Internal mobility in a double-stranded B DNA hexamer and undecamer

A time-dependent proton–proton nuclear Overhauser enhancement study

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The internal mobility of the deoxyribose H2′–H2′′ and base C(H5)–C(H6) and T(CH₃)–T(H6) vectors has been investigated by means of time-dependent nuclear Overhauser enhancement (NOE) measurements in a B DNA hexamer and undecamer. Cross-relaxation rates between these proton pairs are determined from the initial slopes of the time development of the NOEs, and, as the interproton distances between these proton pairs are fixed, apparent correlation times for the 3 interproton vectors are calculated from the cross-relaxation rate data. It is shown that there is little residue to residue variation in the cross-relaxation rates of the interproton vectors within each oligonucleotide, that the mean apparent correlation times of the C(H5)–C(H6) and T(CH₃)–T(H6) vectors are approximately equal and significantly greater than that of the H2′–H2′′ vectors, and that the data for the H2′–H2′′ vectors of both oligonucleotides and the C(H5)–C(H6) and T(CH₃)–T(H6) vectors of the undecamer cannot be accounted for by isotropic tumbling alone. The data are analysed in terms of a two motion model with isotropic tumbling and a single internal motion. The relaxation time of the internal motion at 23°C is ≤ 1 ns for the H2′–H2′′ vectors of both oligonucleotides and ≤ 3 ns for the C(H5)–C(H6) and T(CH₃)–T(H6) vectors of the undecamer. In the case of the H2′–H2′′ vectors, however, the amplitude of the internal motion is found to be too large to be compatible with the known stereochemistry of DNA. This finding can only be explained by invoking additional degrees of internal freedom with a larger number of internal motions of small amplitude of the type deduced from the analysis of crystallographic thermal factors [(1984) J. Mol. Biol. 173, 361–388].

B DNA solution dynamics   *Synthetic oligonucleotide*   *^{1}H-^{1}H NOE*
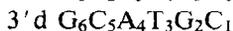
1. INTRODUCTION

Experimental and theoretical studies both indicate that the internal structure of DNA must be regarded as dynamic rather than static [1–15]. Analysis of crystallographic thermal factors can provide information about the directions and magnitudes of the motions of individual atoms. The types of internal motions that have been deduced from such an analysis include base pair propellor twisting, rolling and buckling, coupled

Abbreviations: NOE, nuclear Overhauser enhancement

rotation of the sugar and base as a nucleotide unit, coupled translation of paired bases, fluctuation of the groove sizes and winding/unwinding motions at the ends of helices [15]. Rate information, on the other hand, is accessible through ^{13}C, ^{31}P and ^{1}H-NMR relaxation measurements. To date such NMR studies have been limited to long DNA pieces of greater than 150 base pairs and have suggested the presence of large amplitude internal motions on the nanosecond time scale [1–8]. Long DNA pieces, however, do not permit one to investigate local variations in internal mobility, and for such information it is necessary to turn to short
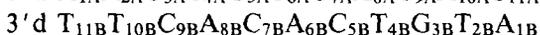
oligonucleotides of defined sequence in which resonances of individual atoms can be observed.

Here we used time-dependent proton–proton NOE measurements to probe the internal mobility of the deoxyribose H2'–H2'', and base C(H5)–C(H6) and T(CH₃)–T(H6) interproton vectors of fixed distance in two B DNA oligonucleotides, namely the self-complementary hexamer:

5'd $C_1G_2T_3A_4C_5G_6$
3'd $G_6C_5A_4T_3G_2C_1$

and the non-self-complementary undecamer:

5'd $A_{1A}A_{2A}G_{3A}T_{4A}G_{5A}T_{6A}G_{7A}A_{8A}C_{9A}A_{10A}T_{11A}$
3'd $T_{11B}T_{10B}C_{9B}A_{8B}C_{7B}A_{6B}C_{5B}T_{4B}G_{3B}T_{2B}A_{1B}$

The ¹H-NMR spectra of both oligonucleotides have been previously assigned by means of pre-steady state NOE measurements [16–18], and the undecamer comprises a portion of the specific DNA target site of the cAMP receptor protein in the *gal* operon [19].

