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Phosphorus-31 transverse relaxation rate measurements by NMR spectroscopy: Insight into conformational exchange along the nucleic acid backbone

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Abstract

Phosphorus (^{31}P) NMR spectroscopy can provide important information about the dynamics of nucleic acids. In this communication, we propose an inversely detected ^{31}P transverse relaxation rate (R_2) measurement experiment. This experiment enables fast measurement of accurate ^{31}P transverse relaxation rates and provides the possibility to detect slow motions mapped by the phosphorus nuclei along the nucleic acid backbone. Dispersion curves show some ^{31}P nuclei experiencing chemical exchange in the millisecond time scale. Under the assumption of a two-state exchange process, the reduced lifetimes of the exchanging sites (τ_{ex}) obtained are in accordance with base pair lifetime estimates deduced from imino proton exchange measurements.

Introduction

Knowledge of the dynamics of biomolecules is essential in order to understand their biophysical properties in addition to their three dimensional static structure in solution (Wand 2001). In this respect, nuclear magnetic resonance (NMR) spin relaxation measurements represent an efficient experimental approach for characterizing molecular motions in solution (Palmer, 1997; Kay, 1998). Moreover, recent NMR experiments enable the exploration of a new range of molecular dynamics phenomena situated in the μs-ms time scale (Akke and Palmer, 1996; Zinn-Justin et al., 1997; Ishima et al., 1998; Konrat and Tallinger, 1999; Mulder, 1999; Loria et al., 1999). This time scale includes major biological events like enzyme catalysis, protein folding, and ligand entry/binding (Cavanagh and Venters, 2001).

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Phosphorus (^{31}P) NMR spectroscopy provides the opportunity to obtain important information about the dynamics of nucleic acids (Hogan and Jardesky, 1979; Keepers and James, 1982; Kan et al., 1987; Williamson and Boxer, 1989; Forster and Lane, 1990; Searle and Lane, 1992; Gorenstein, 1992, 1994, 1996; Odahara et al., 1994; Robinson and Drobny, 1995; Schweitzer et al., 1995; Wijmenga and van Buuren, 1998). So far, relaxation experiments based on inversely detected spectroscopy suffered from poor sensitivity, despite the natural abundance of ^{31}P (Schweitzer et al., 1995). This is largely due to the presence of homonuclear ^{1}H-^{1}H scalar couplings of equivalent magnitude to the heteronuclear ^{1}H-^{31}P couplings, ultimately rendering the ^{1}H-^{31}P magnetization transfer ineffective (Wijmenga and van Buuren, 1998). To overcome this problem, Luy and Marino (2001) developed a heteronuclear single quantum correlation (HSQC) experiment (Muller, 1979; Bodenhausen and Ruben, 1980), featuring a Carr-Purcell-Meiboom-Gill (CPMG) pulse train (Carr and Purcell, 1954; Meiboom and Gill, 1958) expanded in a XY-

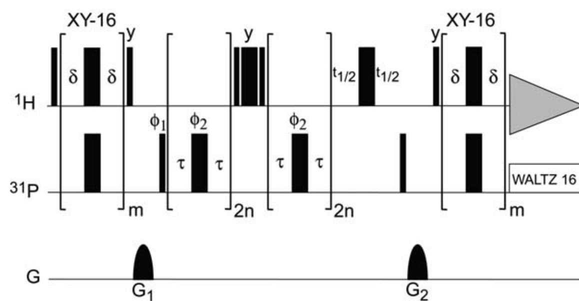

Figure 1. ^{1}H-^{31}P-CPMG pulse sequence scheme for measuring ^{31}P transverse relaxation rates. All pulses are *x* phase unless otherwise indicated. Narrow and wide bars depict 90° and 180° pulses, respectively. During ^{1}H-^{31}P polarization transfer periods, both 180° refocusing pulses are expanded in a (XY-16) supercycle ($m = 8$) (Gullion et al., 1990). During the spin-echo periods, the ^{1}H and ^{31}P pulses 180° were 50 and 53 μs long, respectively. Delays were $\delta = 100$ μs and $\tau = 0.5\ \tau_{CPMG}$. Decoupling during acquisition used WALTZ-16 phase modulation (Shaka et al., 1983) with a field strength of 2.1 kHz. The phase cycle is $\Phi_1 = x, -x$; $\Phi_2 = y$, receiver $= x, -x$. Quadrature detection was obtained in ω_1 by incrementing Φ_1 and Φ_2 in a States-TPPI manner (States et al., 1982). $G1_z$ and $G2_z$ gradient amplitudes were 10 G/cm with 0.5 ms duration. All experiments were collected at 40 °C using a Bruker DRX500 NMR spectrometer with a ^{31}P Larmor frequency of 202.46 MHz.

