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Measurement of the Helix Opening Rate in Z-DNA by ^{1}H Nuclear Magnetic Resonance Relaxation Spectroscopy

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SUMMARY - The exchange rate of the hydrogen-bonded guanine imino protons N(1) in the high-salt form of Poly(dG-dC) was measured by following the non-selective inversion-recovery of their ^{1}H NMR signal at 360 MHz, in the temperature range between 77°C and 90°C. In a 4.5M NaCl solution, Poly(dG-dC) is believed to adapt the left-handed Z-conformation, and the results reported here represent the first quantitative measurements of this rate process for Z-DNA by Nuclear Magnetic Resonance, complementing previous measurements made by tritium exchange at 0°C (Ramstein, J. and Leng, M. (1980) Nature 288, 413-414). The results confirm that this process is much slower in the Z-form, compared to the B-structure, and that this difference in rates results mainly from a large decrease in the entropy of activation for Z-DNA. © 1985 Academic Press, Inc.

Since the existence of a left-handed DNA structure was established in crystals (1), fibers (2), and in solution (3,4), scientists were puzzled and intrigued by the possible role of this Z-DNA conformation in live biological processes. Speculations about the possible significance of this structure were further fueled by the discovery that the conformational transition from the right handed B to the left handed Z structure can happen in supercoiled helices even at physiological salt concentrations. (5-7) A potentially significant distinction of Z-DNA is its apparently much higher stability towards transient openings of the hydrogen bond connected base pairs. The life time of the exchangeable protons within the left-handed double helix was first measured at 0°C by the tritium exchange technique (8), and later by i.r. spectroscopy (9), and was found to be much longer in Z-DNA. Here, we present the first quantitative measurement of the exchange rate of the guanine N(1) imino proton from left-handed Poly(dG-dC) by Nuclear Magnetic Resonance (NMR) spectroscopy. The NMR measurement is important for the quantitative assessment

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of the helix-opening rate, since the imino proton, whose exchange is opening-limited (10,11), is unequivocally identified in the NMR spectrum. Previous attempts to measure the Z-DNA exchange rate by NMR have failed, because the techniques employed were not suitable for the determination of such slow exchange rates (12). In this study, we use a new technique which made such a measurement possible.

The hydrogen bonded imino protons resonate at a down-field position in the NMR spectrum, which is well separated from the spectral region of all the other protons, and are therefore relatively easy to identify even in high molecular weight compounds. Their rate of exchange with the protons of the aqeous solvent is slow compared to the separation between the resonance frequencies of the two species, so that the exchange rate can not be deduced from the linewidth of the N-H signal. On the other hand, the exchange does affect the longitudinal spin-lattice relaxation rate of the imino protons. In a spin-lattice relaxation experiment, the magnetization of the spins is initially perturbed from its thermal equilibrium value. In the presence of exchange, the recovery of the spins at a particular spectral site is determined by two mechanisms. On one hand, the spins undergo magnetic interactions with the surrounding lattice (for protons these are chiefly dipolar interactions), but on the other hand the exchange will physically transfer magnetization into and out of the observed site, and therefore affect the time dependent level of its magnetization.

The exchange-dependent spin-lattice relaxation of the imino protons in double stranded nucleic acids has been monitored so far mainly by so-called selective measurements of the spin-lattice relaxation time T_1 (13-16). With this method, only the magnetization of the N-H protons is initially perturbed (inverted or saturated). The influx of "fresh", fully polarized magnetization from the water will then accelerate the recovery of the N-H spins towards equilibrium, which is measured in the relaxation experiment. One of the drawbacks of this approach, however, is that in order to be reliably determined, the exchange process needs to be the dominant recovery mechanism.

This condition is not always fulfilled by the slowly exchanging imino protons. In particular for Z-DNA (Poly(dG-dC) at 4.5M NaCl concentration), the exchange was found to be too slow for determination by the selective measurement, even at 85°C (12).