2. EXPERIMENTAL

The oligonucleotides 5'd(CGTACG)₂ and 5'd(AAGTGTGACAT).5'd(ATGTCACACTT) were synthesized as in [16] and [18], respectively. After extensive lyophilisation from 99.6% ²H₂O, the oligonucleotides were dissolved in 99.96% ²H₂O containing 1 M KCl, 50 mM potassium phosphate (pH* 6.5, meter reading uncorrected for the isotope effect on the glass electrode) and 0.1 mM EDTA in the case of the hexamer and 300 mM KCl, 15 mM potassium phoshpate (pH* 6.5) and 0.18 mM EDTA in the case of the undecamer. The concentrations of the hexamer and undecamer duplexes were 3.1 and 2.7 mM, respectively.

¹H-NMR measurements at 500 MHz were carried out on a Bruker AM500 spectrometer. Nuclear Overhauser effects were measured from peak areas by using interleaved difference spectroscopy [16–18, 20–22] with presaturation pulses of defined lengths between 0.075 and 0.8 s, and delays of 3 s between scans to permit relaxation of the system. The irradiation power used was sufficient to be in the high power limit so that saturation can effectively be considered to be instantaneous whilst preserving selectivity [22]. Chemical shifts are ex-

pressed relative to 2,2-dimethylsilapentane-5-sulphonate.

3. RESULTS AND DISCUSSION

Fig.1 shows a series of NOE difference spectra for the hexamer illustrating the change in intensity of the $C_5(H2'')$ and $C_1(H5)$ resonances following irradiation of the $C_5(H2')$ and $C_1(H5)$ resonances, respectively, for different lengths of time. A similar set of NOE difference for the undecamer is shown in fig.2, and in fig.3 the magnitudes of selected NOEs are plotted as a function time.

For short irradiation times t, the magnitude of the NOE, $N_{ij}(t)$, observed on resonance i following irradiation of resonance j is directly proportional to the cross-relaxation rate σ_{ij} between protons i and j [20,22]:

$$N_{ij}(t) \sim \sigma_{ij}t \qquad (1)$$

Under these conditions, $N_{ij}(t)$ is completely independent of the spin-lattice relaxation rate $(1/T_1)$ of proton i. In a multiple spin system eq.1 is valid providing that either $\sigma_{ij} \geqslant \sigma_{ik}$ or $\sigma_{ij} \geqslant \sigma_{jk}$ where k is any other proton [22,24]. In the case of the 3 interproton vectors considered here, namely the
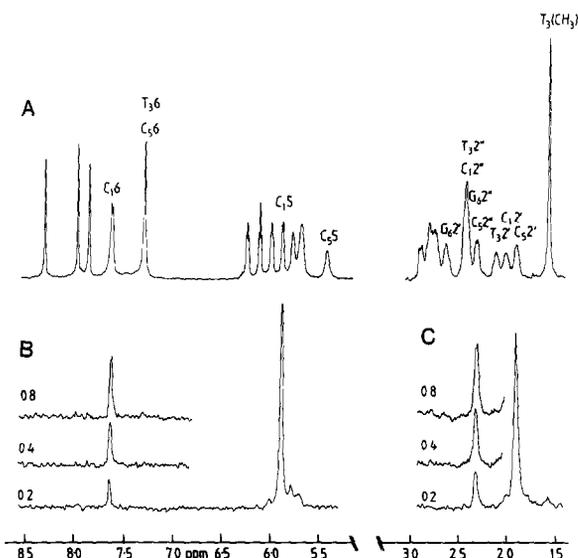

