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Determination of conformational transition rates in the trp promoter by ^{1}H NMR rotating-frame T_1 and cross-relaxation rate measurements

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Abstract. Rotating-frame relaxation measurements have been used in conjunction with spin-spin relaxation rate constants to investigate a conformational transition previously observed in the −10 region of the trp promoter d(CGTACTAGT**TAAC**TAGTACG)$_2$ (Lefèvre, Lane, Jardetzky 1987). The transition is localised to the sub-sequence **TAAC**, and is in fast exchange on the chemical shift time-scale. The rate constant for the exchange process has been determined from measurements of the rotating-frame relaxation rate constant as a function of the spin-lock field strength, and is approximately 5000 s^{-1} at 30 °C. Measurements have also been made as a function of temperature and in two different magnetic fields: the results are fully consistent with those expected for the exchange contribution in a two-site system. A similar transition has been observed in d(GTGATTGA**CAAT**TA).d(CACTAACTGTTAAT), which contains the −35 region of the trp promoter. This has been investigated in the same way, and has been found to undergo exchange at a faster rate under comparable conditions. In addition, the cross-relaxation rate constants for Ade C2H-Ade C2H pairs have been measured as a function of temperature, and these indicate that certain internuclear distances in YAAY subsequences increase with increasing temperature. These changes in distance are consistent with a flattening of propellor twist of the AT base-pairs. The occurrence of conformational transitions in YAAY subsequences depends on the flanking sequence.

Key words: NMR rotating-frame relaxation – Cross-relaxation – Chemical exchange

Introduction

Conformational flexibility is an intrinsic property of macromolecules that must be taken into account in any description of their structural properties, not least because certain kinds of flexibility are central to function. NMR is an important method for studying conformational flexibility because NMR observables such as coupling constants, chemical shifts and relaxation rate constants depend on conformation, and are affected by motions on a variety of time-scales. By the same token, it must be recognised that a solution structure determined by NMR represents an average of the conformations available to the system and that the precise nature of the average depends on the time-scale of any motion. Motional effects are likely to be particularly important for linear nucleic acids since they are generally more flexible than globular proteins, and motions on a wide variety of time-scales have been observed in DNA fragments (Leroy et al. 1988; Lane 1991).

This paper is concerned with slow, large amplitude motions, for example those involved in the interconversion of two or more conformational states that have distinct potential minima. Such processes may manifest themselves in the details of the NMR line-shape. For example, when the rate of exchange is slow compared with the difference in chemical shifts characteristic of the conformational states, two separate resonances are observed, whose widths contain a contribution from the rate constant of the exchange process. As exchange becomes faster, the two resonances coalesce, and the line-shape becomes a complex function of the chemical shifts, intrinsic relaxation rate constants and chemical exchange rate constants. Under these conditions, it is difficult to analyse the conformational transition in detail, especially when the chemical shifts of the pure species are unknown, and the intrinsic temperature-dependence of the resonances cannot be measured. Such a conformational transition has been observed in the trp operator (Lefèvre et al. 1988; Lane 1989), where the line-width of the H2 resonance of an adenine residue increases with increasing temperature to a maximum at about 30 °C, whereupon it then decreases. This behaviour is typical of fast intermediate exchange between two or more conformations. However, the intrinsic shifts of the states being averaged could not be determined, since it was not possible to cool the system to

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below the coalescence temperature. Consequently, it was not possible to determine the exchange rate constant reliably.

Deverell et al. (1970) and Stilbs and Moseley (1978) showed that an alternative method for determining exchange rate constant in the fast exchange regime is provided by measurements of the rotating frame spin-lattice relaxation rate constant ($R_{1\varrho}$) as a function of the spin-lock field-strength (γB_1). In this paper we report applications of this method to the exchange processes observed in the −10 and −35 regions of the trp promoter. These processes have been further elucidated by measurements of the spin-spin relaxation rate constant and the cross-relaxation rate constants for selected protons.

