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Internal mobility of the oligonucleotide duplexes $d(TCGCG)_2$ and $d(CGCGCG)_2$ in aqueous solution from molecular dynamics simulations

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Summary

In this paper we present longitudinal relaxation times, order parameters and effective correlation times for the base and sugar carbons in both strands of the oligonucleotide duplexes $d(TCGCG)_2$ and $d(CGCGCG)_2$, as calculated from 400 ps molecular dynamics trajectories in aqueous solution. The model-free approach (Lipari and Szabo, 1982) was used to determine the amplitudes and time scales of the internal motion. Comparisons were made with NMR relaxation measurements (Borer et al., 1994). The order parameters could acceptably be reproduced, and the effective correlation times were found to be lower than the experimental estimates. Reasonable T_1 relaxation times were obtained in comparison with experiment for the nonterminal nucleosides. The T_1 relaxation times were found to depend mainly on the order parameters and overall rotational correlation time.

Introduction

Nucleic acids are very flexible biomolecules and it is of great interest to study their dynamic behavior to increase our understanding of the intrinsic properties of the DNA double helix, and to aid the interpretation of experimental observations. Both base stacking and base pairing play an important role in the stabilization of the DNA double helix (Saenger, 1988). Unpaired terminal nucleotides, so-called dangling ends, are also very useful to examine, because they increase the stability of oligonucleotide double helices (Sugimoto et al., 1987).

The internal motion of nucleic acids can be studied using nuclear spin relaxation time measurements, but up to now there have been very few investigations concerning the dynamics, i.e., the amplitudes and time scales. Spin-lattice relaxation times (^{13}C T_1) of carbons are predominantly determined by the dipolar interaction of the carbon with its directly bonded proton. However, for base carbons (sp^2) a lesser contribution is due to the chemical shift anisotropy ($\sigma_\parallel - \sigma_\perp$), which has been determined to be 185 ppm for the C6 carbon of a thymine base (Williamson and Boxer, 1988). ^{13}C T_1 relaxation times can also be determined from molecular dynamics (MD) simulations and so far ^{13}C T_1 relaxation times have been calculated for proteins (Balasubramanian et al., 1994; Smith et al., 1995). The overall rotational correlation time of a dinucleoside monophosphate, which is nearly spherical and can therefore be described by a single correlation time, is about 150 ps (Norberg and Nilsson, 1994a). The diameter of a B-DNA duplex is about 20 Å and the axial rise between bases is nearly 3.4 Å. This would suggest that B-DNA duplexes of 5–6 base pairs, such as pentamers and hexamers, are approximately spheroidal. The overall correlation times of various oligonucleotides have been determined by depolarized dynamic light scattering and NMR relaxation measurements (Eimer et al., 1990; Borer et al., 1994). For evaluation of the time scales and

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Abbreviations: MD, molecular dynamics; CSA, chemical shift anisotropy.

amplitudes of the internal motion and for determination of the relaxation times the model-free approach is usually used (Lipari and Szabo, 1982). The model-free approach assumes isotropic overall motion and describes the internal motion by generalized order parameters and effective internal correlation times.

In this MD simulation study of the oligonucleotide duplexes $d(TCGCG)_2$ and $d(CGCGCG)_2$ in aqueous solution we have calculated internal correlation times, generalized order parameters and relaxation times for both strands in the two oligonucleotide duplexes. One advantage of the MD simulations is that both strands can be studied which may also be used as an indication of the precision of the results.

Methods

Correlation function and spectral density

In the model-free approach (Lipari and Szabo, 1982) the ^{13}C NMR relaxation depends on both the internal and overall motion of the macromolecule. For an isotropically rotating macromolecule, the overall motion is assumed to be uncorrelated with the internal motion. The overall motion can therefore be described by a single correlation time, which is in the nanosecond time scale. The analysis of internal motion using ^{13}C NMR relaxation measurements concerns the dipolar relaxation between the carbon atom and its bonded hydrogen atom. Therefore, the motion of the internuclear vector ^{13}C-^{1}H can be analyzed as a function of time using an internal correlation function. The total correlation function of the decoupled overall and internal motion is given by:

$$C(t) = 1/5\, C_R(t)\, C_I(t) \tag{1}$$

where the correlation functions, $C_R(t)$ and $C_I(t)$, describe the overall molecular tumbling and internal motion, respectively. The isotropic overall tumbling of the molecule is given by:

$$C_R(t) = e^{-t/\tau_R} \tag{2}$$

where τ_R is the overall rotational correlation time. The correlation function of the internal motion does not decay to zero, but instead approaches a plateau value defined by the generalized order parameter (S^2), which is due to the restricted motion of the internuclear ^{13}C-^{1}H vector. The internal motion is described by the correlation function of the ^{13}C-^{1}H vector:

$$C_I(t) = S^2 + (1 - S^2)e^{-t/\tau_e} \tag{3}$$

where τ_e is the effective correlation time of internal motion and S^2 is the generalized order parameter, which describes the spatial restriction of the motion. S^2 is close to 1 for considerably restricted motion, but zero for isotropic internal motion where all orientations are equally probable. The effective correlation time, τ_e, of the ^{13}C-^{1}H vector can be calculated by:

$$\tau_e (1-S^2) = \int_0^\infty (C_I(t) - S^2)\,dt \tag{4}$$

If the MD simulation is long enough, so that the ^{13}C-^{1}H vector is accurately sampled, the correlation function $C_I(t)$ can be calculated from an MD trajectory using:

$$C_I(t) = \langle P_2(\cos\theta(\tau,t))\rangle \tag{5}$$

where $\theta(\tau,t)$ is the angle between the ^{13}C-^{1}H vector at a time τ and the same vector at a time t later, and P_2 is the second-order Legendre polynomial.

By integration of the total correlation function the corresponding spectral density can be obtained as follows:

$$J(\omega) = 2\int_0^\infty C(t)\cos(\omega t)\,dt \tag{6}$$

To accurately calculate the spectral density one would like to use the Fourier transformation of the correlation function, but the MD simulations are not long enough to give accurate estimates of the low-frequency region of the spectral density. The spectral density for the model-free approach (Lipari and Szabo, 1982) is:

$$J(\omega) = \frac{2}{5}\left[\frac{S^2\tau_R}{1+(\omega\tau_R)^2} + \frac{(1-S^2)\tau}{1+(\omega\tau)^2}\right] \tag{7}$$

with

$$\frac{1}{\tau} = \frac{1}{\tau_R} + \frac{1}{\tau_e} \tag{8}$$

NMR relaxation

The relaxation rate of the ^{13}C nucleus is due to the dipolar interaction with its directly bonded hydrogen atom. The spin-lattice relaxation rate $1/T_1^{CH}$ (Abragam, 1961) of the ^{13}C nucleus is defined as:

$$1/T_1^{CH} = \frac{1}{10}\left(\frac{\mu_0}{4\pi}\frac{\gamma_C\gamma_H\hbar}{r_{CH}^3}\right)^2 [J(\omega_H-\omega_C)+3J(\omega_C)+6(\omega_H+\omega_C)] \tag{9}$$

where μ_0 is the permeability of vacuum, γ_C and γ_H are the gyromagnetic ratios of the nuclei C and H, respectively, $\hbar$ is the Planck constant divided by 2π, r_{CH} is the bond distance of ^{13}C-^{1}H, and ω_C and ω_H are the Larmor frequencies of the nuclei C and H, respectively. The relaxation of the base carbons are mainly due to the dipolar