Fig.1. (A) 500 MHz ¹H-NMR spectrum of the hexamer. (B,C) NOE difference spectra resulting from irradiation of the $C_1(H5)$ and $C_5(H2')$ resonances, respectively, for 0.2, 0.4 and 0.8 s. Relevant assignments are taken from [16,17]. The temperature is 23°C.
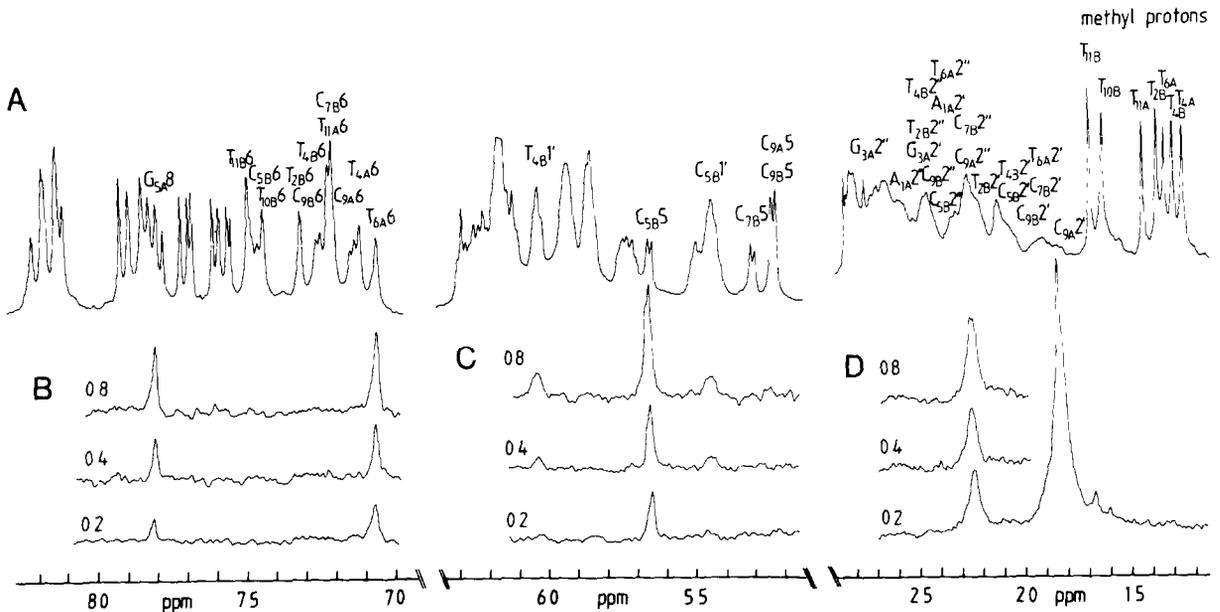
Fig.2. (A) 500 MHz ^{1}H-NMR spectrum of the undecamer. (B,C,D) NOE difference spectra resulting from irradiation of the $T_{6A}(CH_3)$, $C_{5B}(H6)$ and $C_{9A}(H2')$ resonances, respectively, for 0.2, 0.4 and 0.8 s. Relevant assignments are taken from [18]. The temperature is 23°C.

H2'−H2'', C(H5)−C(H6) and T(CH₃)−T(H6) vectors, this condition is satisfied. Thus, the cross-relaxation rate σ_{ij} can simply be determined from the initial slope of the time development of the NOE. The cross-relaxation rate σ_{ij} is in turn proportional to the reciprocal of the sixth power of the distance r_{ij} between the two protons and the apparent correlation time τ_{app} of the $i–j$ interproton vector [23]:

$$\sigma_{ij} = \frac{\gamma^4 \hbar^2}{10 r_{ij}^6} \left(\tau_{app} - \frac{6\tau_{app}}{1 + 4\omega^2 \tau_{app}^2} \right) \qquad (2)$$

where ω is the spectrometer frequency (in rad·s^{-1}), γ the gyromagnetic ratio, and $\hbar$ Planck's constant divided by 2π. The distances of the H2'−H2'', C(H5)−C(H6) and T(CH₃)−T(H6) vectors are fixed by the geometry of the sugar ring and bases themselves and have values of 1.78, 2.46 and 2.70 Å calculated on the basis of standard bond lengths and angles. (The latter value is a $(<r_{ij}^{-6}>)^{-1/6}$ mean calculated on the basis of free rotation of the methyl group.) Consequently the values of τ_{app} for the individual interproton vectors are easily calculated.