In the technique applied here, we exploit the fact that in macromolecules the apparent dipolar spin-lattice relaxation, measured after nonselective inversion of all the spins, is much slower than the actual selective dipolar relaxation rate (17). Thus, much slower exchange processes will not be masked by the magnetization recovery due to the magnetic interactions, and the values of the exchange rate constants can be measured. A detailed derivation of the recovery behaviour of the N-H signal following nonselectivce inversion of all the spins will be presented elsewhere (P. Bendel, submitted for publication). The derived equation, describing the recovery of the longitudinal Z-magnetization for the imino protons is:

$$M_z(t) = M_o\{1 - A[(1-b)\exp(-k_{1I}t) + b\exp(-t/T_{1S})]\} \quad (1)$$

$$\text{with} \quad b = k/(k_{1I}-1/T_{1S}) \quad (1a)$$

where M_o is the N-H proton equilibrium magnetization, k is the exchange rate constant, k_{1I} is the sum of this rate constant and the (nonselective) dipolar relaxation rate, and T_{1S} is the spin-lattice relaxation time for the water signal which should be indepentently determined on the same sample at the same temperature by a standard T_1 measurement. If the waiting time between successive scans (needed for signal averaging) is sufficiently long, then the factor A in equation 1 will be equal to 2, otherwise it will itself be a function of the waiting time and the relaxation rates (18,19):

$$A = 1 + I[1 - (1-b)\exp(-k_{1I}W) - b\exp(-W/T_{1S})] \quad (2)$$

where W equals the waiting time between successivse applications of the inversion recovery sequence, and I represents the r.f. "inhomoogeneity parameter" (I = 1 for perfect inversion).

MATERIALS AND METHODS

Poly(dG-dC) was purchased from Sigma and sonicated under conditions leading to a length distribution of about 50-500 base pairs (20). The polymer solution was dialyzed in a buffer containing 1 mM NaCl, 0.5 mM cacodylic acid and 0.02 mM EDTA at pH 7. After freeze-drying, the sample was reconstituted to final concentrations of 10 mm cacodylate and 0.4 mM EDTA. The desired NaCl concentrations were then reached by adding weighed amounts of the salt. The final NMR sample contained approximately 25 o.d. units (at 260 nm) of Poly(dG-dC) in a volume of 0.3 cc of 95% H_2O/5% D_2O.

NMR measurements were conducted at 360 MHz on a Bruker AM-360 WB instrument, in a 5mm, variable temperature probe. The inversion-recovery rates of the solvent water were determined by the "180°-variable delay - 90°" sequence, replacing the 90° pulse by a solvent-suppressing $\bar{1}33\bar{1}$ sequencse (22), with the transmitter frequency centered at the position of the water peak. Typical acquisition parameters are indicated in the caption of Figure 2.

The peak heights of the phase-corrected spectra were fitted to equation (1), using the nonlinear least-squares fitting routine zxssq of the IMSL library. The independently determined relaxation rate of the water protons, $1/T_{1s}$, was forced upon the solutions, leaving the parameters M_o, k_{1I}, b and I freely variable. Typical resulting values of the inversion parameter I were 0.80 - 0.87.

RESULTS AND DISCUSSION

If the exchange is very slow, equation 1 predicts the recovery to be approximately single-exponential, at the characteristic nonselective dipolar relaxation rate of the imino protons. If k becomes a sizable fraction of k_{1I}, the recovery will be double-exponential, with a second component relaxing at the T_1 rate of the H_2O protons. This behaviour is illustrated in figure 1,

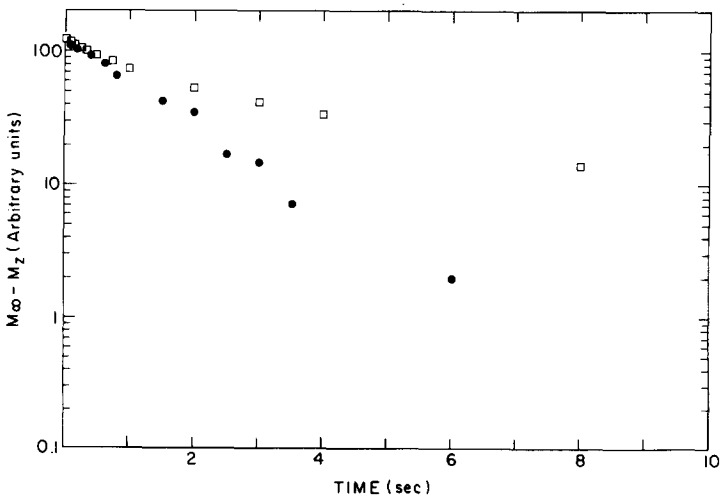

Figure 1. Semi-logarithmic plot of the experimental results of the non-selective inversion recovery measurements for Poly(dG-dC) in 0.1M NaCl (open squares) and 4.5M NaCl (full circles) at 50°C, at a proton larmor frequency of 360 MHz.