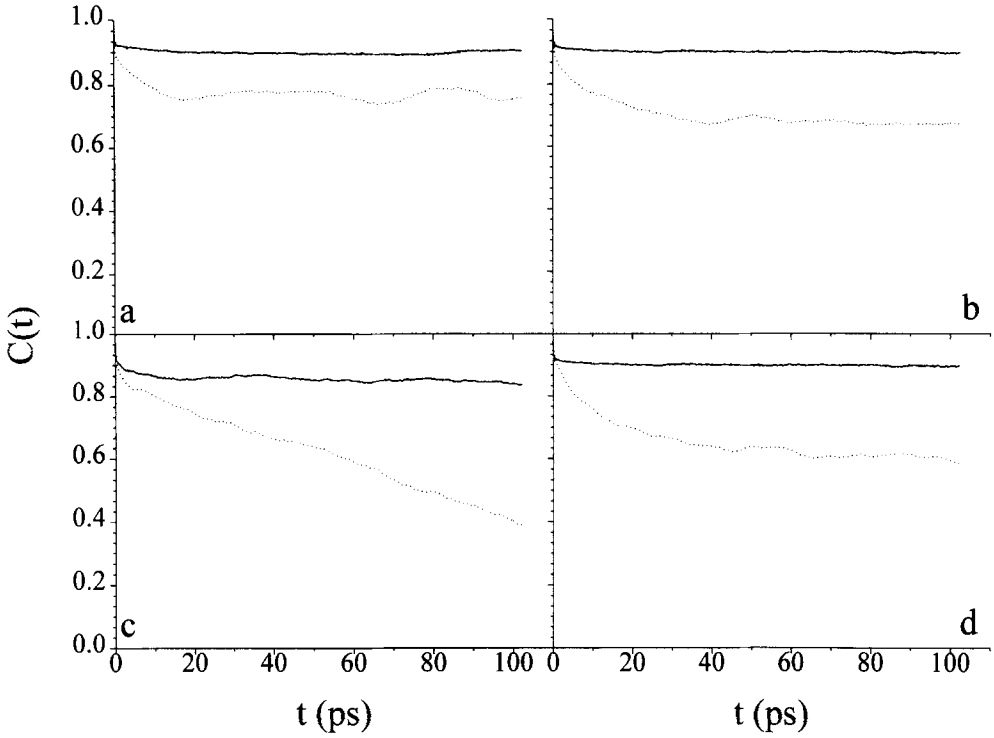

Fig. 1. Internal correlation functions $C_l(t)$ for the ^{13}C-^1H vectors of the d(CGCGCG)$_2$ duplex. Data are shown for the C3,5 (——) and C1,5 (· · · ·) base carbons in strands 1 (a) and 2 (b) and for the C3,5" (——) and G6,2" (· · · ·) sugar carbons in strands 1 (c) and 2 (d).

interaction of the directly attached hydrogen atoms, but with a smaller contribution due to chemical shift anisotropy (Williamson and Boxer, 1989). The $1/T_1^{CH}$ relaxation rate due to the chemical shift anisotropy mechanism is given by:

$$1/T_{1CSA}^{CH} = \frac{2}{15}[\omega_C(\sigma_{\parallel} - \sigma_{\perp})]^2 J(\omega_C) \qquad (10)$$

where $\sigma_{\parallel} - \sigma_{\perp}$ is the chemical shift anisotropy of the C nucleus.

Molecular dynamics simulation protocol

The two oligonucleotide duplexes studied here were generated as right-handed double-stranded standard B-DNA conformations from X-ray fiber diffraction data (Arnott et al., 1976). One was the pentamer d(TCGCG)$_2$, which has a thymidine dangling end, and the other was the hexamer d(CGCGCG)$_2$. To make the pentamer and hexamer oligonucleotide duplexes electrically neutral, eight and ten sodium counterions, respectively, were placed on the bisector of the phosphate oxygens, 4.7 Å from the phosphate atom. Each oligonucleotide duplex was immersed in a sphere built of TIP3P water molecules (Jorgensen et al., 1983) and with a radius of 23.0 Å. To remove bad contacts in the systems, the water molecules were energy-minimized in 200 cycles of steepest descent, while the duplexes were constrained using a harmonic potential with a force constant of 20.0 kcal mol^{-1} Å^{-2}. Stochastic boundary conditions (Brooks and Karplus, 1983) were applied during the simulations; water molecules in the layer outside a radius of 20 Å were propagated with Langevin dynamics using a friction constant of 50.0 ps^{-1} for the oxygen atoms, while all atoms inside this radius were treated with the Verlet algorithm (Verlet, 1967). To allow for a time step of 0.002 ps, all hydrogen-atom–heavy atom bond lengths were constrained using the SHAKE algorithm (Ryckaert et al., 1977). A relative dielectric constant of 1.0 was used and the nonbonded interactions were smoothly shifted to zero at a cutoff of 11.5 Å, using the atom-based force-shift method (Brooks et al., 1985). The nonbonded interactions were updated every 20 steps and the coordinates were saved every 0.2 ps. The systems were heated from 0 to 300 K during the first 30 ps and then equilibrated for 70 ps. Thereafter the simulations were continued up to 500 ps, and the last 400 ps of each trajectory were used for the analysis. The energy minimizations and MD simulations were performed using the CHARMM program (Brooks et al., 1983) with the all-atom nucleic acids parameters (MacKerell et al., 1995) on DEC AXP 3000/400 workstations.

