MAGNETIC RESONANCE IN CHEMISTRY, VOL. 35, 561–565 (1997)

Selectively ^{13}C-Enriched DNA: Dynamics of the C1′H1′ and C5′H5′ or C5′H5″ Vectors in d(CGCAAATTTGCG)$_2$

Florence Gaudin, Luc Chanteloup, N. T. Thuong and G. Lancelot*
Centre de Biophysique Moléculaire, CNRS, Rue Charles Sadron, F-45071 Orléans Cédex 2, France

In order to examine the internal dynamic processes of the dodecamer d(CGCAAATTTGCG)$_2$, the ^{13}C-enriched oligonucleotide was synthesized. The C3, A4 and A6 residues were selectively ^{13}C labeled at the C1′ and C5′ positions and their $R(C_{x,y})$, $R(C_z)$ and $R(H_z \rightarrow C_z)$ relaxation rates were measured. Data variations were observed for the three residues. The analysis of the relaxation rates in the context of the model-free formalism of Lipari and Szabo indicates other pathways than those described by τ_g, S^2 and τ_i. Direct evidence for the presence of a conformational exchange process was obtained for the residue A4 from rotating frame relaxation measurements. Taking into account the exchange process contribution, analysis of these data gave a τ_g value of 3.7 ns at 32 °C, in agreement with those computed from independent ^{13}C relaxation rate measurements on the three thymines or from the H5–H6 NOE measurements. The frequency difference between the exchanging sites was computed in the range 130–250 Hz. This additional relaxation process involves large amplitude and slow motions (τ_{ex} = 130 μs) which can reflect special dynamics of the tract AAATTT which supports the spine of hydration. Analysis of ^{13}C relaxation rates indicated smaller order parameters S^2 for C5′ (0.40) than for C1′ (0.80). These data reflected additional motions undergone by the C5′H5′ or C5″H5″ vectors such as repuckering and motions around the C4′—C5′ bond.

Magn. Reson. Chem. **35**, 561–565 (1997) No. of Figures: 3 No. of Tables: 2 No. of References: 2

Keywords: DNA; dynamics; ^{13}C NMR; A tract

Received 10 June 1996; revised 11 September 1996; accepted 11 September 1996

INTRODUCTION

NMR relaxation experiments can, as a rule, provide a detailed description of nucleic acid dynamics. Precise conclusions are, however, difficult to deduce from ^{1}H spin relaxation rate measurements because several motions implicating a change in orientation and distances of the H–H vectors must be simultaneously taken into account to reflect the experimental data. ^{13}C spin–lattice relaxation whose processes are governed by the changes in orientation of the ^{13}C–^{1}H vector, the length of which is constant, is easier to interpret. Therefore, ^{13}C (or ^{15}N) NMR relaxation studies are the best choice for investigating the dynamic processes. This can be accomplished by measuring the six spin–lattice relaxation rates $R(C_z)$, $R(C_{x,y})$, $R(H_z \rightarrow C_z)$, $R(2H_zC_z)$, $R(2H_zC_{x,y})$ and $R(H_z^c)$, which allows one to compute the spectral density functions for five different frequencies.[1] Nevertheless, the calculated $J(\omega)$ values are altered by accumulation of the uncertainties on each relaxation rate measurement. Consequently, more accurate information on both amplitude and rate of the internal motions will be extracted from the relaxation rates $R(C_z)$, $R(C_{x,y})$ and $R(H_z \rightarrow C_z)$.[2]

In a previous paper,[2] we presented an approach to the internal dynamic processes of the three central thymines in the selectively ^{13}C1′-enriched dodecamer d(CGCAAATTTGCG)$_2$. Experimental spin–lattice relaxation rates were simulated well by computing the effect of local and internal motions of the vector C1′–H1′ on the density functions. Two models for the internal mobility of the base pairs and of sugars were presented for the three thymines. This dodecamer contains an A_3T_3 tract which is known to induce special conformational properties. Inphase ligated A_nT_n segments experience retarded electrophoretic migration which has been widely interpreted as a result from macroscopic DNA curvature[3,4] and the ApT step was distinguished by slow base pair opening kinetics.[5] Moreover, Brown *et al.*[6] reported significantly different mobilities for the adenine and thymine residues in the crystal structure of d(CGCAAATTTGCG)$_2$.