Values of the cross-relaxation rates for all the H2'−H2'', C(H5)−C(H6) and T(CH₃)−T(H6)

vectors that could be measured in both the hexamer (0 and 23°C) and undecamer (23°C) together with the mean apparent correlation times calculated from them are given in tables 1 and 2, respectively. What clearly emerges from the data for both oligonucleotides is that there is little residue to residue variation in the cross-relaxation rates of the interproton vectors within each oligonucleotide, and that the mean apparent correlation times of the C(H5)−C(H6) and T(CH₃)−T(H6) vectors are approximately equal and significantly greater than that of the H2'−H2'' vector. Thus the H2'−H2'' vector must exhibit a higher degree of internal mobility relative to the other two interproton vectors.

Further interpretation of the data requires one to consider the cross-relaxation rate in terms of the spectral density function $J(\omega)$ [23]:

$$\sigma_{ij} = \frac{\gamma^4 \hbar^2}{10 r_{ij}^6} \left[J(0) - 6J(2\omega) \right] \qquad (3)$$

If the only motion experienced by the interproton vector is overall tumbling of the molecule with an isotropic rotational correlation time τ_R, then $J(\omega)$ is given:
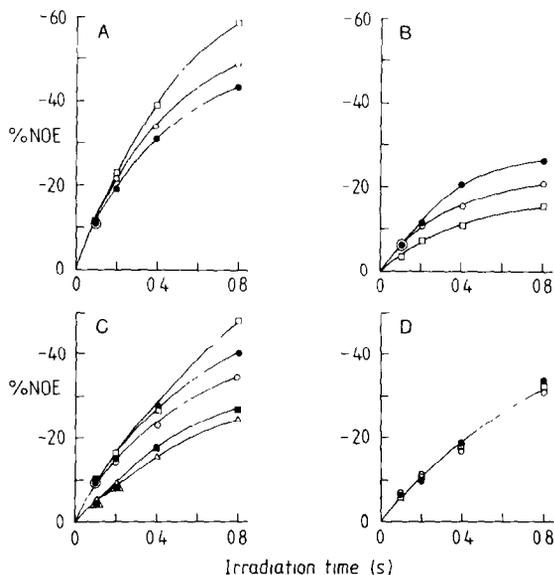
Fig.3. Magnitudes of NOEs as a function of irradiation time for the hexamer (A,B) and undecamer (C,D) at 23°C. (A) H2'' resonances of C_1 (●), C_5 (○) and T_3 (□) following irradiation of their respective H2' resonances. (B) H6 resonances of C_1 (●), C_5 (○) and T_3 (□) following irradiation of the C_1(H5), C_5(H5) and T_3(CH$_3$) resonances. (C) H2'' resonances of C_{9A} (□), C_{5B} (○) and C_{7B} (●) following irradiation of their respective H2' resonances, and H6 resonances of T_{4A} (■) and T_{11B} (△) following irradiation of their respective methyl proton resonances. (D) H6 resonances of C_{9A} (□), C_{5B} (○) and C_{7B} (●) following irradiation of their respective H5 resonances.

$$J(\omega) = \frac{\tau_R}{1 + \omega^2\tau_R^2}$$ (4)

and eq.3 reduced to eq.2. The rotational correlation time τ_R can be calculated from the Stokes-Einstein equation:

$$\tau_R = 8\pi\eta r_o^3/6kT$$ (5)