Relaxation calculation details

The generalized order parameters S^2 were determined by estimating the plateau value from the correlation function $C_l(t)$. For the internuclear distance r_{CH} we used 1.09 Å and for the overall rotational correlation time τ_R the experimental values 0.9 ns (Borer et al., 1994) and

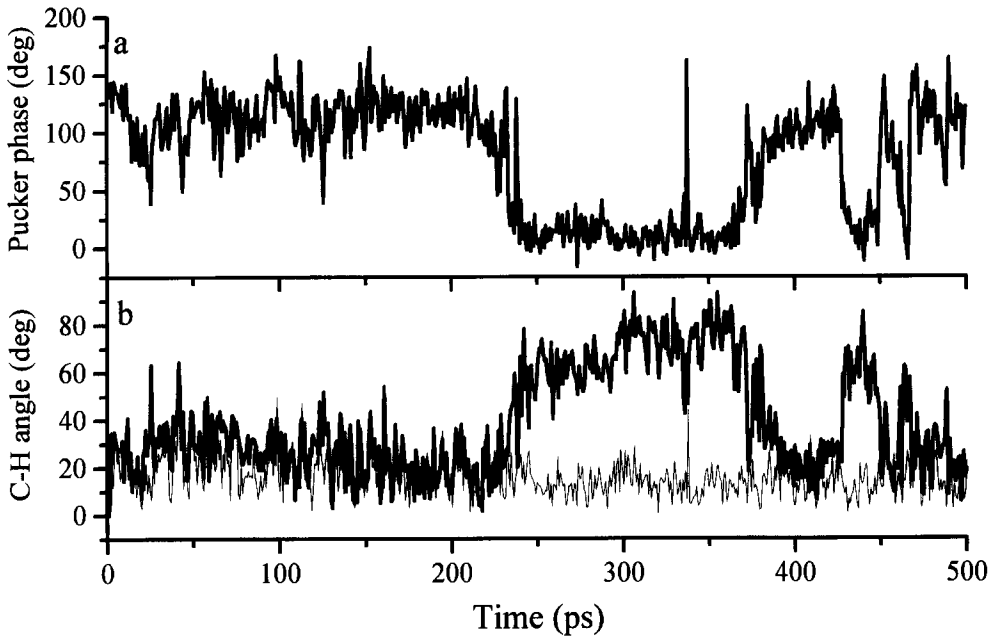

Fig. 2. Time dependence of the conformation of the sugar moiety of G6 in strand 1 of the d(CGCGCG)$_2$ duplex. (a) Sugar pucker phase angle (Altona and Sundaralingam, 1972); (b) the instantaneous angle formed by the C2'-H2" (thick line) and the C4'-H4' (thin line) vectors from their respective starting directions.

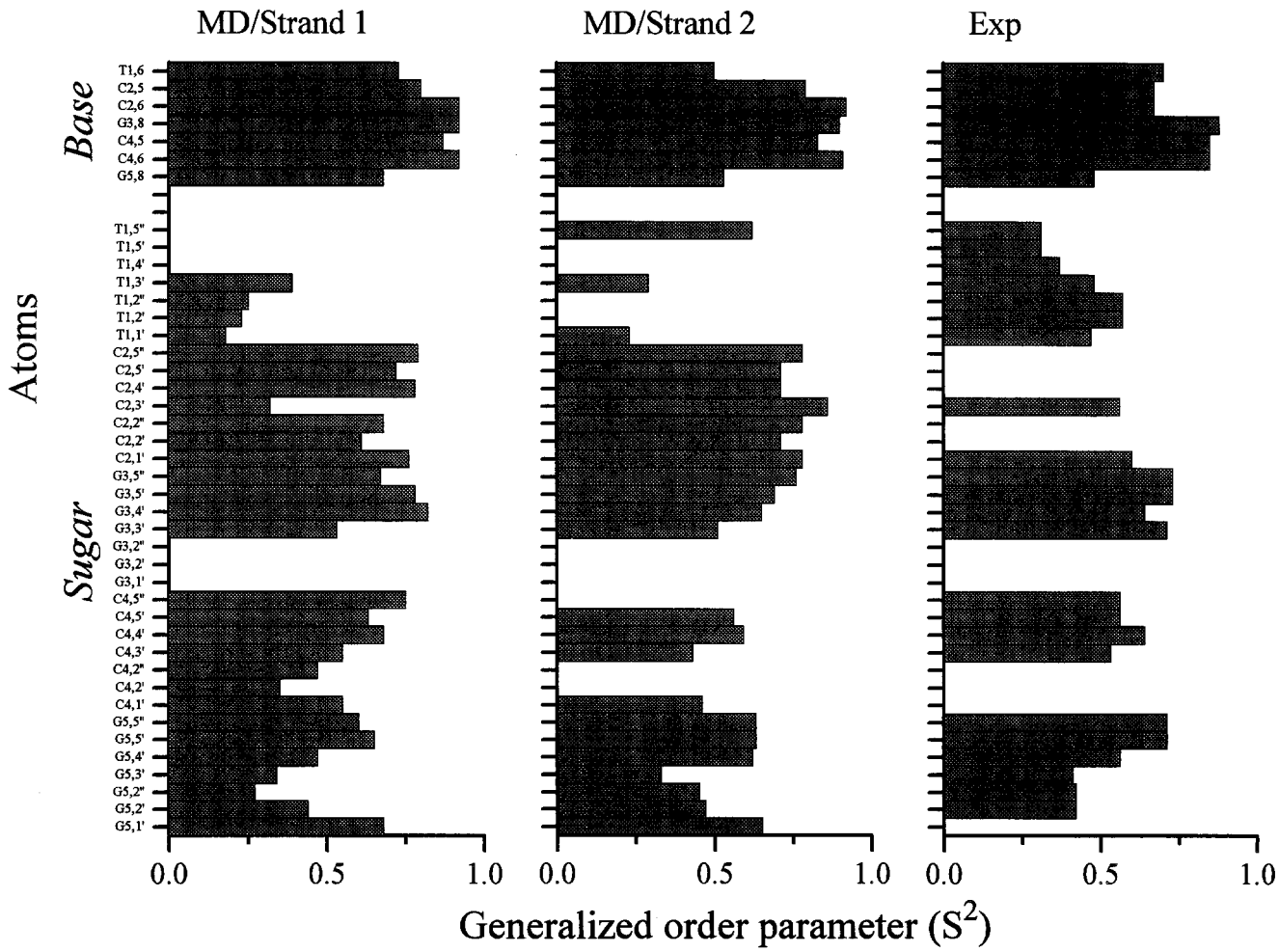

Fig. 3. Generalized order parameters of base and sugar carbons of strands 1 and 2 and experimental values (Borer et al., 1994) of the d(TCGCG)$_2$ duplex.

1.1 ns (approximated from Eimer et al., 1990) for the $d(TCGCG)_2$ duplex, and 1.4 ns (Borer et al., 1994) and 1.5 ns (approximated from Eimer et al., 1990) for the $d(CGCGCG)_2$ duplex were used. A value of 185 ppm (Williamson and Boxer, 1989) was used to calculate the chemical shift anisotropy ($\sigma_{\parallel} - \sigma_{\perp}$) of base carbons. Nuclear g-factors of 5.586 and 1.405 for the ^1H and ^{13}C nuclei, respectively, and a nuclear magneton, μ_N, of 5.05082×10^{-27} A m^2 were employed to determine the gyromagnetic ratio $\gamma_X = g_X\mu_N/\hbar$ (X = ^1H and ^{13}C). The ^{13}C T_1 relaxation times were calculated at 62.9, 90.6 and 125.8 MHz for the $d(TCGCG)_2$ duplex, and at 90.6 and 125.8 MHz for the $d(CGCGCG)_2$ duplex.

