 30 and 31 are based on the decoupling approximation of eq 26 [which rigorously holds only in the uninteresting case that $C_b(\infty) = 0$ for $b = \pm 2, \pm 1$], their statement is inaccurate.

Illustrative Applications

In this section, we analyze, within the framework of the models presented above, the multinuclear relaxation data re-

Table I: ^{1}H Relaxation Data

spectrometer frequency (MHz)	τ_L[a] (μs)	τ_S[a] (μs)	T_1 obsd[a] (s)	T_1 calcd[b] (s)	T_1 calcd[c] (s)	T_1 calcd[d] (s)	T_2 obsd[a] (ms)	T_2 calcd[b] (ms)	T_2 calcd[c] (ms)	T_2 calcd[d] (ms)
360	10	0.1	1.0	1.06	1.06	1.08	3.5	2.44	3.73	3.78
100	10	0.1	0.130	0.128	0.128	0.122	5.0	2.40	3.73	3.67
360	7	0.07	1.1	1.06	1.06	1.08	5.0	3.46	5.32	5.22
360	4	0.04	1.1	1.05	1.05	1.07	8.0	5.92	9.30	8.46

[a] From Hogan & Jardetzky (1980). [b] Calculated by using the jump model with $\Delta\phi = 0, p_{eq}(1) = 1/2, \Delta = 71°, \bar{\theta} = 90°$, and $\tau_{int} = 1.4 \times 10^{-9}$ s. [c] Calculated by using the approximate eq 30 and 31 for the jump model, with the same parameters as in *b*. [d] Calculated by using the cone model with $\beta_{MD} = 55°, \theta_0 = 67°$, and $1/(6D_w) = 1.9 \times 10^{-9}$ s.

cently obtained by Hogan & Jardetzky (1980) for DNA fragments. These authors analyzed their data by using an approximate formulation (eq 30 and 31) of the two-state jump model in the special case that $p_{eq}(1) = p_{eq}(2)$ and $\phi_1 = \phi_2$. In this section, we wish to (1) investigate whether the experimental data can eliminate some model, (2) study the possibility of describing the data by using several models, (3) establish, for a given model, whether a unique set of model parameters can reproduce the data, (4) determine what kind of physical picture (if any) of the internal motions can be extracted from the available data alone, and (5) establish to what extent the results of the analysis of Hogan & Jardetzky (1980) depend on the fact that they used an approximate formulation of the two-state jump model.

^{1}H Relaxation Data. We consider here the data measured for band V, assigned to the protons attached to carbon C2′, in the pentose ring. The relaxation measurements were obtained for the 260 base pair long fraction of DNA fragments at two different resonance frequencies (100 and 360 MHz) and various temperatures. Following Hogan & Jardetzky (1980), we assume that the major contribution to the relaxation is due to the dipolar interaction between adjacent methylene hydrogens $H2'_1$ and $H2'_2$, that is, we neglect coupling between the 2′ protons and the protons attached to neighboring carbons 1′ (H1′) and 3′ (H3′). First, we use the two-state jump model with $r(H2'_1\text{-}H2'_2) = 1.78$ Å, τ_L and τ_S as in Hogan & Jardetzky (1980). This model contains five adjustable parameters: θ_1, θ_2 [or $\bar{\theta} = (\theta_1 + \theta_2)/2$, $\Delta = \theta_1 - \theta_2$], τ_{int}, $\Delta\phi$ ($=\phi_1 - \phi_2$), and $p_{eq}(1)$. If $\Delta\phi = 0$ and $p_{eq}(1) = {}^1/_2$, the two-state jump model reduces to the special case considered by Hogan & Jardetzky (1979, 1980). The best fit to the experimental values gives for this case (see Table I) $\Delta = 71°$, $\bar{\theta} = 90°$, and $\tau_{int} = 1.4 \times 10^{-9}$ s. It is interesting to note that the approximations of eq 30 and 31 with $S^2 = 2.3 \times 10^{-5}$ and $\beta = 0.67$ corresponding to these angles accurately reproduce the predicted values of T_1 but overestimate by more than 50% the values of T_2. Because of this artifact, the relaxation times calculated by using the approximate solution to the model are actually closer to the measured values. For this set of relaxation data, however, the model parameters extracted from the fitting procedure by using the exact solution and the approximate solution agree. As it will be shown below in the discussion of the ^{13}C data, this is not always the case.