In order to compare first the dynamic processes of the C1′ and C5′ atoms and second the internal motions of the adenine residues containing the tract A_3T_3 with those of the other residues, we present here an NMR investigation of the C1′H1′ and of the C5′H5′ or C5′H5″ dynamic processes of the dodecamer d(CGCAAATTTGCG)$_2$ selectively ^{13}C1′ and ^{13}C5′ labeled on the C3, A4 and A6 residues.

EXPERIMENTAL

[1′,5′-^{13}C]-dA and [1′,5′-^{13}C]-dC were prepared by *N*-glycosylation as reported.[7] The required 1′,5′-^{13}C-

* *Correspondence to*: G. Lancelot.

 CCC 0749–1581/97/090561–05 $17.50

labeled oligodeoxynucleotide was prepared on a Pharmacia automatic synthesizer via phosphoramidite chemistry using the classical unlabeled or [1′,5′-^{13}C]-labeled deoxynucleotide 5′-*O*-dimethoxytrityl-3′-*O*-(β-cyanoethyl-*N*,*N*-diisopropylphosphoramidite) building block.[8] After deprotection, the oligodeoxynucleotide was purified by anion-exchange chromatography on a mono-Q column (Pharmacia) and analyzed by reversed-phase HPLC.

NMR samples

Oligonucleotide solutions were passed through a Chelex-100 column to remove paramagnetic impurities and adjusted to pH 7.0, then lyophilized from D_2O and dissolved in D_2O containing 0.1 M NaCl. The NMR samples (1.5 mM) were degassed and were kept in sealed tubes.

NMR spectroscopy and relaxation rate measurements

NMR experiments were performed between 17 and 47 °C on a Bruker AMX-500 spectrometer and processed on an X32 computer. ^{1}H and ^{13}C NMR spectra were recorded at 500.139 and 125.770 MHz, respectively. The ^{1}H and ^{13}C 90° pulses were 10.0 and 27.2 μs, respectively, at 22 °C and were adjusted for each temperature. An increase of 27% in their values was observed between 17 and 47 °C.

The NOESY spectra were recorded with 1024 complex data points and a spectral width of 5500 Hz. The evolution delay in the t_1 dimension was incremented in 512 equal steps of 100 μs, resulting of a 5120 Hz width. Eight NOESY spectra were obtained at 32 °C at mixing times of 0, 30, 50, 75, 100, 150, 200 and 300 ms. The preparation delay was 2 s, which allows at least 80% magnetization recovery of all the protons. Cross peak volumes were measured using the UXNMR software. The intranucleotide H5–H6 NOE of the C3 and C11 residues (overlapped resonances) was computed as the ratio of the corresponding cross peak volume with the volume of the diagonal peak measured on the same row at a zero mixing time. The ^{1}H chemical shifts are given relative to 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) as an internal reference.

The relaxation rates $R(C_z)$, $R(C_{x,y})$ and $R(H_z \rightarrow C_z)$ were measured following the pulse sequences described previously.[1,9] Heteronuclear spin-lock and proton irradiation during the relaxation period were used in order to decouple the cross-relaxation pathways during the relaxation experiments, and to approach mono-exponential behavior as closely as possible.[10,11] T_1 and $T_{1\rho}$ curves were fitted with 12 data points at 0, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 and 600 ms and 0, 20, 40, 60, 80, 100, 130, 180, 220, 250, 300 and 400 ms, respectively. In the $T_{1\rho}$ experiment, the spin-lock was constituted by contiguous phase-alternating 180 °C ^{13}C pulses at low power. These 180° pulses were adjusted at each temperatures. Various r.f. field strengths (ν_{SL} = 746, 1205, 1923, 2416, 3012, 3732 and 4808 Hz) were obtained by adjusting the pulse length at different power levels. The spin-lock carrier frequency was set on the ^{13}C1′ resonance of the A4 residue, leading to an offset of 186 and 23 Hz for the ^{13}Cl′ resonances of the C3 and A6 residues, respectively. The accuracy of $R(C_{x,y})$ was improved by correcting the effective spin-lock field using Eqn (9) in the paper by Peng *et al.*[12] The ^{13}C5′ values were measured with the spin-lock carrier frequency set on the middle of the overlapped ^{13}C5′ resonances and no correction was applied. In order to estimate accurately the heteronuclear NOE value, $R(H_z \rightarrow C_z)$ was measured for cross relaxation delays of 1, 1.2, 1.5 and 2 s and a delay between scans of 4 s. No proton T_1 correction was made to the NOE values. Within the experimental errors, no difference were observed in NOE measured by using the different cross relaxation delays. Areas were calculated with the integration routines of the UXNMR software package. Two series of T_1 and $T_{1\rho}$ measurements and four series of NOE measurements were performed at each temperature.