Atom definitions

All the ^{13}C-^1H vectors are defined by the nucleoside letter, the chain position and the carbon location. For instance, G2,8 in the $d(CGCGCG)_2$ duplex means the C8-H8 vector of the guanine base at the second position of the duplex, and T1,4' in the $d(TCGCG)_2$ duplex means the C4'-H4' vector of the sugar moiety of the thymidine nucleoside at the first position. G3,2' and G3,2" in the $d(TCGCG)_2$ duplex mean the C2'-H2' and C2'-H2" vectors, respectively, of the sugar moiety of the guanosine nucleoside at the third position. The strands of the oligonucleotide duplexes are defined as 1 and 2, and whether it concerns strand 1 or 2 is specified separately.

Results and Discussion

Stability of the trajectory

To evaluate the stability of the trajectory, the time evolution of the all-atom root-mean-square (rms) deviation from the initial structure was calculated. During the heating and equilibration periods of 30 and 70 ps, respectively, the rms deviation of the oligonucleotides increased and reached a plateau value, indicating that the systems were fully equilibrated. The average all-atom rms deviations from the initial structure were 2.80 and 2.53 Å for the $d(CGCGCG)_2$ and the $d(TCGCG)_2$ duplexes, respectively, with rms fluctuations of 0.30 Å for both duplexes during the last 400 ps. If only the heavy atoms were included, rms deviations from the initial structure of 2.52 Å and of 2.24 Å for the $d(CGCGCG)_2$ and $d(TCGCG)_2$

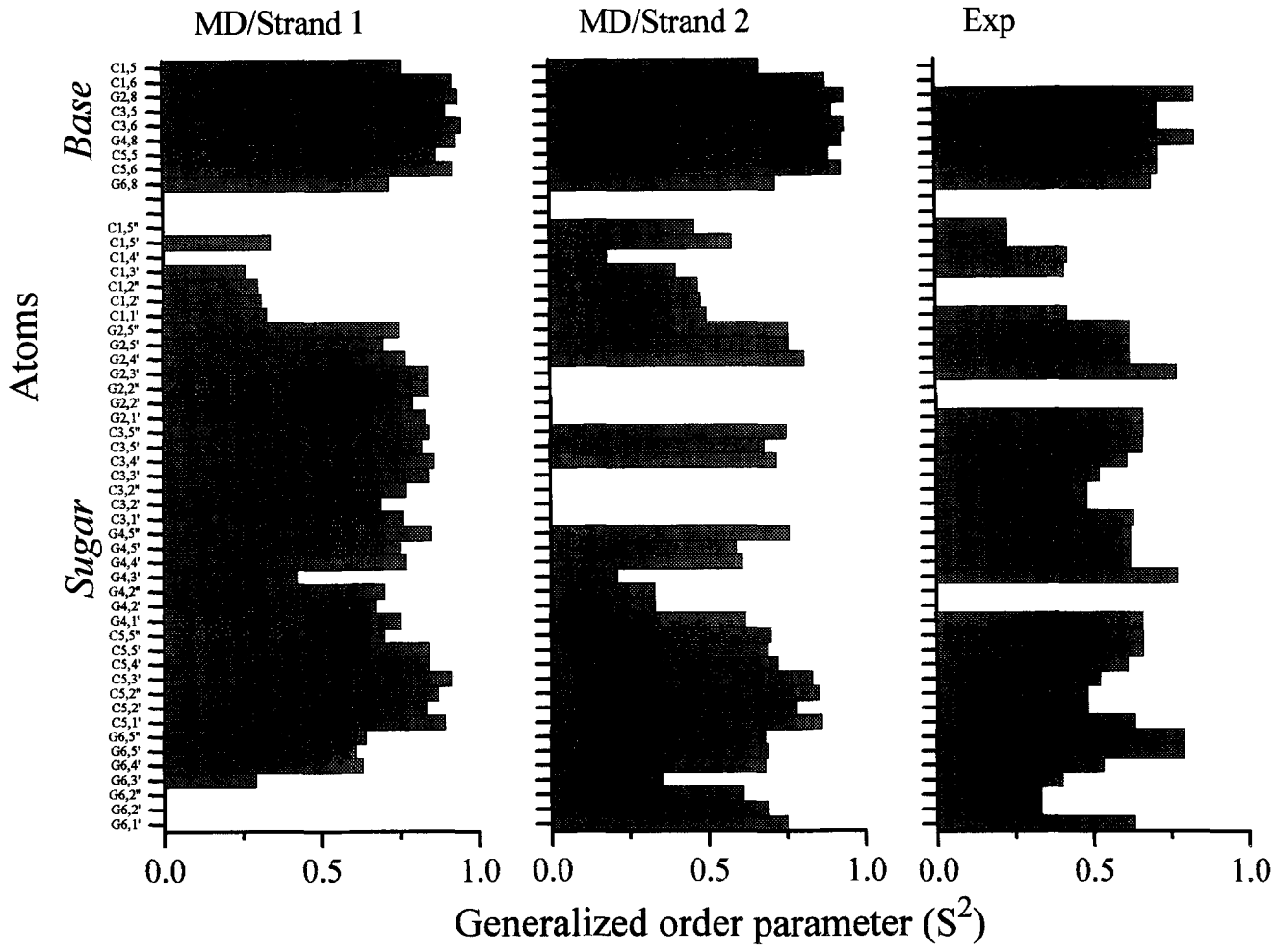

Fig. 4. Generalized order parameters of base and sugar carbons of strands 1 and 2 and experimental values (Borer et al., 1994) of the $d(CGCGCG)_2$ duplex.