where η is the viscosity of the solution, r_o the radius of the molecule approximated as a sphere, and kT the Boltzmann energy factor. The dimensions of the hexamer are $24 \times 24 \times 20.4$ Å and can therefore be considered as a sphere of radius 13 Å with a rotational correlation time of 2.7 ± 0.5 at 23°C and 5.9 ± 1 ns at 0°C. The undecamer, on the other hand, has dimensions of $24 \times 24 \times 37.4$ Å and strictly speaking should be considered as a prolate ellipsoid. However, calculation of the correlation times for orientation about the short and long axes using Perrin's formula [25] indicate that these lie within the expected range of the rotational correlation time of a sphere of equal volume, namely 5 ± 1 ns at 23°C. Thus, in the case of the C(H5)–C(H6) and T(CH$_3$)–T(H6) vectors of the hexamer, isotropic tumbling alone is sufficient to account for the apparent correlation times of 2.2 ± 0.7 ns and 5.5 ± 1 ns at 23°C and 0°C, respectively. In the case of the H2'–H2'' vectors, the apparent correlation times (0.8 ± 0.1 ns and 0.7 ± 0.2 ns for the hexamer and undecamer, respectively, at 23°C, and 1.5 ± 0.3 ns for the hexamer at 0°C) are significantly smaller than the

Table 1

Cross-relaxation rates and mean apparent correlation times for the H2'–H2'', C(H5)–C(H6) and T(CH$_3$)–T(H6) interproton vector of the hexamer at 0 and 23°C

Residue	0°C			23°C		
	$\sigma_{H2'-H2''}$ (s^{-1})	σ_{H5-H6} (s^{-1})	σ_{CH_3-H6} (s^{-1})	$\sigma_{H2'-H2''}$ (s^{-1})	σ_{H5-H6} (s^{-1})	σ_{CH_3-H6} (s^{-1})
C_1	2.6	1.5		1.0	0.6	
C_5	2.5	1.2		1.1	0.5	
T_3	2.7		0.8	1.2		0.35
G_6	2.4			1.1		
σ_{mean} (s^{-1})	2.6 ± 0.1	1.4 ± 0.2	0.8 ± 0.1	1.1 ± 0.1	0.55 ± 0.1	0.35 ± 0.05
r (Å)	1.78 ± 0.05	2.46 ± 0.05	2.70 ± 0.05	1.78 ± 0.05	2.46 ± 0.05	2.70 ± 0.05
τ_{app}mean (ns)	1.5 ± 0.3	5.5 ± 1.5	5.5 ± 1.5	0.8 ± 0.1	2.2 ± 0.7	2.4 ± 0.5

The relative error in the measurement of the cross-relaxation rates is $\leqslant \pm 15\%$

Table 2

Cross-relaxation rates and mean apparent correlation times for the
H2'–H2'', C(H5)–C(H6) and T(CH₃)–T(H6) interproton vectors of
the undecamer at 23°C

Residue	$\sigma_{H2'-H2''}$ (s^{-1})	σ_{H5-H6} (s^{-1})	σ_{CH_3-H6} (s^{-1})
C_{9A}	1.1	0.8	
C_{5B}	0.9	0.7	
C_{7B}	0.8	0.8	
C_{9B}	0.8	0.8	
T_{4A}			0.4
T_{6A}	0.9		0.4
T_{11A}			0.4
T_{2B}	0.9		0.4
T_{4B}	1.1		0.4
T_{10B}			0.4
T_{11B}			0.4
A_{1A}	0.8		
G_{3A}	0.9		
σ_{mean} (s^{-1})	0.9 ± 0.1	0.7 ± 0.1	0.4 ± 0.05
r (Å)	1.78 ± 0.05	2.46 ± 0.05	2.70 ± 0.05
τ_{app}mean (ns)	0.7 ± 0.2	2.8 ± 0.6	2.8 ± 0.6

The relative error in the measurement of the cross-relaxation rates is
$\leqslant \pm 15\%$

rotational correlation times and a large contribution from internal motion needs to be invoked. Similarly, the apparent correlation time of 2.8 ± 0.6 ns for the C(H5)–C(H6) and T(CH₃)–T(H6) vectors of the undecamer also requires a contribution from internal motion.