16 scheme (Gullion et al., 1990) during the periods of magnetization transfer, that delivered substantial sensitivity enhancement.

In this communication, we present an inversely detected ^{31}P transverse relaxation rate (R_2) measurement experiment in the laboratory frame (Figure 1) containing the previously mentioned CPMG-XY-16 pulse train during the two periods of magnetization transfer, with the first CPMG-XY-16 pulse train producing an anti-phase term at the beginning of the first relaxation period. The pulse sequence contains at its centre two additional CPMG pulse trains separated by a ^{1}H 180° pulse to remove any CSA/dipolar relaxation effects (Palmer et al., 1992; Kay et al., 1992). The design of these two CPMG pulse trains is based on previously described ^{15}N relaxation experiments (Loria et al., 1999) but with a variation in the phase cycling: The phase Φ_2 is identical in the two trains and is incremented in conjunction with Φ_1, in a States-TPPI manner (Figure 1). This experiment enables the fast measurement of accurate ^{31}P transverse relaxation rates, and provides the ability to detect conformational exchange mapped by the phosphorus nuclei along the nucleic acid backbone.

The R_2 measurements were performed on a 2 mM DNA duplex sample rich in A•T base pairs (Fig-

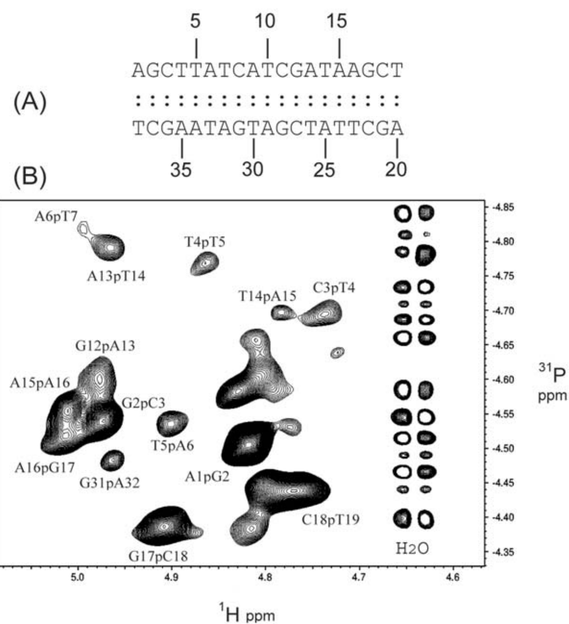

Figure 2. (A) Pseudo-palindromic 19 base pair DNA duplex sample ($T_m \cong 65$ °C) (Fossé et al., 1991). (B) ^{1}H-^{31}P-CPMG spectrum ($n = 1$ and $\tau_{CPMG} = 1$ ms) acquired with (164 × 2048) complex points and spectral widths of (500 × 5000) Hz in the ($t_1 \times t_2$) dimensions. (B) depicts an expansion of the H3′/^{31}P region. The recycle delay was 3 s (averaged T_1 estimated around 0.5 s by a 1D ^{31}P recovery experiment) and a total of 16 and 64 transients were recorded for each complex t_1 point for $\tau_{CPMG} \in$ [0.5; 2 ms] and $\tau_{CPMG} \geq 6$ ms, respectively. ^{1}H and ^{31}P chemical shifts were calibrated with respect to the perdeurated 3-(trimethylsilyl) propionic acid (TSP) and an external trimethylphosphate (TMP) sample. H3′ and ^{31}P chemical shift were assigned with NOESY (Liu et al., 1998) and HP-CPMG-HSQC-NOESY (Luy and Marino, 2001) experiments. Peaks not labeled could not been assigned at these ^{1}H/^{31}P Larmor frequencies. All spectra were processed and displayed using *NMRPipe* software package (Delaglio et al., 1995).