We then investigated the uniqueness of the model parameters extracted from the fitting procedure. If we keep $\Delta\phi$ fixed at 0° and $p_{eq}(1)$ at $^1/_2$, there is no other set of values of τ_{int}, Δ, and $\bar{\theta}$ that reproduces the experimental data with reasonable accuracy. For $\Delta\phi = 0$, the two-state jump model describes motions of the dipole vector in a plane parallel to the long axis of the helix. Intuitively, we would expect the $H2'_1$-$H2'_2$ vector to fluctuate about its equilibrium position. However the value of $\bar{\theta}$ extracted is 90°, vs. 56.5° calculated from the X-ray structure of Arnott et al. (1969), assuming an ideal tetrahedral geometry.

We then performed a series of calculations by varying $\Delta\phi$ along with $\bar{\theta}$, Δ, and τ_{int}. Motions of small amplitude in $\Delta\phi$ ($0 < \Delta\phi < 45°$) do not substantially modify the results obtained for $\Delta\phi = 0$. In the range $50° < \Delta\phi < 130°$, the jump model can reproduce the T_1 values but fails to predict correctly the T_2 data. We can reproduce the data as accurately as in the best fit if $140° < \Delta\phi < 180°$, $\Delta \approx 0$, and $\bar{\theta} \approx 55°$. If we also allow the equilibrium populations $p_{eq}(1)$ to vary, there exists a whole new class of solutions comparable to the best fit for $\Delta\phi = 0$ and $p_{eq}(1) = {}^1/_2$. For example, if $\Delta = 90°$, $\Delta\phi = 90°$, $p_{eq}(1) = 0.34$, $\bar{\theta} = 45°$, and $\tau_{int} = 1.42 \times 10^{-9}$ s, we obtain the same NMR relaxation times as those in Table I. In summary, within the jump model, we can fit the experimental data for several sets of values of the model parameters. Two features are shared by virtually all these solutions: (i) there is an internal motion on a time scale of $\sim 10^{-9}$ s; (ii) motions have a large amplitude. The nature of these motions, however, is not uniquely determined, since substantial fluctuations in ϕ or in θ reproduce the experimental data equally well.

To ensure that large, fast motions of this nature are not an artifact of the two-state jump model, we analyzed the data by using the wobbling in a cone model. The best fit (see Table I) gives $\beta_{MD} = 55°$, $1/(6D_w) = 1.8 \times 10^{-9}$ s, and $\theta_0 = 67.5°$. It is interesting to note that in the cone model the equilibrium orientation angle of the H-H vector extracted from the fitting procedure is very close to the value (56.5°) that we have calculated from the X-ray structure of Arnott et al. (1969). However, there is a whole class of solutions for values of β_{MD}, ranging from 0 to 90°, that reproduce the experimental relaxation times. The values of θ_0 range from 67 to 97°, and $1/(6D_w)$ is between 1.7 and 1.8 ns. Thus, even the cone model predicts fast motions on a time scale of 10^{-9} s and large amplitudes. The angular fluctuations in θ, however, are much bigger (by about a factor of 2) than those predicted by the jump model for $\Delta\phi = 0$ ($\Delta/2 = 35°$).

It appears that the conclusion that there are large-amplitude motions on the 1-ns time scale can be extracted from these data by using almost any model of the motion. For example, let us consider a physically unrealistic model, in which the overall reorientation is isotropic and the internal motion is described as diffusion in a cone of semiangle θ_0. The appropriate correlation function for this model is (Lipari & Szabo, 1980)

$$C(t) = \frac{1}{5}e^{-t/\tau_M}[S_{cone}^2 + (1 - S_{cone}^2)e^{-t/\tau_{eff}}] \qquad (34)$$

where τ_M is the correlation time for overall reorientation, τ_{eff} is an effective correlation time, and S_{cone} is given by eq 14. The relaxation data at 25 °C, measured at 360 and 100 MHz, can be reproduced by using the correlation function of eq 34 if $\tau_M = 81$ ns, $\tau_{eff} = 1.25$ ns, and $\theta_0 = 45°$. It is interesting to note that even this simple-minded model predicts large internal motions on the nanosecond time scale.

