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NOEs in Duplex DNA Depend on Orientations of Internuclear Vectors to the Symmetry Axis

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The determination of the conformations of molecules in solution has been one of the major areas of NMR over the past several years. Such studies on nucleic acids and proteins have relied, to a large extent, on the distance information present in proton–proton NOEs (*1*, *2*).

One of the key questions in this area of research is the accuracy of the distances determined from NOEs. A variety of approaches have been used to estimate the error range for the distances determined from NOEs. It has been widely assumed that the rate of buildup of NOEs can be directly related to the interproton distance through the familiar relationship

$$\sigma_{ij} = \{5.7 \times 10^4/(r_{ij}6)\}\{\tau_c - 6\tau_c/(1 + 4\omega^2\tau_c^2)\},$$

where σ_{ij} is the cross-relaxation rate between protons i and j in seconds, r_{ij} is the distance between protons i and j in nanometers, ω is the Larmor frequency, and τ_c is the correlation time.

A number of studies have shown that to accurately characterize the relaxation of nucleic acids, which is the case of primary interest here, consideration of only pairs of spins is not sufficient and all proton–proton interactions need be taken into account. These approaches using complete relaxation matrix calculations take into account alternate relaxation pathways in addition to the pairwise interaction giving rise to a particular NOE. It is noted that even the complete relaxation matrix can be incomplete in certain cases (*3*).

What all of these studies have had in common is the assumption that the relationship between NOE buildup rates and interproton distance is a *scalar* relationship. However, since double-stranded DNA is an asymmetric molecule under certain conditions the relationship between NOEs and distances is actually a *vector* relationship. That is, the NOE buildup rate depends not only on the interproton distance but also on the orientation of the proton–proton vector relative to the symmetry axis of the DNA.

A DNA double helix can be modeled to a reasonable level of accuracy as a cylinder with a diameter of 2.05 nm and a length of 0.34 nm per base pair. Thus, a dodecamer will have a length to diameter ratio of about 2. The diffusion rate about the long symmetry axis will be different than the diffusion rate about an axis perpendicular to this axis.

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Consider the case of one proton–proton vector pointing along the symmetry axis z and another perpendicular to the z axis along the x axis. Since there will be more rapid motion about the z axis this will cause a proton–proton vector aligned along the x axis to have a shorter effective correlation time than a proton–proton vector aligned along the z axis.

There have been at least three derivations of the effective correlation time of a vector at an angle β to the z axis when the only motion is diffusion of the cylinder and there is no motion of the vector relative to the cylinder. The treatments of Woessner (*4*), Szabo (*5*), and Spiess (*6*) are all quite similar. Woessner's model gives an effective correlation time for a vector at an angle β to the z axis as $3\{\cos[\beta]^2(1-\cos[\beta]^2)/(D_{\|}+5D_{\perp})\}+3\{(1-\cos[\beta]^2)^2/(4(4\mathrm{D}_{\|}+2D_{\perp}))\}+\{(-1+3\cos[\beta]^2)^2/(24D_{\perp}))\}$, where $D_{\|}$ is the diffusion constant about the z axis, $D_{\perp}$ is the diffusion constant perpendicular to the z axis, and β is the angle between the internuclear vector and the z axis.

This expression has been used to calculate the dependence of the effective correlation time on orientation relative to the z axis which is shown in Fig. 1 for the range of $D_{\|}/D_{\perp}$ of 1 to 5, which corresponds roughly to double-stranded DNA helixes of length 6 to about 20. The results indicate that the effective correlation time, and hence rate of NOE buildup, can vary by about 2.5-fold depending on the orientation of the proton–proton vector to the helical z axis and the ratio $D_{\|}/D_{\perp}$. A 2.5-fold variation in NOE buildup rate corresponds to about a 17% error in distance which means a 0.4 nm distance might be in error by more than 0.06 nm.

It is noted that the H5–H6 vector of a cytosine is more or less perpendicular to the z axis, 86° in canonical B-form, whereas a H2′-H2″ vector is at an angle of 55° in