Table 1. ^{31}P spin-spin relaxation rates, R_2 ($\tau_{CPMG} = 1$ ms) (s^{-1})

Nucleus	R_2 (s^{-1})	Nucleus	R_2 (s^{-1})
A1pG2	16.40 ± 0.15	A13pT14	24.59 ± 0.45
G2pG3	17.89 ± 0.17	T14pA15	23.88 ± 1.14
C3pT4	20.58 ± 0.57	A15pA16	25.14 ± 0.32
T4pT5	24.03 ± 0.50	A16pG17	22.44 ± 0.36
T5pA6	23.46 ± 0.42	G17pC18	19.47 ± 0.35
A6pT7	–	C18pT19	14.17 ± 0.21
G12pA13	18.82 ± 0.59	G31pA32	21.79 ± 0.69

ure 2 and Table 1) in 35 mM NaCl and 99.996% D_2O (pH = 7). Relaxation data are not available for ^{31}P nuclei situated between residues T_7 and G_{12}, because the reduced spectral dispersion at both 500.13 and 202.46 MHz (^1H and ^{31}P Larmor frequencies, respectively) precluded assignment. Globally, the R_2 rate tends to increase from the end of the strands toward the centre of the sequence. As expected, this corresponds to a stiffening of the DNA backbone towards the centre of the duplex, however, a more rigorous interpretation would require a spectral density function calculation. Nonetheless, it is interesting to note a levelling of the R_2 rate from both ends after only the third residue, with an average of 23.6 ± 1.8 s^{-1}. The most likely explanation would lie in the fraying of the base pairs situated at both ends of the oligonucleotide that would affect, in a decreasing manner, the backbone dynamics until the third phosphorus nucleus. One exception on these observations concerns $G_{12}pA_{13}$ nucleus situated next to the non-palindromic region at the centre of the sequence. This nucleus displays a noticeably lower value ($R_2 = 18.8$ s^{-1}) compared to the average value mentioned above, illustrating a greater proportion of faster motion at this site.

Chemical exchange in the μs-ms time scale contributes to the transverse relaxation rate (Orekhov et al., 1994). This contribution to R_2, defined as R_{ex}, can be evaluated by CPMG dispersion curve analysis (Palmer et al., 1996; Loria et al., 1999). However, the evolution during the relaxation delay under the scalar coupling Hamiltonian of the in-phase coherence toward an anti-phase term -or *vice-versa*- could render the R_{ex} extraction irrelevant (Palmer et al., 2001). To avoid such an effect, τ_{CPMG} has to be smaller that the inverse of the sum of the scalar coupling constants. Alternatively, *relaxation-compensated* experiments could be performed (Loria et al., 1999). In the case of the ^{31}P nucleus belonging to a nucleic acid, an optimum average of the in-phase and anti-phase coherences would be approximate considering the multiple ^1H partners. Within a B-DNA, the sum of the ^1H-^{31}P scalar coupling constants typically does not exceed 20–25 Hz (Hilbers and Wijmenga, 1996), thereby making the in-phase contribution to the transverse relaxation negligible. Thus, the rate constants for anti-phase and in-phase coherences should not need to be averaged in a relaxation-compensated manner (Loria et al., 1999), even for τ_{CPMG} values above 10 ms.

Figure 3 depicts several CPMG dispersion curves obtained by varying the interpulse delay of the experi-