Theory

For an uncoupled two-site exchange process at temperatures above the coalescence point, the exchange contribution to the rotating-frame spin-lattice relaxation rate constant, $R_{1\varrho}^{ex}$, is given by (Deverell et al. 1970):

$$R_{1\varrho}^{ex} = 0.5 \int d\tau \, \langle \delta\omega(t) \, \delta\omega(\tau - t) \rangle \cos(\omega_1 \tau) \tag{1}$$

where ω_1 is the spin-lock field strength measured in angular frequency units ($\omega_1 = \gamma B_1$). $\langle \delta\omega(t) \, \delta\omega(t-\tau) \rangle$ is a correlation function that describes the time-dependent fluctuation in the frequency produced by the exchange process. The exchange between the two sites having different chemical shifts results in a fluctuation in the z-component of the magnetization: this causes relaxation perpendicular to z, and in particular for spins locked in the transverse plane. Deverell et al. (1970) have given the correlation function for exchange between two equally populated sites at equilibrium. In the two-site process:

$$A \underset{k_{-1}}{\overset{k_1}{\Leftrightarrow}} B$$

k_1 and k_{-1} are the forward and reverse rate constants, respectively, and are in general not identical. The correlation function for the unequally populated case is $p_a \, p_b \, \Delta\omega^2 \exp(-k\,\tau)$ (Marshall 1970), where p_a, p_b are the mole fractions of states A and B, $\Delta\omega$ is the frequency difference between them, and k is the exchange rate constant ($= k_1 + k_{-1}$).

For this correlation function, (1) becomes:

$$R_{1\varrho}^{ex} = p_a \, p_b \, \Delta\omega^2 \, k/(k^2 + \omega_1^2) \tag{2}$$

The measured value of the spin-lattice relaxation rate constant in the rotating frame, $R_{1\varrho}$, will contain an additional term, R_1^{∞}, which describes relaxation from mechanisms other than exchange. Hence,

$$R_{1\varrho} = R_1^{\infty} + 4\pi^2 \, p_a \, p_b \, \Delta\nu^2 \, k/(k^2 + \omega_1^2) \tag{3}$$

where $\Delta\nu$ is the frequency difference expressed in Hz. Hence, measurement of $R_{1\varrho}$ as a function of ω_1 allows both k and the product $p_a \, p_b \, \Delta\nu^2$ to be determined.

Equation (3) shows that, at sufficiently high spin-lock field strengths, the exchange contribution becomes negligible, whereas at low spin-lock field strengths (i.e. $\omega_1^2 \ll k^2$), the value of $R_{1\varrho}$ reaches its maximum value. In this limit, $R_{1\varrho}$ is essentially equal to the measured value of R_2 (Allerhand et al. 1965). Equation (3) also predicts that the exchange contribution to the line-width (in Hz) at a given temperature will be proportional to the square of the external magnetic field strength. Also, the exchange contribution falls to half of its maximum value when $\omega_1 = k$.

The rate constant k can be written as $k_1 (1+K)/K$ where K is the equilibrium constant. At a spin-lock field strength $\omega_1 = 0$, and making use of the identity $p_b = K/(1+K)$, (3) becomes:

$$R_{1\varrho}^{(\omega_1 = 0)} = R_2 = 4\pi^2 \, \Delta\nu^2 \, p_a \, p_b^2/k_1 + R_1^{\infty} \tag{4}$$

The first term on the right hand side of (4) is the exchange contribution to the relaxation that would affect the line-width, while the second term describes the exchange-independent contribution. The resulting dependence of the line-width on frequency, populations and rate constant is identical to that for the expression given in Sandström (1982).

Experimental

The self-complementary 20-mer, d(CGTACTAGTTAACTAGTACG)$_2$, which contains the −10 region of the trp promoter was synthesized and purified as previously described (Lane 1989). This sequence will be referred to as trp −10 20-mer. The relaxation measurements were made on two independently synthesized samples. The ^{1}H NMR assignments of this oligomer have been previously reported at 25 °C (Lefèvre et al. 1987), and independently checked at 40 °C (Lane, unpublished data). The non-self complementary 14-mer containing the −35 site of the wild-type trp promoter-35, d(GCTGTTGACAATTA). d(TAATTGTCAACAGC) (trp −35 14-mer) was prepared as previously described (Birchall and Lane 1990). The ^{1}H NMR assignments for this oligonucleotide and the T6A transversion are given elsewhere (Lane et al., submitted).

Relaxation rate measurements

Spin-spin relaxation rate constants were measured at different temperatures at 9.4 T on a Bruker AM 400 instrument and at 14.1 T on a Varian Unity 600 using a simple 180° spin-echo method. Rate constants are reported only for singlets, which include the H8 of purines and the C2H of adenine residues.