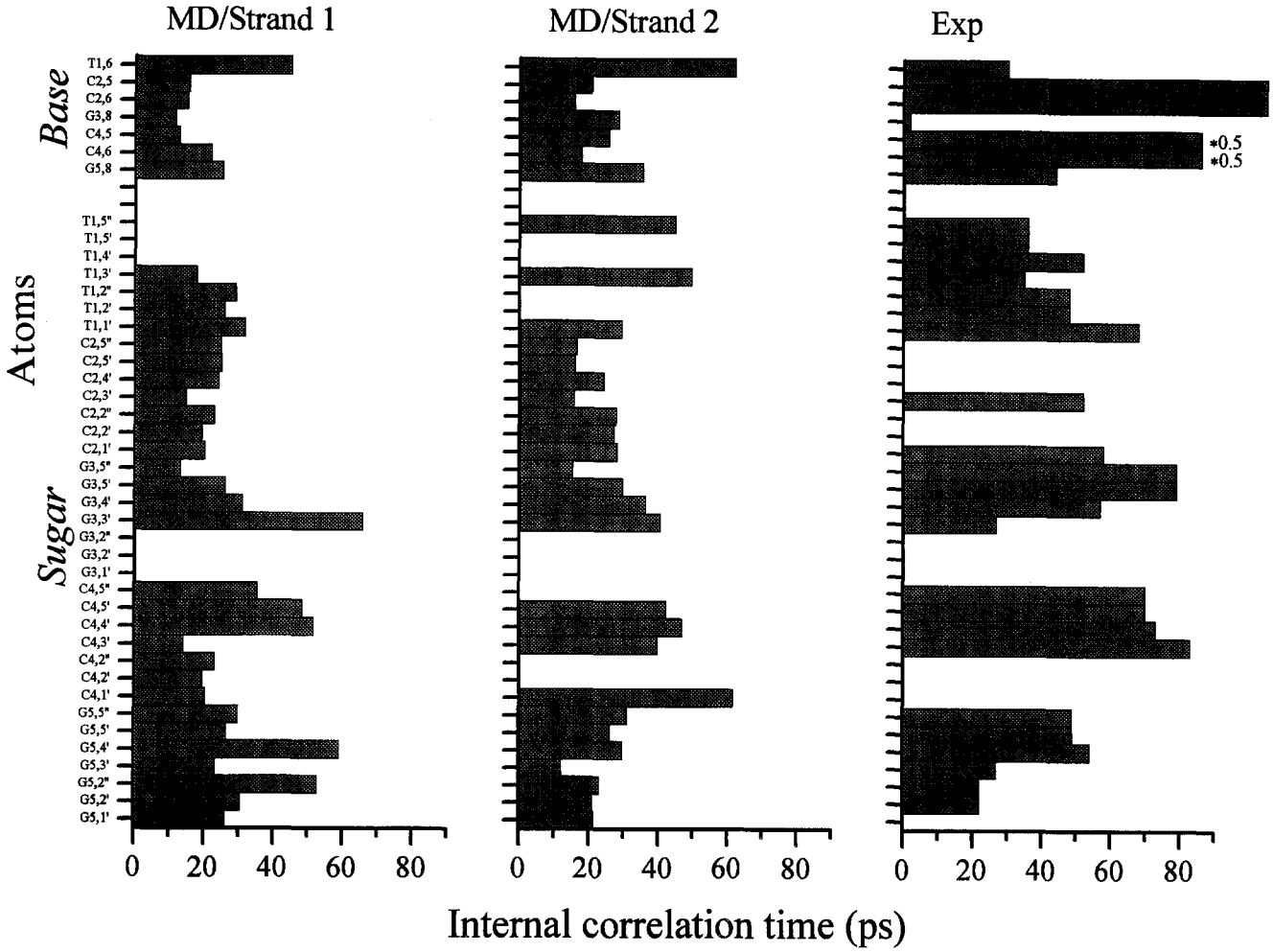

Fig. 5. Internal correlation times of base and sugar carbons of strands 1 and 2 and experimental values (Borer et al., 1994) of the d(TCGCG)$_2$ duplex. The experimental values of C4,5 and C4,6 are multiplied by 0.5.

duplexes, respectively, were obtained. In the analysis below we present results for the two strands separately, even though they are equivalent and should not be seen as separate entities in an experiment. We do this nevertheless to give an indication of the precision of the simulation data.

Correlation functions and generalized order parameters

The internal reorientational correlation functions were calculated for the ^{13}C-^{1}H vectors of the two strands in the d(CGCGCG)$_2$ and the d(TCGCG)$_2$ duplexes using Eq. 5. The correlation functions were not calculated for the methyl group of the thymine bases. Representative examples of the internal correlation functions $C_I(t)$ are displayed for the oligonucleotide d(CGCGCG)$_2$ in Fig. 1. For both duplexes $C_I(t)$ shows a very fast initial decay and then a stable plateau value (solid lines in Figs. 1a, b, c and d). From this behavior it was easy to estimate the generalized order parameters. Other $C_I(t)$ values showed a much slower initial decay and then a plateau value, which in some cases was stable and in others noisy (dotted lines in Figs. 1a, b and d). For a few other ^{13}C-^{1}H vectors no plateau value was reached (dotted line in Fig. 1c) and therefore the order parameters could not be determined. For the G3,1', G3,2' and G3,2" sugar carbons in both strands of the d(TCGCG)$_2$ duplex we were not able to estimate the plateau value of the correlation functions; experimentally these sugar carbons were also behaving difficult, so no order parameter could be determined (Borer et al., 1994). For many nucleotides the 2' and 2" sugar carbons it proved to be difficult to obtain the plateau value.

The difficult behavior of the sugar carbon correlation functions may be related to sugar repuckering events, which are not adequately sampled during the simulations, as can be seen in Fig. 2. The sugar-pucker phase angle for G6 in strand 1 of the d(CGCGCG)$_2$ duplex is shown together with $\phi(t)$, the angle formed by a CH vector at time t with itself at the beginning of the simulation, for the C2'-H2" and C4'-H4' vectors of the same sugar; the angles were calculated after removal of the overall rotation of the duplex. The repuckering that occurs after 220 ps leads to a significant change in the direction of the C2'-H2" vector, which is the direct cause of the 'not-so-

well-behaving' correlation function in Fig. 1c. The C4'-H4' vector of the same sugar is however not at all affected by the sugar repuckering, and thus the correlation function can be calculated and used to extract a generalized order parameter for some sugar carbons, even if the sugar pucker has changed during the simulation. We found (data not shown) the directions of the H2', H2" and H3' vectors to be the most sensitive to the sugar pucker, which is why it was more difficult to determine the plateau values for these correlation functions.

In the 400 ps d(CGCGCG)$_2$ duplex simulation the sugar repuckering was found to occur between 0 (for the C5 sugar moieties) and 10 (for the C1 sugar moiety of strand 1) times.

In general, we observed the same shape of the correlation function for a specific ^{13}C-^{1}H vector in both strands, but significant differences were also seen (dotted lines in Figs. 1c and d). A lower level of the plateau value and therefore a lower order parameter was found for the terminal bases (Fig. 1a).