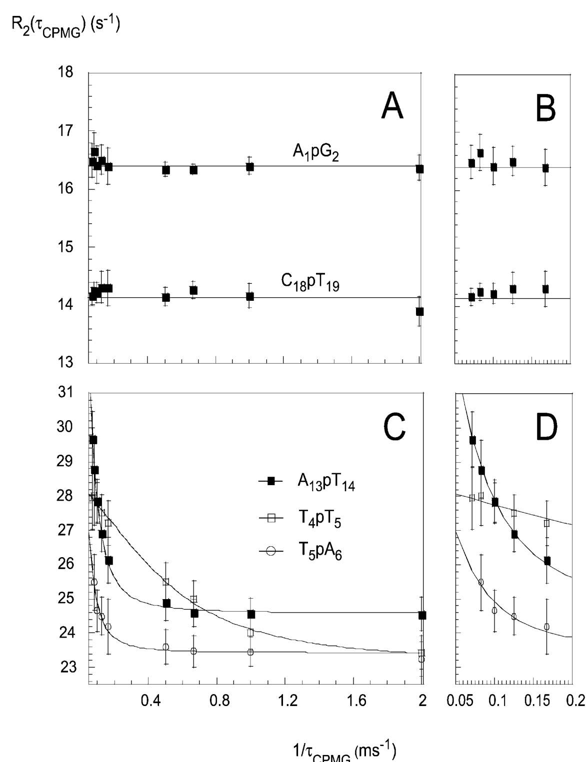

Figure 3. CPMG dispersion curves. Values of $R_2(\tau_{CPMG})$ are plotted versus $1/\tau_{CPMG}$. Intensities were evaluated with *NMRView* (Johnson and Blevins, 1994) and the R_2 rates and error bars were calculated using *CurveFit* (Palmer, A.G.) with 10 individual time points and two duplicates. For $\tau_{CPMG} \geqslant 6$ ms, $R_2(\tau_{CPMG})$ values were evaluated with two points (experiment repeated 3 times); intensities at zero time were obtained after omitting the relaxation periods and ^1H 180° refocusing pulse shown in Figure 1. Non linear fits were performed with the *Grace* software package (Turner, P. J.). Fitted parameters are given in Table 2 (see Equation 2, Loria et al., 1999). R_{ex} and $R_2(1/\tau_{CPMG} \to \infty)$ represent the chemical exchange contribution to R_2 and the intrinsic transverse relaxation rate, respectively (see text for τ_{ex}). (A) and (B) report on ^{31}P nuclei situated at both ends, while (C) and (D) show ^{31}P nuclei experiencing some conformational exchange; (B) and (D) represent an expanded region of (A) and (C), respectively.

Table 2. Conformational exchange parameters

^{31}P nucleus	R_{ex} (s^{-1})	$R_2(1/\tau_{CPMG} \to \infty)$ (s^{-1})	τ_{ex} (ms)
T4pT5	5.26 ± 0.24	23.10 ± 0.47	0.58 ± 0.04
T5pA6	7.08 ± 1.24	23.42 ± 0.49	5.45 ± 0.13
A13pT14	12.99 ± 0.85	24.57 ± 0.44	4.76 ± 0.16

ment described in Figure 1. As expected, the constant R_2 values indicate that the nuclei close to the ends of the duplex do not exhibit any conformational exchange in the 0.5–10 ms time scale (Figures 3A and 3B). This is the generally accepted behavior of base pairs located at both ends of a duplex whose termini are likely to be frayed (Nonin et al., 1995). This observation also tends to confirm the hypothesis made previously regarding the negligible in-phase contribution to the transverse relaxation within the τ_{CPMG} scale used (0.5–14 ms). Figures 3C and 3D highlight the presence of chemical exchange for T_4pT_5, T_5pA_6 and $A_{13}pT_{14}$ ^{31}P nuclei. In contrast, no ^{31}P nucleus located next to either a G or C nucleotide shows any variation in its R_2 rate. This observation does not contradict with the lifetimes of G•C base pairs which are typically three times longer and have a dissociation constant ten times smaller than A•T base pair (Guéron et al., 1987).

On a NMR chemical shift time scale for the exchange process, the fast-limit approximation (Luz and Meiboom, 1963) leads to a good fit of the experimental data (Figures 3C and 3D, Table 2) under the assumption of a two-state exchange process. In addition, the reduced lifetimes of the exchanging sites (τ_{ex}) are in accordance with base pair lifetimes estimated from imino proton exchange measurements (Guéron et al., 1987; Kochoyan et al., 1987; Leroy et al., 1988). These chemical exchange data could illustrate the bending oscillations of the DNA. Furthermore, it has been demonstrated that the base pair opening and the DNA bending are strongly correlated (Ramstein and Lavery, 1988). On the other hand, since no stable B_{II} population on a NMR time scale were detected (*vide infra*), these R_{ex} measurements cannot report on $B_I \Leftrightarrow B_{II}$ transitions (Gupta et al., 1980; Privé et al., 1987), which are short lived transitions occurring on the picosecond time scale (Hartmann et al., 1993; Gorenstein 1996).