Table II: ^{31}P Relaxation Data[a]

τ_L[a] (μs)	τ_S[a] (μs)	T_1 obsd[a] (s)	T_1 calcd[b] (s)	T_1 calcd[c] (s)	T_2 obsd[a] (ms)	T_2 calcd[b] (ms)	T_2 calcd[c] (ms)	NOE obsd[a] ($1 + \eta$)	NOE calcd[b] ($1 + \eta$)
3	0.05	3.2	3.54	3.46	32.0	35.3	82.5	1.35	1.37
10	0.1	3.6	3.61	3.58	13.0	12.2	32.0	1.37	1.38
26	0.26	3.9	3.66	3.65	4.2	4.7	12.4	1.40	1.38
52	0.52	3.8	3.68	3.67	3.1	2.4	6.2	1.41	1.38

[a] From Hogan & Jardetzky (1980); spectra were measured at 40.5 MHz. [b] Calculated by using the jump model with r(P-H) = 2.8 Å, $\Delta\phi = 0$, $p_{eq}(1) = {}^1/_2$, $\Delta = 56°$, $\bar{\theta} = 46°$, and $\tau_{int} = 2.25 \times 10^{-9}$ s. [c] Calculated by assuming that each actual P-H vector moves about its equilibrium position as the effective vector does, with $r_{P\text{-}H} = 2.8$ Å, $\bar{\theta} = 90°, 70°$, and 55°, and the other parameters as in *b*.

Table III: ^{13}C Relaxation Data

τ_L[a] (μs)	τ_S[a] (μs)	T_1 obsd[a] (ms)	T_1 calcd[b] (ms)	T_1 calcd[c] (ms)	T_1 calcd[d] (ms)	$\Delta\nu_{1/2}$ obsd[a] (Hz)	$\Delta\nu_{1/2}$ calcd[b] (Hz)	$\Delta\nu_{1/2}$ calcd[c] (Hz)	$\Delta\nu_{1/2}$ calcd[d] (Hz)	NOE obsd[a] ($1 + \eta$)	NOE calcd[b] ($1 + \eta$)	NOE calcd[c] ($1 + \eta$)
10	0.1	80.0	80.0	78.6	74.0	220	220	220	127	2.1	2.1	2.84

[a] From Hogan & Jardetzky (1980); spectra were measured at 25.1 MHz. The relaxation data are for C2′. [b] Calculated by using the isotropic model (correlation function of eq 34) for $\tau_M = 102$ ns, $\tau_{eff} = 1.4$ ns, and $\theta_0 = 23°$. [c] Calculated by using the jump model with r(c-H) = 1.09 Å, $\Delta\phi = 0$, $p_{eq}(1) = {}^1/_2$, $\Delta = 65°$, $\bar{\theta} = 75°$, and $\tau_{int} = 4.7 \times 10^{-10}$ s. [d] Calculated by using the approximate eq 30 and 31 for the jump model with the same parameters as in *c*.

^{31}P NMR Relaxation. Data for ^{31}P NMR relaxation were measured for the fractions of 140 and 260 base pair long DNA at a resonance frequency of 40.5 MHz. Hogan & Jardetzky (1979, 1980) assumed that the dominant relaxation mechanism is a dipolar interaction between the ^{31}P nucleus and the protons H5′$_1$, H5′$_2$, and H3′, neglecting the contribution to the relaxation due to chemical shift anisotropy. Since the relaxation times are proportional to the inverse of the spectral densities, we have

$$(T_i^{\text{obsd}})^{-1} = \sum_{j=1}^{3} (T_i^{(j)})^{-1}$$

where $i = 1, 2$ and j refers to the contribution to the relaxation of the jth interaction vector. If we introduce an effective interaction vector, we can set