Curve fitting was performed using a least-squares program[13] to minimize the value of χ^2. Uncertainties were obtained from the covariance matrix as already described[2] and given at a 90% confidence interval. The $R(C_z)$, $R(C_{x,y})$ and $R(H_z \rightarrow C_z)$ values are given with a relative precision of 3, 6, and 8%, respectively. All the deviations of the data between the series of T_1, $T_{1\rho}$ and NOE experiments with the same relaxation delay or cross relaxation delays were running within the corresponding indicated precision.

RESULTS

Assignment of the ^{1}H1′ resonances

Figure 1 shows an expansion of the H8/H6–H1′ NOESY connectivities of the dodecamer d(CGCAAATTTGCG)$_2$ at 22 °C. All the aromatic and H1′ resonances were easily assigned by using the proximities of the aromatic protons H6/H8 with the H1′ protons belonging to their own residue or to their 5′-neighboring residues. The connectivities involving the other sugars and the H5(n)/CH3(n)–H6($n-1$)/H8($n-1$) cross peaks are in agreement with this assignment.

^{13}C-1′ spin–lattice relaxation rate measurements

The spin–lattice relaxation rates of the C3, A4 and A6 residues were measured at different temperatures between 17 and 47 °C. Table 1 shows that below 29 °C, the $R(C_{x,y})$ value of the C3 residue was always weaker than the adenine residues values by a difference of 2.7 ± 0.1 s^{-1} and that above 29 °C, the $R(C_{x,y})$ value of the A4 residue was always higher than the other values of C3 and A6. Moreover, the $R(H_z \rightarrow C_z)$ value was always different between the three labeled residues. These data provide evidence for a different dynamic behavior for the three residues. Table 1 gives the best fit obtained by analyzing these data with the two-parameter Lipari and Szabo approach.[14,15] For all the temperatures, except 47 °C, the order parameter S^2 is

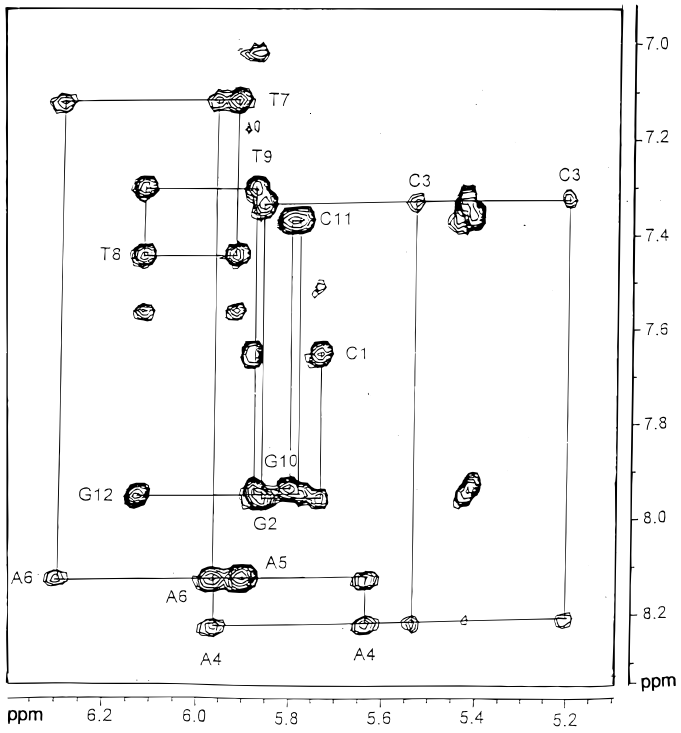

Figure 1. Expension of the NOESY spectra of the ^{13}C-labeled duplex d(CGCAAATTTGCG)$_2$ in D_2O, 1.5 mM at 22 °C, 0.1 M NaCl. The mixing time was 200 ms. The double connectivities implicating the C3, A4 and A6 resonances indicate the ^{13}C labeling of these residues.