Relaxation times were determind by non-linear regression to the equation:

$$M(t) = M^0 \exp(-R_2 \, t) \tag{5}$$

where $M(t)$ is the magnetization at time t, M^0 is the magnetization at $t = 0$ and R_2 is the spin-spin relaxation rate constant. 12 to 16 time points were used to sample the magnetization decays. Line-widths were measured as a function of temperature at 4.7 T on a Bruker WM 200 spectrometer. The contribution from inhomogeneity of

the magnetic field was corrected for by subtracting the width of the methyl resonance of internal 2,2′-dimethylsilapentane-5-sulphonate measured under the same conditions.

Rotating-frame T_1 experiments were done in the pulsed Fourier transform mode (Freeman and Hill 1971) on the 9.4 T Bruker instrument using a high-power 90° decoupler pulse to excite all the spins in the sample, and then switching to lower power (using the minimum delay of 0.3 ms for power switching on the Bruker instrument) for the spin-lock. The carrier was set on-resonance with the signal of interest to minimise off-resonance effects. The transmitter was used for both high and low power pulses on the 14.1 T instrument. The spin-lock field strength was varied from 1 to 9 kHz. The duration of the spin-lock period was varied from 1 ms to 500 ms in 12 to 16 unequally spaced steps. The data were analysed by non-linear regression to (5), with $R_{1\varrho}$ replacing R_2. For the weakest B_1 fields, the pulsed spin-lock method was used, and the identity $R_2 = R_{1\varrho}$ assumed (Allerhand et al. 1965, Farmer et al. 1988).

$\Delta\nu$ (app) and k were determined at each temperature and B_0 by non-linear regression to:

$$R_{1\varrho} = R_{1\varrho}^{\infty} + \pi^2 \, \Delta\nu(\text{app})^2 \, k/(k^2 + \omega_1^2) \qquad (6)$$

where $\Delta\nu$(app) is the apparent frequency difference = $\Delta\nu \, (p_a \, p_b)^{1/2}$ (cf. (3)).

Cross-relaxation rate constants were measured at 400 MHz using the truncated NOE experiment described by Wagner and Wüthrich (1979), using a recycle time of 8 to 9 seconds. Correlation times for the cytosine H6-H5 vectors were taken from Lane et al. (1986).

Analysis of errors

The standard deviations of the relaxation rate constants estimated from the curvature matrix in the non-linear regression algorithm are small, of the order ±2–3%. However, replicate determinations at a given spin-lock field-strength indicate a reproducibility of about ±10%. Also, there is some uncertainty in the value of the spin-lock field-strength. Hence, the standard errors determined from the curvature matrix may underestimate the true uncertainties. To account for the experimental errors, we have used Monte-Carlo simulations in which data sets were generated with random variations of γB_1 and $R_{1\varrho}$ drawn from normal distributions having the estimated standard deviations, and means equal to the experimental values of γB_1 and the values of $R_{1\varrho}$ calculated from the 'best-fit' parameters, respectively (Press et al. 1986). The variation in the fitted parameters to each of 500 data sets then gives a more realistic estimate of the mean and variance of the parameters taking into account the experimental uncertainties.

Results

Dependence of line width on external magnetic field strength and temperature

The line-width of the C2H resonance of Ade11 in the trp −10 20-mer has a maximum at 30 °C (not shown),