$$(T_i^{\text{obsd}})^{-1} = 3/\langle T_i \rangle$$

or

$$\langle T_i \rangle = 3T_i^{\text{obsd}} \qquad (35)$$

where $\langle T_i \rangle$ is the relaxation time associated with the effective interaction vector. Following Hogan & Jardetzky (1980), we use 2.8 Å for the P–H internuclear distance. Fitting the data with the two-state jump model for $\Delta\phi = 0$ and $p_{eq} = {}^1/_2$ [τ_L and τ_S as in Hogan & Jardetzky (1980)], we obtain $\Delta = 56°$, $\bar{\theta} = 46°$, and $\tau_{int} = 2.25 \times 10^{-9}$ s, as shown in Table II. These parameters are in good agreement with the values extracted by using the approximate eq 30 and 31. As found for the proton relaxation data, the jump model can reproduce the observed data for various sets of the model parameters if we let $\Delta\phi$ and/or $p_{eq}(1)$ vary along with Δ, $\bar{\theta}$, and τ_{int}. In all cases, τ_{int} is in the nanosecond range, and large amplitudes in Δ and/or $\Delta\phi$ are required. The wobbling in a cone model gives for the best fit $\beta_{MD} = 36.8°$, $\theta_0 = 46°$, and $1/(6D_w) = 2.6 \times 10^{-9}$ s. A whole class of solutions for various values of β_{MD} exists, except for the range $40° < \beta_{MD} < 70°$, i.e., close to the "magic angle"; in all cases, $1/(6D_w) \sim 10^{-9}$ s and $30° < \theta_0 < 50°$. As in the case of ^{1}H relaxation, the experimental data can be reproduced even by using the wobbling in a cone model superimposed on an isotropic overall reorientation (correlation function given by eq 34). For example, measurements for the 260 base pair long fragment can be reproduced for $\tau_M = 1.4$ μs, $\tau_{eff} = 2.3$ ns, and $\theta_0 = 39°$.

Within the jump model, we now consider whether the motion of an assumed effective interaction vector can be related to the motion of the individual P-H vectors. The simplest possibility is to assume that each of the P-H interaction vectors fluctuates about its equilibrium position by an amount Δ and with an internal correlation time τ_{int} determined from the fitting procedure for the effective interaction vector. To determine the feasibility of this interpretation, one has to perform three separate calculations fixing $\bar{\theta}$ at the equilibrium position calculated from the X-ray structure. If the above assumptions are justified, one should find, considering dipolar interactions as simply additive

$$\left[\sum_{j=1}^{3} 1/T_i^{(j)}\right]^{-1} = T_i^{\text{calcd}}/3 \qquad (36)$$

where $i = 1,2$, j refers to the jth P-H vector, and T_i^{calcd} is the value calculated in the best fit, i.e., the predicted value of $\langle T_i \rangle = 3T_i^{\text{obsd}}$. We evaluated the left-hand side of eq 36 by using for the values of the equilibrium angles quoted by Hogan & Jardetzky (1979) between the P-H vectors and the long axis 90°, 70°, and 55° and a P-H distance of 2.8 Å. The results are shown in Table II. For the values of T_1, eq 36 holds very well. For the T_2 values, however, the values obtained by adding the contributions of the individual P-H vectors differ from the values calculated for a single "effective" interaction vector by almost an order of magnitude. We then investigated the geometrical parameters involved in the calculation. For the X-ray data for a B-DNA helix of Arnott et al. (1969), we find that the values given by Hogan & Jardetzky (1979) are incorrect in that one of the angles should be 64° rather than 90°. This does not modify substantially the results summarized in Table II. If we use the more recent X-ray data of Arnott & Hukins (1972), we find that the H3′–P, H5′$_1$–P, and H5′$_2$–P distances are 3.04, 2.66, and 3.14 Å, respectively. The values of $\bar{\theta}$ are 52°, 64°, and 63°, respectively. The internuclear distances are quite different; a simple average would give 2.95 Å. Since the dipole–dipole interaction is proportional to the inverse sixth power of the internuclear distance, another possibility is to determine an "average" internuclear distance, $\langle r \rangle$, for the "effective" interaction vector as

$$\langle r \rangle^{-6} = \frac{1}{3}\sum_{i=1}^{3} r_i^{-6} \qquad (37)$$

where r_i is the internuclear distance for the ith P-H vector. We calculate $\langle r \rangle = 2.89$ Å. If we fit the data by using the two-state jump model for $\Delta\phi = 0$ and $p_{eq}(1) = {}^1/_2$ with $\langle r \rangle$

= 2.89 Å, we obtain $\bar{\theta}$ = 42°, Δ = 66°, and $\tau_{\mathrm{int}} = 2.3 \times 10^{-9}$ s. Even in this case, eq 36, using the calculated P–H distances and $\bar{\theta}$ angle for each dipole, does not hold for T_2 values, which again are approximately an order of magnitude larger than the values obtained by dividing the effective interaction vector result by a factor of three. Hence, we conclude that, even in the context of the simple two-state jump model, it is not possible to relate in a simple way the motion of an assumed effective interaction vector to the actual motions of the P-H vectors.