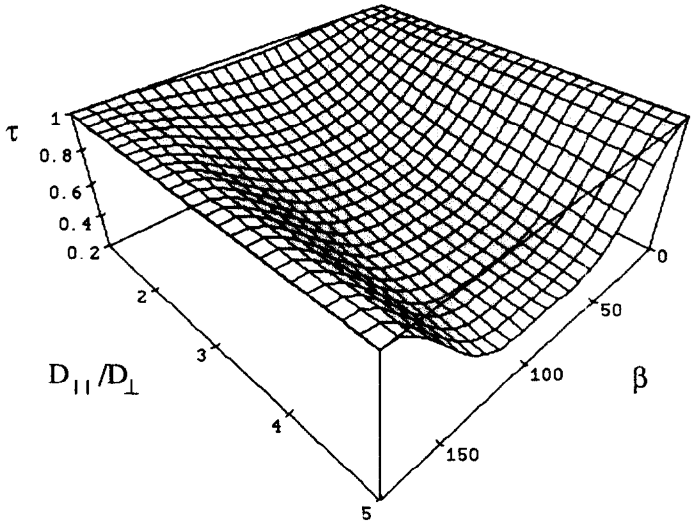

FIG. 1. The three-dimensional plot shows the dependence of the effective correlation time on the angle, β, of a vector to the z axis of a cylinder with diffusion rates $D_{\|}$ about the symmetry axis and $D_{\perp}$ perpendicular to the symmetry axis in the absence of internal motion. All plots were generated using the program Mathematica (*11*).

canonical B-form DNA. Therefore, in the absence of internal motion, the NOE buildup rates of these two pairs of protons cannot scale with internuclear distance. A recent report by Reid *et al.* (*7*) claims that there is no internal motion in duplex DNA since the NOE buildup rates for these two pairs of protons, in a dodecamer sample, scale to the sixth power of their respective interproton distances. This claim is not consistent with the cylindrical shape of duplex DNA.

The actual value of $D_{\|}/D_{\perp}$ for a double-stranded DNA such as a dodecamer is not known. Simple hydrodynamics would suggest that $D_{\|}/D_{\perp}$ goes as L^2/D^2. More sophisticated hydrodynamic theories would give corrections to this straightforward expression (*8–10*) but do not dramatically change the value of $D_{\|}/D_{\perp}$ from the simple model.

Figure 2 contains plots of σ_{ij} versus β for the case of $\omega\tau_{\perp} = 19$ which corresponds to a dodecamer investigated at 500 MHz with $\tau_{\perp}$ being 6 ns which is the value experimentally determined by dynamic depolarized light scattering (*10*) and $\tau_{\perp}$

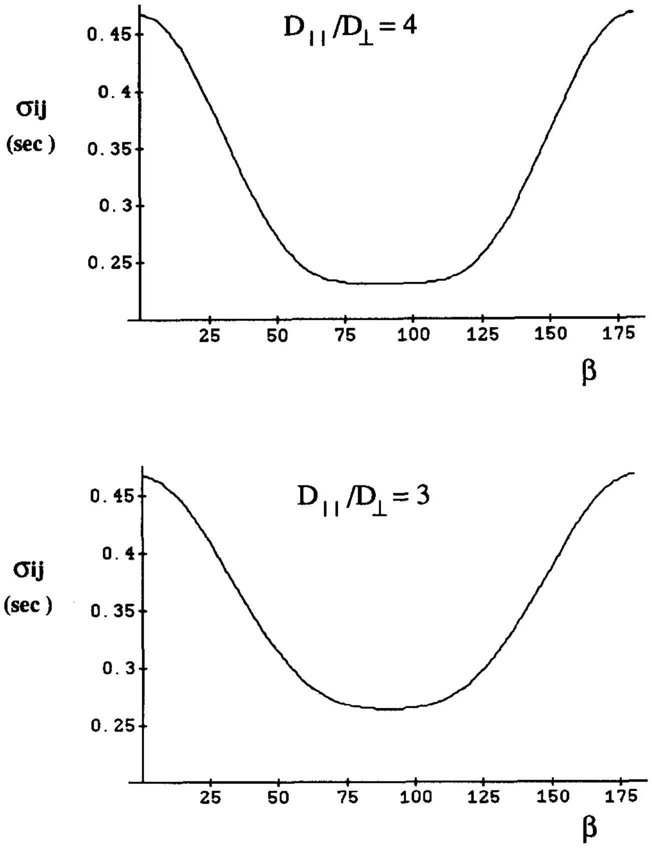

FIG. 2. The curves show the dependence of σ_{ij} for a proton–proton vector on the angle β to the z axis of a cylinder for the values of $D_{\|}/D_{\perp}$ indicated in the absence of internal motion. The σ_{ij} were calculated using the equation for σ_{ij} given in the text for a proton–proton distance of 0.3 nm and $\omega\tau_{\perp} = 19$. This corresponds to a field strength of 500 MHz and having $\tau_{\perp} = 6$ ns. $\tau_{\perp}$ is equal to $1/6D_{\perp}$.

$= 1/6D_{\perp}$. It is seen that σ_{ij} versus β is about the same for both $D_{\parallel}/D_{\perp} = 3$ and $D_{\parallel}/D_{\perp} = 4$.