0.80 ± 0.02 for A4 and A6 and 0.65–0.73 for C3. The effective correlation time is in the range 15–31 ps, but these values were spoiled by too large an uncertainty to make each difference indicative. Nevertheless, τ_e increased significantly with increase in temperature for the three residues. It can be pointed out that, reflecting the difference between the $R(C_{x,y})$ values, the global correlation time changed significantly from the adenine to the cytosine residues at the same temperature.

Table 1. Experimental values of the ^{13}C1′ relaxation rates in d(GCGAAATTTGCG)$_2$ at various temperatures with, τ_g, S^2 and τ_e computed by using the Lipari–Szabo relationship[14,15]

T (°C)	Residue	$R(C_z)$	$R(C_{x,y})$	$R(H_z \rightarrow C_z)$	τ_g (ns)	S^2	τ_e (ps)
17	C3	1.75	21.9	0.14	5.85	0.74	26
	A4	1.69	24.5	0.11	6.21	0.75	18
	A6	1.79	24.5	0.10	5.96	0.82	15
22	C3	2.09	18.3	0.17	4.79	0.74	31
	A4	1.96	21.0	0.13	5.25	0.79	20
	A6	2.04	20.9	0.12	5.09	0.81	16
24.5	C3	2.18	16.6	0.18	4.42	0.72	30
	A4	2.14	19.5	0.14	4.80	0.79	23
	A6	2.26	19.3	0.13	4.58	0.82	19
27	C3	2.38	15.0	0.20	3.94	0.72	31
	A4	2.42	18.5	0.16	4.33	0.82	29
	A6	2.47	18.1	0.14	4.19	0.72	31
29.5	C3	2.39	13.9	0.23	3.82	0.68	37
	A4	2.44	17.5	0.18	4.22	0.79	35
	A6	2.64	16.8	0.19	3.92	0.81	39
32	C3	2.54	13.0	0.24	3.52	0.68	38
	A4	2.60	16.8	0.20	3.98	0.80	42
	A6	2.85	15.7	0.20	3.58	0.82	40
37	C3	2.75	11.3	0.26	3.04	0.66	38
	A4	2.86	15.2	0.23	3.54	0.79	49
	A6	3.15	14.0	0.25	3.15	0.80	54
42	C3	2.92	10.4	0.28	2.75	0.64	39
	A4	3.12	13.7	0.27	3.15	0.78	59
	A6	3.36	12.3	0.32	2.81	0.76	69
47	C3	2.79	10.7	0.27	2.91	0.64	38
	A4	2.94	13.9	0.27	3.33	0.76	56
	A6	3.16	11.6	0.32	2.84	0.71	55

Are the three relaxation rates governed by the same relaxation processes? Before drawing a general conclusion from these results, the influence of the asymmetric shape of DNA needs to be tested. The duplex was modeled by a cylinder with a length ($12 \times 3.4 = 40.8$ Å) twice the diameter (20.5 Å). Then the ratio between the translational $D_{\|}$ and rotational $D_{\perp}$ diffusion coefficient was taken as 2.2.[16,17] The spectral density $J(\omega)$ were computed with the equation[18]

$$J(\omega) = S^2/4[(3\cos^2\theta - 1)j(\tau_1) + 3\cos^2\theta\sin^2\theta j(\tau_2) + 3/4\sin^2\theta j(\tau_3)] + (1 - S^2)j(\tau_i) \quad (1)$$

with $j(\tau) = 2/5\tau/(1 + \omega^2\tau^2)$, $\tau_1 = 6D_{\|}$, $\tau_2 = D_{\|} + 5D_{\perp}$ and $\tau_3 = 4D_{\|} + 2D_{\perp}$.

Using the solution structure of d(CGCAAATTTGCG)$_2$,[19] we computed a polar angle θ value of 83, 76 and 88° for the C1′H1′ vector of C3, A4 and A6 residues, respectively. These angles, which led to small variations (5%) of the density functions, are not able to explain the difference in the $R(C_{x,y})$ values of the different residues.

We concluded that our data were not fitted well with a single value of τ_g using the two-parameter model-free formalism. A good fit of the data of the three residues needs a 12–20% variation of the global correlation time, depending of the temperature, whereas it should be invariant along the sequence for the same temperature. Hence these simple equations cannot agree with the whole set of experimental data.