The generalized order parameters of the ^{13}C-^{1}H vectors of both strands in the d(TCGCG)$_2$ and d(CGCGCG)$_2$ duplexes and the experimental data (estimated from Borer et al., 1994) of these duplexes are presented in Figs. 3 and 4. Some order parameters could not be determined in the experiment due to overlapping or ambiguously assigned carbon resonances. For the base carbons we observed the highest order parameters for the internal bases of both duplexes. In the d(TCGCG)$_2$ duplex the thymine base (dangling end) was found to stack quite well on the base-paired core. This was observed in the calculation of the distance, R_{NN}, between the glycosidic nitrogens of bases adjacent in the sequence, which in MD simulations (Norberg and Nilsson, 1994a,b) had previously been seen to represent the degree of stacking well. The R_{NN} values for the two TC steps in d(TCGCG)$_2$ were 4.4 Å (±0.4 Å) and 4.8 Å (±0.7 Å), whereas the averages for all CG and GC steps in d(TCGCG)$_2$ and d(CGCGCG)$_2$ were 4.9 and 4.4 Å, respectively. The shorter R_{NN} value for the GC step is in agreement with results obtained from potential of mean-force calculations of deoxyribodinucleoside monophosphates (Norberg and Nilsson, 1995), but the dangling end thymine in the present simulation seems, according to this distance criterion, to stack better on the CGCG du-

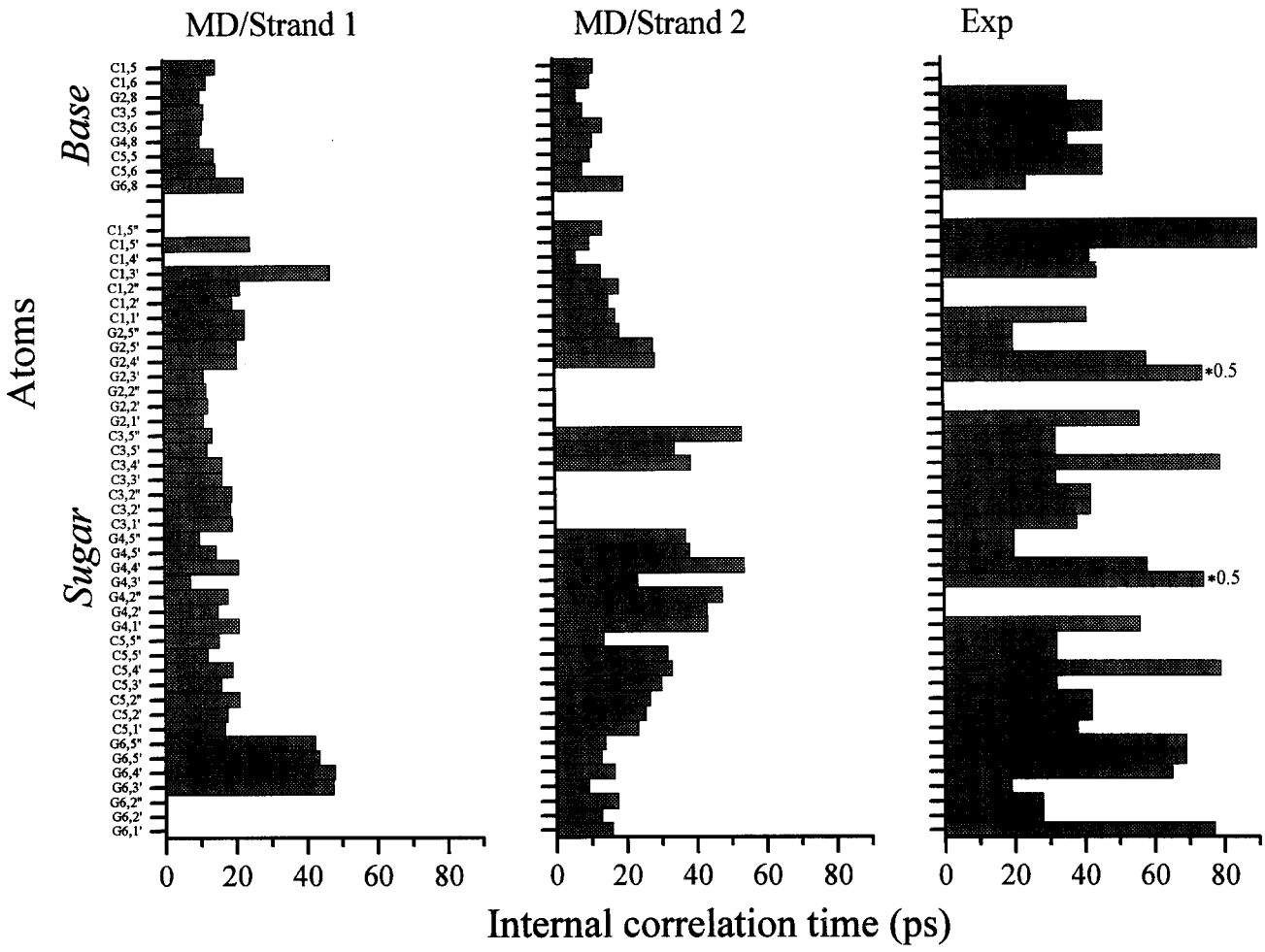

Fig. 6. Internal correlation times of base and sugar carbons of strands 1 and 2 and experimental values (Borer et al., 1994) of the d(CGCGCG)$_2$ duplex. The experimental values of G2,3' and G4,3' are multiplied by 0.5.