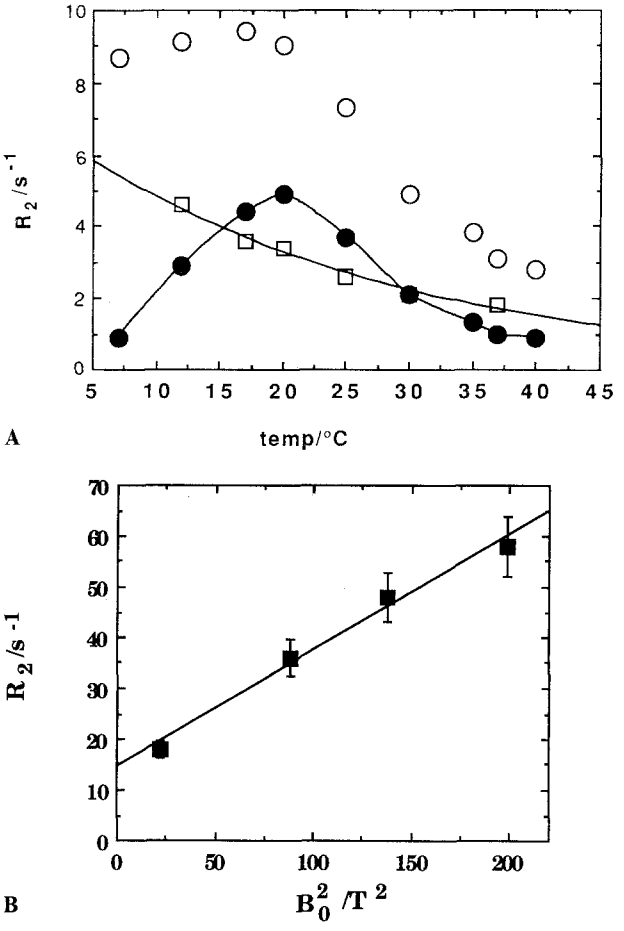

Fig. 1 A, B. Dependence of R_2 on temperature and applied magnetic field strength. **A)** Variation of R_2 of protons in the trp −35 14-mer on temperature at 9.4 T. Open circles denote the measured R_2 values of A10 C2H. Closed circles represent the values after subtracting the intrinsic temperature dependence due to overall rotation. The open squares show the temperature dependence of A8 C2H for comparative purposes. **B)** Magnetic field dependence of R_2 at 303 K for the C2H of A11 in the trp −10 20-mer

confirming previous measurements (Lefèvre et al. 1988). In the trp −35 14-mer, the value of R_2 for A10 C2H passes through a maximum at 20 °C, whereas R_2 for other protons decreases monotonically (Fig. 1 A). However, the maximum value of R_2 at 9.4 T is only 9 s^{-1} (Fig. 1 A), compared with 38 s^{-1} for the C2H of A11 in the trp −20-mer.

Equation (4) indicates that the line-width, ($R_{1\varrho}$ at $\omega_1 = 0$) of a resonance in fast exchange should increase with the square of the external magnetic field strength through the term $\Delta\nu^2$. We have measured R_2 of A11 C2H in the wild-type trp −10 20-mer at four external magnetic field strengths at 303 K, where the maximum line-width was found. We find that R_2 increases from 17 s^{-1} at 4.7 T to 55 s^{-1} at 14.1 T. However, the measured value of R_2 contains an exchange-independent contribution (3). Assuming, a dipole-dipole relaxation mechanism, it can easily be shown that the line-width from this contribution decreases by less than 4% over the range 4.7 T to 14.1 T, which is insignificant compared with the large increase that can be attributed to the exchange contribution. As