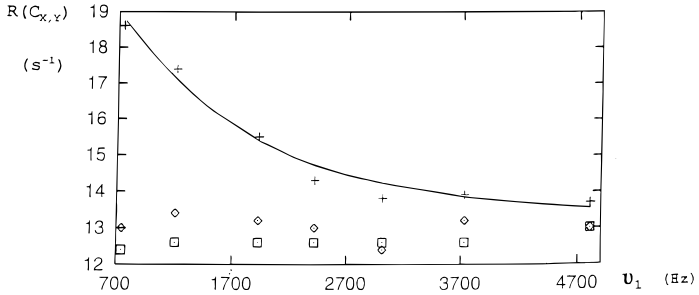

Figure 2. Plot of $R(C_{x,y})$ at 32 °C *vs.* the spin–lock power ν_1. The curve represents the fit of Eqn (3) to the experimental data. $R_d(C_{x,y})$ = 13.0 s^{-1}, τ_{ex} = 130 μs, $\Delta\omega^2 p_1 p_2$ = 61 000 rad^2 Hz^2. (□) C3; (+) A4; (◇) A6.

Additional relaxation pathways induced by slow motions. In order to explain the relaxation rate difference along the sequence of the duplex d(CGCAAATTTGCG)$_2$, the relative contribution of each possible relaxation process requires estimation. Taking into account that paramagnetic impurities such as cations or oxygen were carefully removed, relaxation processes involving paramagnetic impurities can be ruled out. Scalar relaxation of the second kind[20] could affect the spin–spin relaxation of the C1′through an indirect one-bond interaction if N1 (in the pyrimidines) or N9 (in the purines) relaxed unusually rapidly. However, ^{13}C spin-lock during the relaxation delay destroyed any scalar spin coupling effects. Consequently, scalar relaxation of the second kind cannot be taken into consideration under our experimental conditions. Chemical shift anisotropy relaxation, which has already been calculated to contribute only 2% to the total relaxation,[2] cannot markedly increase the $R(C_{x,y})$ relaxation rate. Consequently, differences in the three relaxation rates, and more particularly in $R(C_{x,y})$, for different residues in the same oligonucleotide could be attributed to additional slow motions or to chemical exchange.

Exchange relaxation. NMR chemical exchange processes are efficient when the exchange time-scale is in the range of the inverse of the resonance frequency difference between the two exchanging sites. This leads to exchange time-scales in the range of microseconds to seconds. Consequently, only components implicating low frequency are modified and the term R_{ex} is efficient on the spin–lattice relaxation rate $R(C_{x,y})$ only. For a two-step exchange process, the exchange contribution to $R_{ex}(C_{x,y})$ is related by the equation[21]

$$R_{ex} = (\Delta\omega)^2 p_1 p_2 \tau_{ex}/(1 + \omega_1{}^2\tau_{ex}^2) \qquad (2)$$

where p_1 and p_2 are the populations of the two conformers, $\Delta\omega$ the resonance frequency difference between the exchanging sites and ω_1 the equivalent frequency of the applied r.f. field. In this case, $R(C_{x,y})$ is the sum of the dipolar contribution $R_d(C_{x,y})$ and of the exchange contribution:

$$R(C_{x,y}) = R_d(C_{x,y}) + R_{ex} \qquad (3)$$

The dependence of $R(C_{x,y})$ on the applied r.f. field strength $\omega_1 = -\gamma B_1$ for the C3, A4 and A6 residues at 32 °C is shown in Fig. 2. The $R(C_{x,y})$ values of C3 and A6 were constant within the experimental uncertainty. On the other hand, the $R(C_{x,y})$ values of A4 decreased with ω_1 and tended toward a plateau for $\nu_1 > 3000$ Hz, giving direct evidence of exchange processes for the $^{13}C1'$ resonance of A4. The exchange lifetime τ_{ex} was derived by fitting $R(C_{x,y})$ with a least-squares program[13] computing Eqn (3) (Fig. 2). The best fit was obtained with $\tau_{ex} = 130$ μs, $(\Delta\omega)^2 p_1 p_2 = 61\,000$ rad^2 Hz^2 and $R_d(C_{x,y}) = 13\ s^{-1}$.