TABLE 1
^{13}C RELAXATION TIMES OF THE OLIGONUCLEOTIDE d(TCGCG)$_2$

Carbon	T_1 (s) at 62.9 MHz			T_1 (s) at 90.6 MHz			T_1 (s) at 125.8 MHz		
	Strand 1	Strand 2	Exp.	Strand 1	Strand 2	Exp.	Strand 1	Strand 2	Exp.
T1,6	0.19	0.27	0.12	0.21	0.30	0.15	0.25	0.34	0.23
C2,5	0.18	0.18	nd[a]	0.20	0.20	nd	0.23	0.23	nd
C2,6	0.16	0.16	0.14	0.17	0.17	0.16	0.20	0.20	0.19
G3,8	0.16	0.16	0.14	0.17	0.18	0.13	0.20	0.20	0.18
C4,5	0.17	0.17	nd	0.18	0.19	nd	0.21	0.22	nd
C4,6	0.16	0.16	0.10	0.17	0.18	0.13	0.20	0.20	0.17
G5,8	0.21	0.26	0.19	0.23	0.29	0.21	0.27	0.33	0.28
T1,5"	nd	0.23	0.33	nd	0.25	0.40	nd	0.28	0.46
T1,5'	nd	nd	0.33	nd	nd	0.40	nd	nd	0.46
T1,4'	nd	nd	nd	nd	nd	0.30	nd	nd	0.41
T1,3'	0.36	0.44	0.24	0.40	0.48	0.25	0.45	0.54	0.39
T1,2"	0.52	nd	0.18	0.57	nd	0.22	0.65	nd	0.39
T1,2'	0.57	nd	0.18	0.62	nd	0.22	0.71	nd	0.39
T1,1'	0.69	0.56	nd	0.75	0.62	0.22	0.84	0.69	0.41
C2,5"	0.18	0.18	nd	0.20	0.20	nd	0.23	0.23	nd
C2,5'	0.20	0.20	nd	0.22	0.22	nd	0.25	0.26	nd
C2,4'	0.18	0.20	nd	0.20	0.22	nd	0.23	0.25	nd
C2,3'	0.44	0.17	0.20	0.48	0.19	0.22	0.55	0.21	0.32
C2,2"	0.21	0.18	nd	0.23	0.20	nd	0.27	0.23	nd
C2,2'	0.23	0.20	nd	0.26	0.22	nd	0.30	0.25	nd
C2,1'	0.19	0.18	nd	0.21	0.20	0.22	0.24	0.23	0.28
G3,5"	0.21	0.19	0.13	0.24	0.21	0.18	0.27	0.24	0.25
G3,5'	0.18	0.21	0.13	0.20	0.23	0.18	0.23	0.26	0.25
G3,4'	0.18	0.22	nd	0.19	0.24	0.21	0.22	0.27	0.27
G3,3'	0.26	0.27	0.15	0.28	0.30	0.21	0.32	0.34	0.25
G3,2"	nd	nd	nd	nd	nd	nd	nd	nd	nd
G3,2'	nd	nd	nd	nd	nd	nd	nd	nd	nd
G3,1'	nd	nd	nd	nd	nd	nd	nd	nd	nd
C4,5"	0.19	nd	0.19	0.21	nd	0.23	0.24	nd	0.28
C4,5'	0.22	0.25	0.19	0.24	0.27	0.23	0.28	0.31	0.28
C4,4'	0.21	0.24	0.14	0.23	0.26	0.18	0.26	0.30	0.32
C4,3'	0.26	0.32	0.18	0.29	0.35	0.22	0.33	0.40	0.30
C4,2"	0.30	nd	nd	0.33	nd	nd	0.38	nd	nd
C4,2'	0.40	nd	nd	0.44	nd	nd	0.50	nd	nd
C4,1'	0.26	0.29	nd	0.28	0.32	nd	0.33	0.36	nd
G5,5"	0.24	0.23	0.15	0.26	0.25	0.19	0.30	0.28	0.25
G5,5'	0.22	0.23	0.15	0.24	0.25	0.19	0.28	0.28	0.25
G5,4'	0.29	0.23	nd	0.31	0.25	0.22	0.36	0.29	0.32
G5,3'	0.40	0.43	0.27	0.44	0.47	0.31	0.51	0.54	0.43
G5,2"	0.46	0.31	0.26	0.50	0.34	0.32	0.56	0.39	0.43
G5,2'	0.31	0.30	0.26	0.35	0.33	0.32	0.39	0.38	0.43
G5,1'	0.21	0.22	nd	0.23	0.24	nd	0.27	0.28	nd

For strands 1 and 2 the MD simulation results are shown; experimental data were taken from Borer et al. (1994).
[a] nd = not determined.

plex than it does on a single C. We obtained order parameters of the same level for the T1 base and the G5 base, but experimentally the T1 base was more densely stacked than the G5 base (Borer et al., 1994). The order parameters obtained for the terminal base carbons were slightly higher in one of the strands. As expected, the terminal bases of the duplexes were more flexible than the internal bases, which are not exposed to water. The internal base carbons of the d(CGCGCG)$_2$ duplex were very well ordered in both of the strands. Order parameters of similar magnitude were obtained for the base carbons of the two strands and good agreements with experiment were found.

Very low order parameters were determined for the T1 sugar carbons in both strands of the d(TCGCG)$_2$ duplex. This was also examined by calculating the rms fluctuations of every nucleotide for the last 400 ps and we found clearly higher fluctuations of the thymidine dangling ends than for other nucleotides.

Higher order parameters were obtained for the 5', 4' and 1' sugar carbons than for the 3' and 2', except for the C2,2', C2,2" and C2,3' sugar carbons in strand 2. Borer et

al. (1994) found this behavior only for the terminal bases T1 and G5, whereas we observed it for most of the nucleotides.

High order parameters were found for the base-paired core of the $d(CGCGCG)_2$ duplex. The behavior of high order parameters of the 5', 4' and 1' sugar carbons and low order ones of the 3' and 2' carbons was mainly found for the terminal nucleotides in both strands. Borer et al. (1994) observed this trend for the G6 nucleotide, and the C1 nucleotide also had low order parameters. Low order parameters were obtained for the terminal C1 nucleotide, indicating larger flexibility than for the terminal 3'-nucleotide G6. The C1 terminal nucleotides also showed higher rms fluctuations (~1.28 Å) than the G6 terminal nucleotides (~0.98 Å).

In an error analysis the order parameters in the $d(TCGCG)_2$ duplex were found to be well-defined with an estimated average relative error of 15% (Yu et al., 1995).

The effective correlation times calculated using Eq. 4 are displayed for both strands of the $d(TCGCG)_2$ and the $d(CGCGCG)_2$ duplexes in Figs. 5 and 6, respectively, together with the experimental values (from Borer et al., 1994) for these duplexes. The effective correlation times strongly depend on the accuracy of the order parameters and therefore the uncertainties are high. We found the effective correlation times to be in the range of 0–60 ps for both the base and sugar carbons, whereas the experimental values of Borer et al. (1994) varied from 20 to 150 ps for the sugar carbons with uncertainties as large as 100 ps. Most of the effective correlation times are about 20 ps for both duplexes. No real comparison could be made, because of the large error bars in the experimental data, but values from the MD simulation data presented are clearly smaller than the experimental values. The average relative error of the effective correlation times in the $d(TCGCG)_2$ duplex has been estimated to be 85% (Yu et al., 1995).