Internal motion in an asymmetric molecule like double-stranded DNA can have pronounced effects on NOE buildup rates as well as relative NOE buildup rates. Using an approach similar to that recently proposed by Eimer *et al.* (*10*) an expression for the effective correlation time of an internuclear vector can be obtained, which is $\{\frac{1}{4} + 9/(8\exp(4\theta^2)) + (3\cos[2\beta]/2\exp[2\theta^2]) + (9\cos[4\beta]/8\exp[4\theta^2])\}/24D_{\perp} + 3\{(1 + 1/2\exp[4\theta^2]) - (2\cos[2\beta]/\exp[2\theta^2]) + (\cos[4\beta]\exp[-4\theta^2 - 4\zeta^2]/2)\}/(16(2D_{\perp} + 4D_{\parallel})) + 3\{(\exp[-4\theta^2]) - (\exp[-4\theta^2 - \zeta^2]\cos[4\beta])\}/(8(5D_{\perp} + D_{\parallel}))$. Here $D_{\parallel}$ is the diffusion constant about the z axis, $D_{\perp}$ is the diffusion constant perpendicular to the z axis, β is the angle between the internuclear vector and the z axis, θ is the square root of the root mean square polar angle of motion, and ζ is the square root of the root mean square azimuthal angle of motion. When there is no internal motion the above equation reduces to Woessner's equation, given above, for the effective correlation time.

The above expression has been used to calculate the effective correlation time for a cylinder having $D_{\parallel}/D_{\perp} = 3.5$ as a function of β and θ with the results shown in Fig. 3. These results show that in the presence of internal motion the orientational dependence of the effective correlation time diminishes. When the extent of internal motion approaches 25° or so the orientational dependence of the correlation time is somewhat diminished and when it approaches 45° it is negligible. Thus, in the presence of sufficient internal motion proton–proton pairs with different β could have NOE buildup rates which scale as a function of the internuclear distance alone. It is also noted that the effective correlation times for all vectors regardless of β decrease as the internal motion increases.

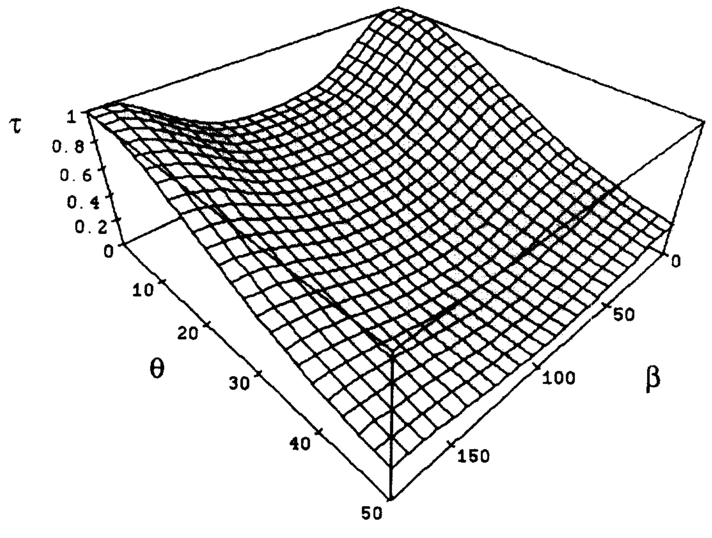

FIG. 3. The three-dimensional plot shows the dependence of the effective correlation time on the angle, β, of a vector to the z axis of a cylinder; θ is the square root of the root mean square polar angle of motion for the case of $D_{\parallel}/D_{\perp} = 3.5$.

At the present time there is not sufficient data to estimate reliably the extent of internal motion in DNA duplexes and in particular the extent of internal motion for different proton–proton vectors. The existing data, from a number of experiments many of which are cited in (*10*), are consistent with internal motion of most proton–proton and carbon–proton vectors on the order of 10–20°. More reliable experimental information could be obtained from detailed study of ^{13}C and ^{15}N single- and multiple-quantum relaxation parameters of both the sugars and bases of DNA duplexes. Another source of information about the extent of internal motion could be molecular dynamics trajectories of DNA in solution.

The asymmetry of duplex DNA complicates the straightforward analysis of NOE data in terms of conformational analysis. However, if independent information about correlation times can be obtained then the vectorial nature of the NOE information may actually prove to be an aid to conformational studies. For example, the orientation and internal motion effects on NOEs could be incorporated into relaxation matrix and distance geometry calculations in a straightforward manner.

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11. Mathematica is a trademark of Wolfram Research, Inc., Champaign, Illinois.