In the presence of internal motion, the spectral density function $J(\omega)$ can always be expressed as a sum of Lorentzians [26,27]:

$$J(\omega) = \sum_{j=1}^{j=n} \frac{\alpha_j \tau_j}{1 + \omega^2 \tau_j^2} \qquad (6)$$

where n is the number of motions, and τ_j and α_j are the relaxation time and amplitude factor, respectively, of the jth motion. The amplitude factors are subject to the conditions $\alpha_j \geqslant 0$ and $\Sigma \alpha_j = 1$, and the first motion is overall tumbling of the molecule (i.e., $\tau_1 = \tau_R$). It is important to note that the formulation of $J(\omega)$ in terms of eq.6 is independent of the nature of the motion and thus does not require one to assume any particular type of motion. Given that only a single experimental parameter

could be measured, namely the cross-relaxation rate, it is evident that the data can only be interpreted in terms of two motions: overall tumbling and a single internal motion. As $\omega^2 \tau_R^2 \gg 1$, $J(\omega)$ is then given by:

$$J(\omega) = \alpha\tau_R + (1 - \alpha) \left(\tau_i - \frac{6\tau_i}{1 + \omega^2\tau_i^2} \right) \qquad (7)$$

where τ_i is the relaxation time of the internal motion. Clearly unique values of α and τ_i cannot be determined from the experimental data. Nevertheless, a phase diagram can be constructed as α must lie in the range $0 \leqslant \alpha \leqslant 1$. Phase diagrams for the various interproton vectors are shown in fig.4.

Inspection of fig.4 enables one to deduce the following points:

(i) The cross-relaxation data can be accounted for by internal motion with a relaxation time of $\leqslant 1$ ns for the H2'–H2'' vectors of the hexamer and undecamer at 23°C, $\leqslant 2$ ns for the H2'–H2'' vectors of the hexamer at 0°C, and $\leqslant 3$ ns for the C(H5)–C(H6) and T(CH₃)–T(H6) vectors of the undecamer at 23°C.
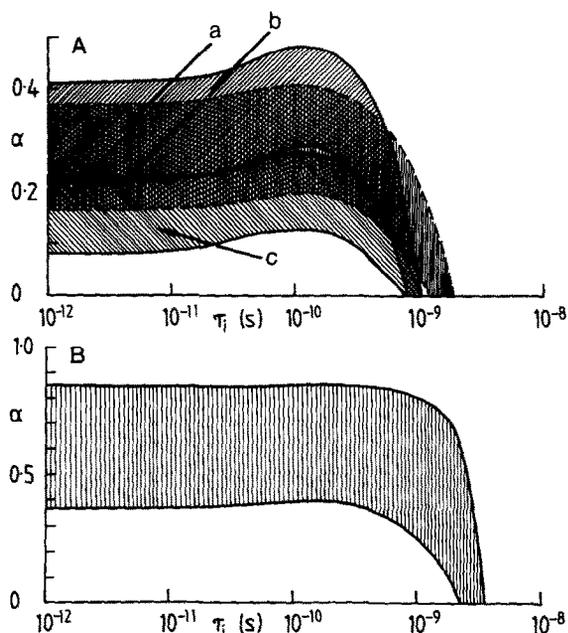
Fig.4. Phase diagrams relating the relaxation time τ_i of internal motion to the amplitude factor α defined in eq.7 for: (A) the H2'−H2'' vectors of the hexamer at 0°C (a) and 23°C (b) and the undecamer at 23°C (c); (B) the C(H5)–C(H6) and T(CH$_3$)–T(H6) vectors of the undecamer at 23°C. The phase diagrams are calculated from the data in tables 1 and 2 and the rotational correlation times given in the text using eq.3 and 7. Note that the amplitude of the internal motion is $(1 - \alpha)$.

(ii) If it is further assumed that the amplitude of the internal motion of the H2'−H2'' vectors is the same for both the hexamer and undecamer at 23°C, then the relaxation time for this motion can be limited to two time domains, namely 1–0.35 ns and ⩽ 10 ps.

(iii) In the case of the undecamer, the amplitude $(1 - \alpha)$ of the internal motion for the C(H5)–C(H6) and T(CH$_3$)–T(H6) vectors is significantly smaller than that of the H2'−H2'' vectors for all values of τ_i.