^{13}C T_1 relaxation times

The calculated and experimental (Borer et al., 1994) values of the longitudinal relaxation times T_1 are shown in Table 1 for the $d(TCGCG)_2$ duplex at 62.9, 90.6 and 125.8 MHz, and in Table 2 for the $d(CGCGCG)_2$ duplex at 90.6 and 125.8 MHz. These T_1 relaxation times were calculated using the experimentally determined (Borer et al., 1994) overall rotational correlation times of 0.9 and 1.4 ns for the $d(TCGCG)_2$ and the $d(CGCGCG)_2$ duplexes, respectively. For the nonterminal carbons of the $d(TCGCG)_2$ duplex we found reasonable agreement with experiment, but exceptions were also observed, for instance for C2,3' in strand 1. The T_1 relaxation times of the base carbons were of the same magnitude as the experimental values, but for the terminal sugar carbons we obtained too large T_1 relaxation times. In the terminal sugar carbons, major variations in the T_1 relaxation times

TABLE 2

^{13}C RELAXATION TIMES OF THE OLIGONUCLEOTIDE $d(CGCGCG)_2$

Carbon	T_1 (s) at 90.6 MHz			T_1 (s) at 125.8 MHz		
	Strand 1	Strand 2	Exp.	Strand 1	Strand 2	Exp.
C1,5	0.16	0.24	nd[a]	0.20	0.27	nd
C1,6	0.14	0.18	nd	0.17	0.21	nd
G2,8	0.13	0.17	0.15	0.16	0.20	0.17
C3,5	0.14	0.18	nd	0.17	0.20	nd
C3,6	0.13	0.17	0.14	0.16	0.20	0.13
G4,8	0.13	0.17	0.15	0.16	0.20	0.17
C5,5	0.14	0.18	nd	0.18	0.21	nd
C5,6	0.14	0.17	0.14	0.17	0.20	0.13
G6,8	0.17	0.22	0.17	0.21	0.25	0.21
C1,5"	nd	0.34	0.38	nd	0.39	0.38
C1,5'	0.35	0.27	0.38	0.42	0.31	0.38
C1,4'	nd	0.86	0.29	nd	0.98	0.39
C1,3'	0.42	0.39	0.27	0.50	0.46	0.45
C1,2"	0.40	0.33	nd	0.48	0.38	nd
C1,2'	0.39	0.33	nd	0.47	0.37	nd
C1,1'	0.36	0.31	0.29	0.44	0.36	0.40
G2,5"	0.17	0.21	0.22	0.20	0.24	0.32
G2,5'	0.18	0.21	0.22	0.22	0.24	0.32
G2,4'	0.16	0.20	0.22	0.20	0.22	0.28
G2,3'	0.15	nd	0.16	0.18	nd	0.25
G2,2"	0.15	nd	nd	0.18	nd	nd
G2,2'	0.16	nd	nd	0.19	nd	nd
G2,1'	0.15	nd	0.20	0.18	nd	0.28
C3,5"	0.15	0.21	0.20	0.18	0.24	0.30
C3,5'	0.15	0.23	0.20	0.19	0.26	0.30
C3,4'	0.15	0.22	0.20	0.18	0.25	0.29
C3,3'	0.15	nd	0.28	0.18	nd	0.32
C3,2"	0.16	nd	0.28	0.20	nd	0.34
C3,2'	0.18	nd	0.28	0.22	nd	0.34
C3,1'	0.16	nd	0.21	0.20	nd	0.30
G4,5"	0.15	0.21	0.22	0.18	0.24	0.32
G4,5'	0.17	0.26	0.22	0.20	0.30	0.32
G4,4'	0.16	0.25	0.22	0.20	0.29	0.28
G4,3'	0.30	0.68	0.16	0.36	0.77	0.25
G4,2"	0.18	0.43	nd	0.22	0.49	nd
G4,2'	0.19	0.44	nd	0.23	0.49	nd
G4,1'	0.17	0.25	0.20	0.20	0.29	0.28
C5,5"	0.18	0.23	0.20	0.22	0.26	0.30
C5,5'	0.15	0.23	0.20	0.18	0.26	0.30
C5,4'	0.15	0.22	0.20	0.18	0.25	0.29
C5,3'	0.14	0.19	0.28	0.17	0.22	0.32
C5,2"	0.14	0.19	0.28	0.18	0.21	0.34
C5,2'	0.15	0.20	0.28	0.18	0.23	0.34
C5,1'	0.14	0.19	0.21	0.17	0.21	0.30
G6,5"	0.19	0.23	0.20	0.23	0.27	0.22
G6,5'	0.20	0.23	0.20	0.24	0.26	0.22
G6,4'	0.19	0.23	0.27	0.23	0.27	0.28
G6,3'	0.39	0.45	0.33	0.46	0.51	0.45
G6,2"	nd	0.26	0.38	nd	0.30	0.48
G6,2'	nd	0.23	0.38	nd	0.26	0.48
G6,1'	nd	0.21	0.20	nd	0.24	0.28

For strands 1 and 2 the MD simulation results are shown; experimental data were taken from Borer et al. (1994).

[a] nd = not determined.

were observed. For the $d(CGCGCG)_2$ duplex a few T_1 relaxation times were found to be too long in the terminal nucleosides and for the nonterminal C1,4' and G4,3' sugar carbons. The T_1 relaxation time was found to increase with the frequency for both duplexes. The effect of including the chemical shift anisotropy was very small on the base carbons. For both duplexes we observed differences of various magnitude for the T_1 relaxation times calculated for strands 1 and 2. This indicates that small conformational changes may produce differences between the strands that result in different T_1 relaxation times.

Model dependence

To investigate the dependence on different parameters in the model-free approach (Lipari and Szabo, 1982), we calculated the T_1 relaxation times for the two experimentally determined overall rotational correlation times of 0.9 and 1.1 ns for the $d(TCGCG)_2$ duplex and 1.4 and 1.5 ns for the $d(CGCGCG)_2$ duplex (Eimer et al., 1990; Borer et al., 1994). Slightly smaller T_1 relaxation times were found when increasing the overall rotational correlation time from 1.4 to 1.5 ns. A larger reduction of the T_1 relaxation times was observed for all base and sugar carbons if the carbon–hydrogen bond length was shortened to 1.07 Å. The $1/T_1$ relaxation rates were partitioned into two contributions, from the overall rotational correlation and from the effective internal correlation of a ^{13}C-^1H vector. The contribution from the overall rotation was calculated by only including the first term in Eq. 7 and the other contribution was therefore determined by using the second term in Eq. 7. We found that the overall rotational correlation time and the order parameter to a large degree determined the $1/T_1$ relaxation rates.

Conclusions

The results presented here for two oligonucleotides, the $d(TCGCG)_2$ duplex with a thymine dangling end and the $d(CGCGCG)_2$ duplex, from MD trajectories were found to correlate with experimental results. The advantage of being able to make predictions from MD simulations is that both strands and all kinds of oligonucleotides (not only symmetric ones) can be studied, and that there is no problem with overlapping resonances. The dangling thymine at both ends of $d(TCGCG)_2$ remained nicely stacked on the duplex core of the molecule throughout the simulation. To accurately be able to predict dynamic properties, like magnitudes and time scales of the internal motions of nucleic acids, the MD simulations should considerably be lengthened to sample all kinds of motion reliably. Sugar repuckering was found to influence the correlations of the C2' and C3' sugar carbons, leading to lower generalized order parameters for these motions. The overall agreement of the order parameters was reasonable, but large differences were observed for the effective correlation times, which also showed high experimental uncertainties. The T_1 relaxation times from the MD trajectories were observed to be mainly due to the applied overall rotational correlation time and the calculated order parameter, and was found to agree acceptably with experiment.

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