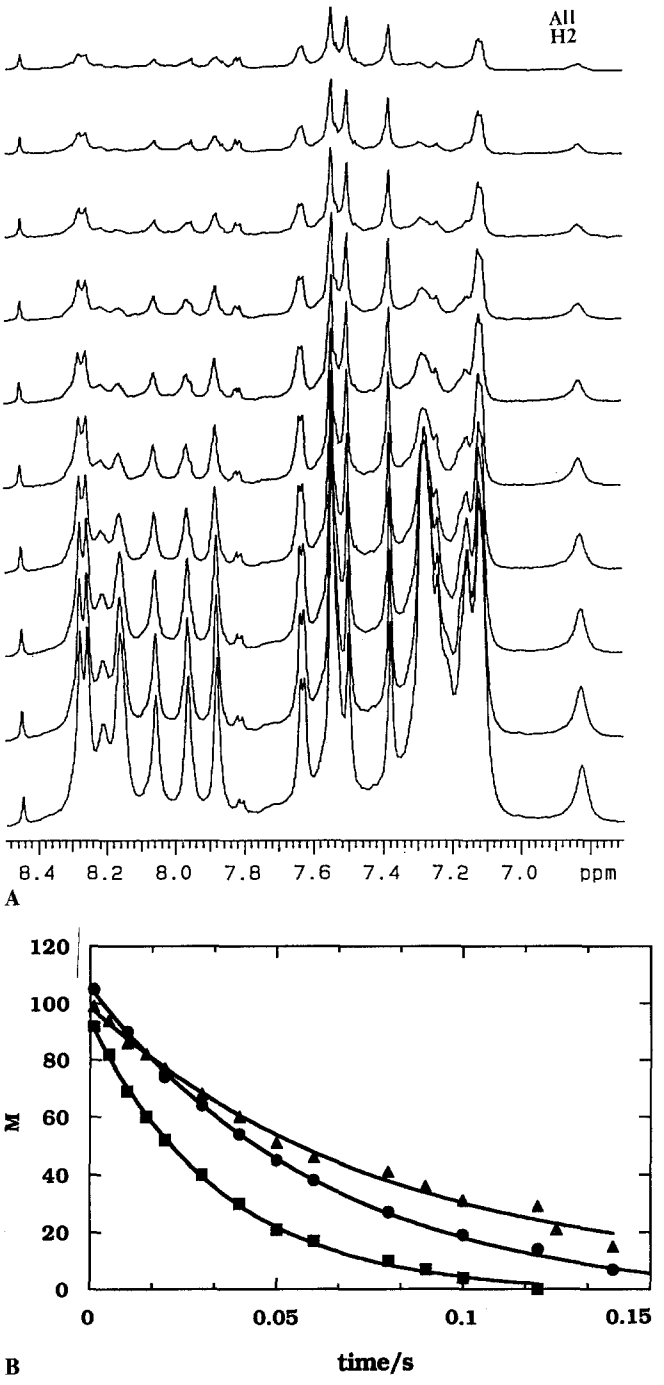

Fig. 2A, B. Rotating frame relaxation at 14.1 T. The $T_{1\varrho}$ experiments were performed at 14.1 T as described in the Experimental section. The temperature was 303 K. **A)** Spectra showing the decay of magnetization of the trp −10 20-mer during spin-locking for periods of 1, 10, 20, 40, 60, 80, 100, 125, 150, 175, 200 ms. The spin-lock field strength was 6 kHz. The resonance of A11 C2H is marked. **B)** Exponential fit according to (2). The values of γB_1 were 860 Hz (■), 2 000 Hz (●) and 8 600 Hz (▲)

Fig. 1 B shows, the measured value of R_2 is proportional to the square of the applied magnetic field strength over the range 4.7 T to 14.1 T, in agreement with (4). We conclude that the nucleotide A11 is undergoing a chemical exchange process that is fast on the chemical shift timescale. However, according to (4), the line-width data are insufficient to separate the chemical shift difference from the rate constant for the exchange process.

Table 1. Exchange parameters for the trp promoter −10 and −35 regions. Rate constants and frequency differences were determined as described in the text. k is the sum of the forward and reverse rate constants $k_1 + k_{-1}$, and $\Delta\nu_{app}$ is the apparent separation in Hz not corrected for the unknown populations, $= \Delta\nu\,(p_a\,p_b)^{1/2}$. Errors were estimated as described in the text

T K	k s^{-1}		$\Delta\nu_{app}$ Hz	
	9.4 T	14.1 T	9.4 T	14.1 T
trp −10 20-mer				
293	nd[a]	2 500±180	nd	62±4
303	5 900± 630	5 100±190	95±13	102±5
313	8 300±1 000	nd	100±13	nd
trp −35 14-mer				
293	nd	5 000±400	nd	50±5

Rotating frame T_1 measurements

According to the treatment given in the Theory section, the rate constant for the transition can be separated from the chemical shift differences by measuring the rotating frame spin-lattice relaxation rate constant, $R_{1\varrho}$, as a function of γB_1. We have measured $R_{1\varrho}$ at different temperatures and field strengths for the C2H of A11 in the trp −10 20-mer. Figure 2A shows a series of rotating frame spectra recorded at 14.1 T, as a function of the duration of the spin-lock. The dependence of the area of the A11 C2H resonance on duration for different values of γB_1 is shown in Fig. 2B, clearly indicating the exponential dependence of the magnetization on spin-lock time (c.f. (5)).

Figure 3 shows the dependence of $R_{1\varrho}$ on γB_1 for A11 C2H of the trp −10 20-mer at 293 K and 303 K. The value of $R_{1\varrho}$ drops rapidly between $\gamma B_1 = 0$ and $\gamma B_1 = 2$ kHz, and approaches a constant value at the highest field strengths. This implies that ω_1^2/k^2 varies from zero to $\gg 1$ over this range of γB_1 (cf. (3)). The value of $R_{1\varrho}$ at large γB_1 is the exchange-independent contribution to $R_{1\varrho}$ (i.e. $R_{1\varrho}^{\infty}$) and is of similar magnitude to the value of R_2 determined for other Ade C2H resonances that show no evidence of significant exchange (Lefèvre et al. 1988). For example, the values of R_2 and $R_{1\varrho}$ for Ade 4 C2H at 303 K are 7 and 8 s^{-1}, respectively. The similarity of these values for Ade 4 C2H indicates that the exchange contribution to the relaxation is negligible (c.f. (6)). As expected, the value of $R_{1\varrho}^{\infty}$ decreases with increasing temperature (see below).