(iv) The amplitude $(1 - \alpha)$ of the internal motion for the H2'−H2'' vectors is unusually large and requires large angular fluctuations. This is illustrated in fig.5 for three different models of internal motion; a two state jump model, restricted internal diffusion and wobbling within a cone. It will be noted that the maximum value of $(1 - \alpha)$ for the first two models is 0.75 whereas in the third
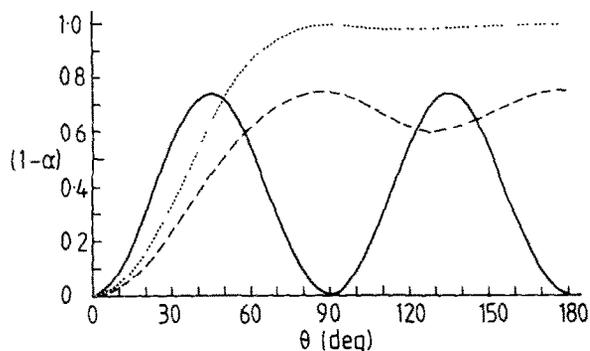

Fig.5. Dependence of the amplitude factor $(1 - \alpha)$ of internal motion defined in eq.7 on the half angular magnitude θ of the internal motion of the H2'−H2'' vector for 3 different models of internal motion: a two state jump model (———), restricted internal diffusion (– – –) and wobbling within a cone (- - -). The angle which the H2'−H2'' vector makes with the axis of rotation about the C3'−C2' and C2'−C1' bonds is 90° (this angle is only relevant to the first two models); α is given by $0.75[1 - \cos 2\theta][2 - (1 - \cos 2\theta)]$ in the case of the two state jump model, by $0.25 + 0.75(\sin 2\theta/2\theta)^2$ in the case of the limited internal diffusion model, and by $\cos^2\theta \sin^4\theta/[4(1 - \cos\theta)^2]$ in the case of the wobbling within a cone model [28].

model $(1 - \alpha)$ can have a maximum value of 1. The magnitudes of these motions are too large to be compatible with the known stereochemistry of DNA. Consequently, the small values of α obtained for the H2'−H2'' vectors require one to invoke further degrees of internal freedom with a larger number of correlated and uncorrelated internal motions of small amplitude of the type deduced from the analysis of crystallographic thermal factors [15].

From the experimental viewpoint, further analysis will require the measurement of additional relaxation parameters. In this respect ^{13}C T_1, T_2 and NOE data at several spectrometer frequencies should prove most useful. A complementary approach, and in the long term potentially the most informative, would be to use molecular dynamics simulations to calculate NMR relaxation parameters and compare these to experimental ones. In terms of the internal motions which are accessible to molecular dynamics, namely those with correlation times of < 100 ps, it is possible to calculate correlation functions from the trajec-

tories which yield a generalised order parameter S^2 defined by [12,29].

$$S^2 = \alpha = \tau_{app}/\tau_R = \sigma_{obs}/\sigma_R \qquad (8)$$

where σ_{obs} is the observed cross-relaxation rate for a particular interproton vector, and σ_R the corresponding calculated cross-relaxation rate for a rigid system undergoing only isotropic tumbling. From fig.4, it can be seen that at 23°C S^2 has values of 0.32 ± 0.1 and 0.16 ± 0.08 for the H2'-H2'' vectors of the hexamer and undecamer, respectively, and a value of 0.60 ± 0.15 for the C(H5)-C(H6) and T(CH₃)-T(H6) vectors of the undecamer. These compare to calculated values of 0.87-0.92 for the P-C3', P-C5' and C4'-C5' heavy atom vectors of $5'd(CGCGCG)_2$ obtained from molecular dynamics [12]. Thus, the molecular dynamics calculations presently available underestimate the extent of internal mobility as observed experimentally in solution. In this respect it is also of interest to note that the thermal factors calculated from the molecular dynamics are also a factor of 5-8 times smaller than the experimental ones determined by X-ray crystallography [13].

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