If we assume that p_1 is in the range 0.1–0.5, the apparent frequency difference between the two exchanging sites of A4 is estimated to be in the range 130–250 Hz. These frequency differences between the different conformational states implicate large amplitude motions such as several tens of a degree or several tenths of an angstrom. They can be favored by the strong ring current effects of the adenines which governed the $^{13}C1'$ chemical shift variations of their neighboring bases. Such a dynamic process can be related to a special structure characterized by a gradual and increasingly compressed minor groove which reached a minimum at the ApT step.[22]

Analysis of the spin–lattice relaxation rate including the exchange. The spin–lattice relaxation rates of the three residues were fitted after subtraction of R_{ex} from $R(C_{x,y})$ for A4. The best fit was obtained with a unique correlation time of 3.66 ns and an order parameter of 0.73, 0.75 and 0.83 for C3, A4 and A6, respectively, at 32 °C. In order to validate this analysis, an independent measure of τ_g using a proton cross relaxation rate was made. The H5–H6 NOE build-up rates of C3 and C11 (overlapped resonances) (Fig. 1) were measured at eight different mixing times. The simulated NOE were made by using the RELAZ program,[23] which computes the NOE after diagonalization of the complete relaxation matrix.[24] The best fit (Fig. 3) was obtained with a global correlation time of 3.4 ± 0.4 ns by using the conformation and the surrounding of the C3 residue computed in

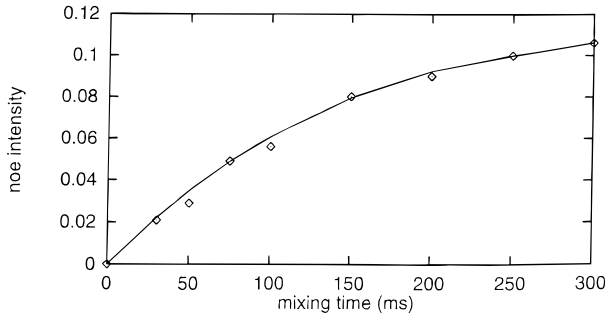

Figure 3. Plot of the NOE between H5 and H6 for C3 and C11 (average of the two values) measured at 32 °C *vs.* the mixing time. The curve represents the computed NOE (see text) with a global correlation time of 3.4 ns.

Table 2. Experimental values of the ^{13}C5′ relaxation rates in d(GCGAAATTTGCG)$_2$ at various temperatures, with τ_g, S^2 and τ_e computed by using the Lipari–Szabo relationship[14,15]

T (°C)	$R(C_z)$	$R(C_{x,y})$	$R(H_z \rightarrow C_z)$	τ_s (ns)	S^2	τ_e (ns)
22	1.85	7.40	0.25	3.15	0.41	26
27	2.19	6.21	0.31	2.46	0.41	32
32	2.38	5.67	0.29	2.05	0.42	27
37	2.57	4.83	0.43	1.71	0.37	44

the solution structure.[19] This value is in agreement with $\tau_g = 3.66$ ns computed via the ^{13}C relaxation rates and with the already published ^{13}C relaxation rate measurements on the three central thymines of the same oligonucleotide which gave $\tau_g = 3.3$ ns at 32 °C.

^{13}C-5′ spin–lattice relaxation rate measurements

The H5′–H5″ resonances and the ^{1}H–^{13}C5′ correlations appeared too overlapped to allow separate relaxation rate measurements for each residue. Consequently, Table 2 gives the average of the $R(C_z)$, $R(C_{x,y})$ and $R(H_z \rightarrow C_z)$ values for C3, A4 and A6. Taking into account that the carbon C5′ is bound to two hydrogen atoms, $R(C_z)$ and $R(C_{x,y})$ were computed by dividing the inverse of T_1 and $T_{1\rho}$ by two. The analysis of these data with the two parameter Lipari and Szabo relationship showed order parameters weaker than those of the ^{13}C1′ resonances for the same temperature. Nevertheless, the global correlation time computed at 32 °C (2 ns) is different than those computed for the C1′ resonance (3.7 ns), indicating supplementary pathways of relaxation. Introduction of an additional slow motion[25] led to $S_f^2 = 0.50$, $S_s^2 = 0.61$, $\tau_s = 1$ ns, $\tau_f = 2.5$ ps with τ_g fixed at 3.7 ns. It can be pointed out that this analysis was made by assuming that the relaxation rates of the three residues are close. This assumption may not be valid, in which case the curves will not be exponential and the fit error will be substantially higher. Consequently, attempts to relate these data with more accurate analysis seems hazardous at this time.