The rate constant k and the apparent separation of the resonances in states A and B can be determined by fitting the data to (2). At 303 K we obtain a value for k of 5 100 s^{-1} at 14.1 T, and 5 900 s^{-1} at 9.4 T (Table 1). Within the limits of the estimated errors on k, these two values are not significantly different. The values of k at 20 °C and 40 °C are 2 500 s^{-1} and 8 000 s^{-1}, respectively

(Table 1). We have also measured the rate constant at 20 °C for A10 C2H in the trp −35 14-mer in the same way (Table 1). The value of k for A10 C2H at this temperature is higher than that measured for A11 C2H in the trp −10 20-mer, and the apparent frequency difference is smaller. This accounts for the smaller exchange contribution to the line-width observed in the trp −35 14-mer.

$R_{1\varrho}$ has also been determined for A11 C2H of the trp −10 20-mer at $\gamma B_1 = 8$ kHz as a function of temperature at both 9.4 T and 14.1 T. Under these conditions, the exchange contribution is largely quenched (see above), and $R_{1\varrho}$ was found to be independent of the external magnetic field strength. It was found that the value of $R_{1\varrho}$ decreases monotonically with increasing temperature, in contrast to R_2, which reaches a maximum at about 30 °C. The apparent activation energy of the 'intrinsic' $R_{1\varrho}$ is 24 kJ mol^{-1}, which is slightly higher than the activation energy for the viscosity of D_2O (ca. 19 kJ mol^{-1}, Wilbur et al. 1979) (Table 1). We have previously shown that the cross-relaxation rate constant for the cytosine H6-H5 vectors scale with viscosity in the same way (Birchall and Lane 1990).

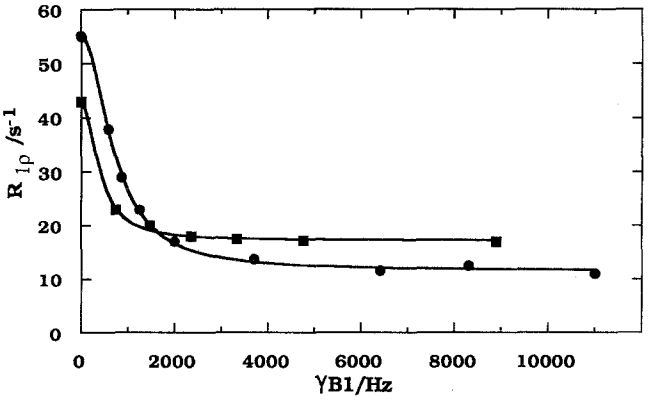

Fig. 3. Dependence of $R_{1\varrho}$ on B_1 at 14.1 T. Values of $R_{1\varrho}$ were determined at different spin-lock field strengths as in Fig. 2. The lines are the best regression fits to (3). ■ 293 K, ● 303 K

Cross-relaxation rates for Ade C2H-Ade C2H spins

The experiments described above demonstrate that both A11 in the trp −10 20-mer and A10 in the trp −35 14-mer are involved in chemical exchange process on the sub-millisecond time scale. There is no evidence for similar exchange processes involving other adenine residues. This could be because the chemical shift difference between states is vanishingly small, or because the rate constant is very large at accessible temperatures (or both).

The exchange process indicates that there are at least two distinct conformations. Additional information may therefore be obtained from measurements of NOEs. For example, in both the wild-type trp −10 20-mer and a mutant version (Lefèvre et al. 1988; Lane 1989) there is a conformational transition in the subsequences TAAC and TAAT, in which the apparent distance between the neighbouring Ade C2 protons in the minor groove increase with increasing temperature. As the NOE (or the cross-relaxation rate constant) is very sensitive to the distance, the NOE experiment can be used to detect relatively small differences in distance. Figure 4 shows the base-proton region of a NOESY spectrum recorded at 10 °C on the trp promoter-35 14-mer. The labelled cross-peaks demonstrate the presence of significant NOEs between all pairs of neighbouring Ade C2 protons in this molecule, both intra and interstrand. In addition, we have determined the cross-relaxation rate constants, σ, at several temperatures from time-dependent truncated NOE experiments, for both the wild-type sequence and the T6A transvertant (Lane et al., submitted). The cross-relaxation rate constants for the various Ade C2H-C2H vectors decrease with increasing temperature, as would be expected from the effects of temperature on the rotational correlation time. However, the observed decrease in the cross-relaxation rate constants is greater than would be expected from the influence of temperature on