A_6pT_7 and $T_{14}pA_{15}$ nuclei, and a few other unassigned correlation peaks, display a very low signal intensity (Figure 2B). As such, the R_2 rate estimate is difficult to obtain under these conditions. Nevertheless, comments can be made relative to the dynamics of these nuclei despite the absence of R_2 and/or R_{ex} measurements. In fact, these low intensities cannot report on $^{31}P\text{-}H_{3'}$ scalar coupling constant $-{}^3J(^{31}P,H_{3'})-$ variations, considering the narrow range experienced by $^3J(^{31}P,H_{3'})$ in the case of a B-DNA : 1.3 to 10 Hz (Gorenstein, 1996). In addition, this variability is closely linked to the DNA backbone conformations B_I and B_{II} (Gorenstein, 1996), and no NMR evidences of any population in a stable B_{II} conformation state were found with the oligonucleotide employed in this study: (1) Narrow ^{31}P chemical shifts dispersion centered around -4.6 ppm ($\Delta\delta \approx 0.5$ ppm, cf. Figure 2B), i.e. no substantial ^{31}P signal shifts toward higher frequencies (Roongta et al., 1990; Gorenstein, 1992, 1996; Tisné et al, 1998), (2) no significant lower NOE peak intensities between H_6/H_8 and $H_{1'}$, H_6/H_8 and $H_{2'}$ and H_6/H_8 and $H_{2''}$ characterizing the higher energy B_{II} conformation (Tisné et al., 1998).

As a consequence, these weaker signals are most likely the outcome of greater R_2 values. This could arise from an increase in the *intrinsic* part, R_2^{int}, defined as R_2 in the absence of chemical exchange, or from a greater exchange contribution to R_2. R_2^{int} reports on fast motion (ps-ns time scale) while R_{ex} rather illustrates the presence of slower motion (μs-ms time scale). Regarding R_2^{int}, despite the absence of any B_{II} population on a NMR time scale, *short* $B_I \Leftrightarrow B_{II}$ transitions could be invoked, reflecting substantial changes in the ε and ζ torsional angles (Dickerson and Drew, 1981; Dickerson, 1983), and/or other backbone modifications through changes in the α, β, and γ torsional angles, as much as furanose puckering fluctuations. Theoretical studies by molecular dynamics computations describe these various fluctuations as very sharp transitions, i.e., effective within a few picosecondes (Swaminathan et al., 1991; Brahms et al., 1992; Hartmann et al., 1993). However, in the absence of chemical exchange, the spectral density function at zero frequency is the predominant term in the transverse-relaxation process, describing motion on the nanosecond time scale, making any faster internal motion inconsistent with a large R_2^{int} enhancement. Moreover, in spite of the little data available on chemical exchange, data in Table 2 reveal that R_2^{int} does not vary as much ($\Delta R_2(1/\tau_{CPMG}\rightarrow\infty) = 1.5\ s^{-1}$) as the contribution to R_2 from chemical exchange ($R_{ex} \in [5 - 13\ s^{-1}]$). Thereby, if no substantial variation in the backbone dynamics on a nanosecond time scale occur, no significant R_2^{int} variation would become apparent, so the low intensities observed on Figure 2B, including the nuclei A_6pT_7 and $T_{14}pA_{15}$, would result from chemical exchange.

In conclusion, the inverse-detected ^{31}P CPMG experiment proposed herein enables precise R_2 rate measurements, and as a consequence, provides the possibility to detect conformational exchange on the millisecond time scale. More data would be needed

to assert any relationship between ^{31}P chemical exchange highlighted in the present study and the two-step opening base pair events. This could be achieved by collecting data at multiple temperatures and magnetic fields (Millet et al., 2000) in parallel with imino proton exchange measurements. Furthermore, conformational exchange processes inaccessible to the present technique, i.e., below 0.5 ms and above 10 ms, could also be investigated using other previously described NMR experiments modified in a similar fashion as described above (Akke and Palmer, 1996; Zinn-Justin et al., 1997; Montelione and Wagner, 1989; Wider et al., 1991; Farrow et al., 1994). Examination of exchange phenomena through ^{31}P NMR could also assist the study of the opening/closing dynamics of double-stranded DNA molecules, the protein-nucleic acid interactions and the investigation of ligand binding for drug design.

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