^{13}C Relaxation Data. The ^{13}C relaxation data were obtained for the 260 base pair long fraction of DNA fragments. The relaxation times and NOE are similar for all protonated sugar carbons to within the appropriate normalization factor for the number of interactions. Therefore, we only considered the data for C2′ (a nucleus with two protons directly attached to it). The individual resonance (peak 6) for this carbon was resolved, and since above we studied the relaxation of protons bound to C2′, it is particularly interesting to investigate the relaxation for this nucleus. Using 1.09 Å for the C–H distance and τ_L and τ_S as in Hogan & Jardetzky (1980), we tried to fit the data by using the jump model for $\Delta\phi = 0$ and $p_{\mathrm{eq}}(1) = {}^1/_2$. It is not possible to fit all the relaxation data simultaneously (see Table III). If $\bar{\theta}$ = 73°, Δ = 65°, and $\tau_{\mathrm{int}} = 4.7 \times 10^{-10}$ s, we can reproduce T_1 and $\Delta\nu_{1/2}$, but the predicted NOE is too high. Also, the internal correlation time is an order of magnitude shorter than the values predicted by a number of different models for ^{31}P and ^{1}H relaxation. Hogan & Jardetzky (1980) reproduce the ^{13}C data for α (or S^2) = 0, β = 0.3, and $\tau_{\mathrm{int}} = 10^{-9}$ s by using the approximate eq 30 and 31, which give T_1 = 76 ms, NOE = 2.13, and $\Delta\nu_{1/2}$ = 191 Hz. However, their good fit is spurious. Using eq 29 for β and our revised expression for α given by eq 32, their fit corresponds to Δ = 39.2° and $\bar{\theta}$ = 57.7°. With these values, the exact formulation of the two-state model predicts T_1 = 89 ms, NOE = 2.34, and $\Delta\nu_{1/2}$ = 707 Hz. Thus, the line width is overestimated by almost a factor of four. The data cannot be reproduced within the jump model even if $p_{\mathrm{eq}}(1)$ and/or $\Delta\phi$ is allowed to vary. The T_1 and NOE data alone can be fitted with $\bar{\theta}$ = 56°, Δ = 36°, and $\tau_{\mathrm{int}} = 1.9 \times 10^{-9}$ s. These parameters predict that $\Delta\nu_{1/2}$ = 816 Hz, which is much too large. The situation in analogous for the wobbling in a cone model: there is no set of values of D_w, β, and θ_0 for which the experimental data can be reproduced simultaneously. For β = 41°, θ_0 = 75° and $1/(6D_w) = 2.2 \times 10^{-10}$ s, the T_1 and line-width values are predicted accurately, but the theoretical NOE is too high (2.9). Considering the fact that ^{1}H and ^{31}P data can be reproduced by using a variety of parameters and models for which the values of τ_L and τ_S are determined from hydrodynamics for a fragment of a given size, it is surprising that the limited ^{13}C data cannot be fitted at all by using such models. As in the case of ^{1}H and ^{31}P relaxation, however, the physically unrealistic model in which the overall reorientation is isotropic and the internal motion is described as diffusion in a cone can reproduce ^{13}C data if the single correlation time describing the macromolecular motion is allowed to vary. The experimental data can be reproduced by τ_M = 102 ns, τ_{eff} = 1.4 ns, and θ_0 = 23°. Considering that the isotropic model is physically unrealistic, it is not surprising that τ_M for different nuclei varies over an order of magnitude. It is remarkable, however, that the correlation times for internal motions are all in the 1–2-ns range.

In summary, although all the models considered here predict large-amplitude motions on the nanosecond time scale, the data of Hogan & Jardetzky (1980) seem too limited to lead to a unique physical picture of internal motions in DNA fragments. It is hoped that as more extensive data at different magnetic fields become available, especially for protonated carbons for which the relaxation mechanism is simple, the models formulated in this paper will prove useful, and a coherent physical picture of the internal dynamics of polynucleotides will emerge.

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