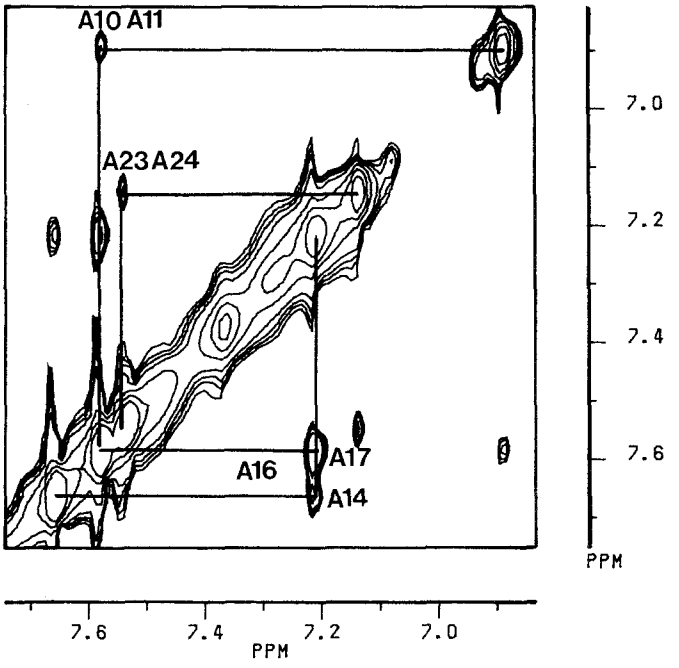

Fig. 4. Cross-relaxation between Ade C2 protons. The NOESY spectrum was recorded at 283 K at 9.4 T with a mixing time of 100 ms. The data matrix consisted of 2 048 K by 512 complex data points, filled with zeroes to 4 096 by 2 048 points prior to apodisation with a 90°-shifted sine-squared function in both dimensions

the correlation time alone. We have therefore calculated the apparent distance, r_{app}, from the cross-relaxation rate constants, σ, using the previously determined correlation times at the different temperatures (Birchall and Lane 1990), according to:

$$r_{app} = [56.92\,(6J(2\omega) - J(0))/\sigma]^{1/6} \quad (7)$$

The spectral density functions $J(2\omega)$ and $J(0)$ were calculated using the correlation time for end-over-end tumbling, as the C2H-C2H vectors are nearly parallel to the long axis of the DNA duplex. As shown in Table 2, the values of r_{app} increase with increasing temperature. The increase is only about 0.2 Å for A23 C2H-A24 C2H, but

Table 2. Apparent distances for Ade C2H-Ade C2H vectors in the trp −35 14-mer. Apparent distances, r_{app}, were calculated from cross-relaxation rate constants determined from NOE time-courses at each temperature as described in the text. A10* A11* refer to the bases in a mutant T6A trp −35 14-mer

T °C	r_{app} (Å)				
	A10–A11	A23–A24	A16–A17	A14–A16	A10*–A11*
10	3.3	3.3	2.9	3.2	3.4
20	3.50	3.45	3.3	3.5	nd
30	3.55	3.4	3.45	3.55	nd
40	3.6	3.40	nd	nd	3.8
Δr_{max}	0.3	0.1	0.55	0.4	0.4

is nearer 0.5 Å for A10 C2H-A11 C2H and A16 C2H-A17 C2H. Further, r_{app} for A14 C2H-A16 C2H, which is between the strands, also increases with increasing temperature. The agreement between cross-relaxation rate constants determined on both the wild-type and mutant trp −35 14-mers is within 30%, which is equivalent to an error in the apparent distance of up to 0.15 Å. The data in Table 2 show that the steps A10–A11 and A16–A17 undergo conformational transitions in the −35 region of the trp promoter. These nucleotides are found in the subsequences CAAT and TAAT. The line-shape of the C2H of A10 also shows exchange behaviour (see above). Interestingly, the subsequence CAAC does not undergo a significant conformational transition under these conditions. We note that similar effects were observed in the mutant trp −10 20-mer in the subsequence TAAT in the range 5 to 45 °C (Lane 1989), and in the wild-type trp −10 20-mer in the subsequence TAAC (Lefèvre et al. 1988; Lane, unpublished data).