CONCLUSION

The three relaxation rates $R(C_z)$, $R(_{x,y})$ and $R(H_z \rightarrow C_z)$ were accurately measured for three residues in the duplex d(CGCAAATTTGCG)$_2$. For each temperature, the three residues exhibited fast motions on the timescale of a few picoseconds. This fast process could correspond to the motion of the sugar around the glycosidic bond.[2] The r.f. field strength dependence of the $R(C_{x,y})$ values of C1′ provided evidence for an exchange process for the A4 residue at 32 °C. It can be pointed out that no additional relaxation pathways induced by slow motions have been observed for the other labeled residues (C3, A6, T7, T8 and T9).[2] The exchange process of the adenine residue is favored by the important ring current of this base. This additional relaxation process was analyzed in terms of chemical exchange between distinct conformational states involving large amplitude and slow (130 μs) motions which can reflect special dynamics of the tract A_3T_3 which supports the spine of hydration.[26] The relaxation rates of the C5′ carbon provided evidence for internal motions with higher amplitude than for C1′. These weak order parameters are in agreement with the additional motions undergone by the C5′H5′ or C5′H5″ vectors, such as repuckering of sugars and rotation around the C4′—C5′ bond.

REFERENCES

1. J. W. Peng and G. Wagner, *J. Magn. Reson.* **98**, 308 (1992).
2. F. Gaudin, F. Paquet, L. Chanteloup, J. M. Beau, N. T. Thuong and G. Lancelot, *J. Biomol. NMR* **5**, 49 (1995).
3. D. M. Crothers, T. E. Haran and J. G. Nadeau, *J. Biol. Chem.* **265**, 7093 (1990).
4. P. J. Hagerman, *Nature* (*London*) **321**, 449 (1986).
5. J. L. Leroy, E. Charretier, E. Kochoyan and M. Guéron, *Biochemistry* **27**, 8894 (1988).
6. D. G. Brown, M. S. Sanderson, E. Garman and S. Neidle, *J. Mol. Biol.* **226**, 481 (1992).
7. L. Chanteloup and J. M. Beau, *Tetrahedron Lett.* **33**, 5347 (1992).
8. H. Vorbrüggen, K. Krolikiewizc and B. Bennua, *Chem. Ber.* **114**, 1234 (1981).
9. L. E. Kay, L. K. Nicholson, F. Delaglio, A. Bax and D. A. Torchia, *J. Magn. Reson.* **97**, 359 (1992).
10. J. Boyd, U. Hommel and I. D. Campbell, *Chem. Phys. Lett.* **175**, 477 (1990).
11. A. G. Palmer, M. Rance and P. E. Wright, *J. Am. Chem. Soc.* **113**, 4372 (1991).
12. J. W. Peng, V. Thanabal and G. Wagner, *J. Magn. Reson.* **94**, 82 (1991).
13. G. Lancelot, *Biochimie* **59**, 587 (1977).
14. G. Lipari and A. Szabo, *J. Am. Chem. Soc.* **104**, 4546 (1982).
15. G. Lipari and A. Szabo, *J. Am. Chem. Soc.* **104**, 4559 (1982).
16. M. M. Tirado and J. Garcia de la Torre, *J. Chem. Phys.* **71**, 2581 (1979).
17. M. M. Tirado and J. Garcia de la Torre, *J. Chem. Phys.* **73**, 1986 (1980).
18. D. E. Woessner, *J. Chem. Phys.* **37**, 647 (1962).
19. F. Gaudin, D. Genest and G. Lancelot, to be published.
20. A. Abragam, *The Principles of Nuclear Magnetism*. Oxford University Press, Oxford (1961).
21. M. J. Blackledge, R. Brüschweiler, C. Griesinger, J. M. Schmidt, P. Xu and R. R. Ernst, *Biochemistry* **32**, 10960 (1993).
22. A. A. Lipanov and V. P. Chuprina, *Nucleic Acids Res.* **15**, 5833 (1987).
23. G. Lancelot, J. L. Guesnet and F. Vovelle, *Biochemistry* **28**, 4982 (1990).
24. J. W. Keepers and T. L. James, *J. Magn. Reson.* **57**, 404 (1984).
25. G. M. Clore, P. C. Driscoll, P. T. Wingfield and A. M. Gronenborn, *Biochemistry* **29**, 7387 (1990).
26. H. R. Drew and R. E. Dickerson, *J. Mol. Biol.* **151**, 535 (1981).