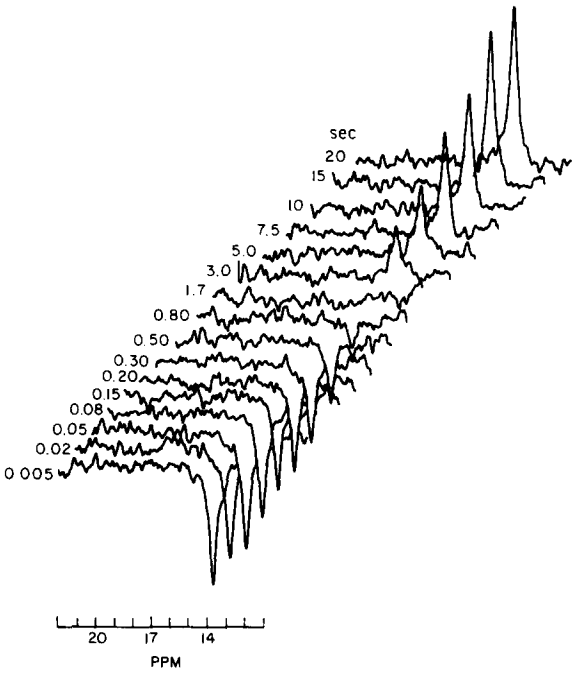

Figure 2. Sections of the NMR spectra displaying the results of the non-selective relaxation experiments for the high-salt form of Poly(dG-dC) at 90°C. The pulse sequence for the experiment contained a composite inversion pulse, followed by a variable delay (the values of which are indicated to the right of the spectra), and a "1331" solvent-suppressing readout pulse, with the transmitter frequency centered at the position of the water peak at 4.8 ppm. 800 scans were signal-averaged for each spectrum, and the recycle time between scans was 8.205 sec. The spectra are presented after an exponential sensitivity-enhancement (with line-broadening of 50 Hz) and base-line correction. The ppm scale is referenced to external TSP.

displaying a semi-logarithmic plot of the experimental relaxation results for Poly(dG-dC) in the B-form (0.1M NaCl), and in the Z-form (4.5M NaCl), at 50°C, clearly indicating a double exponential behaviour for the former and apparently single-exponential for the latter. The relaxation rate for the water protons at this temperature was 0.262 s^{-1} in the low-salt and 0.238 s^{-1} in the high-salt sample. The exchange in Z-DNA is thus too slow to be measured by this technique at 50°C, while the derived exchange rate for the imino protons in the low-salt conformation polymer is 1.1 s^{-1} . (The computer fit to the double exponential function yielded the best fit parameters of k_{II}= 1.73 s^{-1} , and b = 0.734).

Figure 2 displays most of the spectral traces obtained from the non-selective inversion-recovery experiment for the high-salt sample at 90°C. This

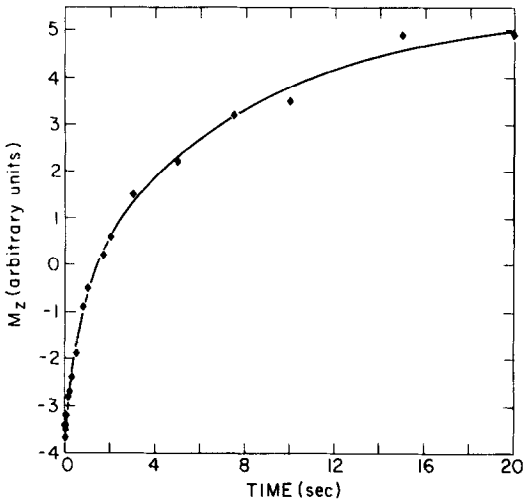

Figure 3. The experimental intensities of the experiment at 90°C for the high-salt form of Poly(dG-dC). The solid curve is the best fit to the double exponential function in equation (1). The value of one of the exponential rates was fixed to 0.131 s^{-1}, which was the $1/T_1$ result for the solvent water peak in this sample, as determined in a separate experiment. From the fitted value of the other exponential rate and from the relative weights of the two components, the derived value of the exchange rate constant is 0.79 s^{-1}, and the dipolar relaxation rate is 0.53 s^{-1}.