Discussion

All of the data are consistent with a two-site exchange process that occurs on the sub-millisecond time-scale. However, more complicated mechanisms involving exchange among more than two sites cannot be ruled out. The measured rates are significantly faster than that associated with the 'breathing' that leads to exchange of iminoprotons with solvent, which is expected to occur at $k \approx 200$ *to* 300 s^{-1} under these condition for AT base-pairs (Leroy et al. 1988).

The value of k increases with increasing temperature, which for a constant chemical shift difference implies a decreasing contribution to the line-width (cf (3)). The observation of a maximum line-width therefore indicates that the populations of states A and B change with increasing temperature. Specifically, the product $p_a\, p_b$ must reach a maximum in the experimental temperature range. If R_x is the exchange contribution to the line-width, then the product $k \cdot R_x$ is proportional to $\Delta\omega^2\, p_a (1-p_a)$. Assuming that the chemical shift difference is independent of temperature, this function has a maximum at $p_a = 0.5$. Published data for the temperature-dependence of R_2 ($= R_x$) of A11 C2H of the trp −10 20-mer (Lefèvre et al. 1988) and the rate constants given in Table 1 show that $p_a = 0.5$ at about 40 °C. The value of p_a reaches a value of 0.5 only at 40 °C; its value therefore varies at most between 0 and 0.5 over a temperature interval of 30 °C.

We have previously observed that the chemical shift of A11 C2H of the trp −10 20-mer varies essentially linearly over from 10 °C to 45 °C, with a slope of 6.5 ppb K^{-1} which is significantly larger than that observed for the other Ade C2H resonances (typically 1 to 2 ppb/K) (Lefèvre et al. 1988; Lane 1989). This behaviour can be accounted for as follows. Assuming that the NMR signals remain in fast exchange over the experimental temperature range, the observed signal, S, is given by the weighted average (Neuhaus and Williamson 1989):

$$S = p_a\, S_a + (1-p_a)\, S_b \tag{8}$$

If p_a varies relatively slowly over the experimental range, the NMR signal S will also appear to vary almost linearly with temperature. Simulations of the line-shape as a function of temperature, using values for the shift differences and rate constants near those given in Table 1, show that for a variation of p_a from ca. 0.1 to 0.6, the temperature-dependence of the chemical shift of A11 C2H in the range 10 to 50 °C would be essentially linear, and the temperature coefficient would be relatively large, which is in agreement with the observations.

As similar behaviour was also observed for A10 H2 of the trp −35 14-mer, the above argument would also account for the observed variation of the cross-relaxation rate constant which, when corrected for the intrinsic temperature-dependence, decreases about 3-fold over this temperature range. The apparent distances given in Table 2 for the trp −35 14-mer are then averages over the two conformational states (cf. (8)); only the distances at low temperatures are likely to approach those corresponding to a single conformation (i.e. in state A). The apparent distances determined at higher temperatures must therefore underestimate the true distance appropriate to the second conformation.

The detailed nature of the conformational transitions observed in these molecules cannot be determined with the present data. However, simulations in which helical parameters were systematically varied (not shown) demonstrate that the distance between C2H on neighbouring adenines is most sensitive to base-pair roll or propellor twist, and weakly dependent on other helical parameters in the neighbourhood of the B-family of conformations. In the B-conformation, this distance is 3.85 Å for a propellor twist of 0°; a propellor twist of around 20° reduces the distance to about 3 Å, which accounts for the data at low temperature. The distances at higher temperatures can be accounted for by a decrease in the propellor twist to around 5°. This range of propellor twists is typical of AT base-pairs in crystal structures of oligonucleotides, especially in AA sequences (Yanagi et al. 1991).

The bases that have been observed to undergo exchange processes are all in the subsequences YAAY where Y is a pyrimidine. In the −10 region of the trp promoter, it is TAAC, TAAT in the mutant trp promoter, and TAAC in the −35 region of the trp promoter. Further, both the

apparent frequency difference and the rate constant for the transition differ for these subsequences, indicating that flanking bases influence the dynamic properties of YAAY subsequences.

These experiments are also applicable in principle to proteins. However, the greater spectral complexity may make two-dimensional methods necessary. There is currently considerable interest in the dynamics of the backbone and side-chains as the introduction of isotopic labelling has made relaxation studies more accessible. Generally it is found that sub-nanosecond fluctuations that affect T_1 are of small amplitude, whereas slower processes that affect T_2 have been observed, particularly in loops (Kay et al. 1989; Clore et al. 1990; Kördel et al. 1992). The rotating frame measurements can provide additional information about both shift differences and exchange rate constants because of the additional variable, B_1, that can be used.

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