time the recovery was clearly double exponential, and the best fit to the appropriate function is shown in figure 3. The resulting value of the exchange rate constant for the exchange of the imino protons (and thus of the helix opening rate) for Poly(dG-dC) in the Z conformation at 90°C is 0.8 s^{-1}. Additional measurements were conducted at 84°C and at 77°C, and the experimental results are summarized in table 1. At these elevated temperatures the exchange rates for the B-Form at 0.1 M NaCl were too fast to be measured by the present technique, yielding single-exponential recovery curves with the rates characteristic of the solvent protons ($1/T_{1S}$), as predicted by equation 1, if k makes a dominant contribution to the spin-lattice recovery of the imino-protons. This could of course be foreseen based upon the exchange rates measured by Mirau and Kearns (12), using selective inversion-recovery techniques.

The rates at which the nitrogen-connected protons in the B- and Z- forms of Poly(dG-dC) exchange with the solvent were previously measured at low temperatures, using real-time tracing techniques, such as tritium exchange or

Table 1

Conformation	Temperature(^{o}C)	$1/T_{1S}(sec^{-1})$	$k_{1I}(sec^{-1})$	k (sec^{-1})
B	50	0.262	1.73	1.08(1.1 ± 0.1)
Z	50	0.238	0.71	too slow to determine
Z	77	0.165	0.84	0.29(0.3 ± 0.05)
Z	84	0.154	1.20	0.59(0.6 ± 0.05)
Z	90	0.131	1.33	0.79(0.8 ± 0.08)

Parameters resulting from the best fit to the nonselective ^{1}H NMR inversion recovery of the N(1) proton in Poly(dG-dC) at 360 MHz, and the protons in H_2O. k is the exchange rate constant and k_{1I} the total spin-lattice relaxation rates (exchange + nonselective dipolar relaxation) for the imino-protons, and $1/T_{1S}$ is the longitudinal relaxation rate for the solvent water. The approximate confidence margins for k are indicated in parentheses.

infrared spectroscopy (8,9). One problem associated with these measurements is that different types of exchangeable protons (e.g., imino and amino of different groups) all contribute to the observed signals with different rates, although the assignment for the specific exchange rate of the imino-protons could be made with reasonable confidence. An NMR measurement of the imino-proton exchange rate in the Z-form at 85^{o}C was then attempted by a selective T_1 measurement, but only an upper limit of 2 sec^{-1} could be given (12), which now turns out to be not too far from the actual values. Assuming that the imino-proton exchange rates at low temperatures were correctly identified by Ramstein and Leng (8) and Hartmann et al. (9) as belonging to the faster class of exchanging protons, we can combine the results of their measurements with those presented here to estimate the apparent Arrhenius parameters for the process, in which the local opening of the double helix is believed to be the rate-determining step. Combining the tritium exchange-determined value of $5.8x10^{-4} sec^{-1}$ at 0^{o}C with our results at 77^{o}C, 84^{o}C, and 90^{o}C yields k_o = $2.3x10^{9}$ and Ea = 15.7 Kcal/mole for the equation: $k = k_o \exp(-Ea/RT)$, with a linear correlation factor of -0.9995. Using the i.r. determined value of $1.7x10^{-4}$ S^{-1} at 6^{o}C, results in $k_o = 1.66x10^{12}$, and Ea = 20.5 Kcal/mole (r = -0.9998). This compares with the parameters of $k_o = 1.3x10^{20}$ and Ea=28.8 Kcal/mole, derived by Mirau and Kearns for the B-form of Poly(dG-dC). While

the significance of the apparently lower activation energy in the Z-form remains to be investigated, it is clear that the very much slower exchange rate for the Z-form in aqueous solutions results from the drastically reduced pre-exponential factor in the Arrhenius equation.

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