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Conformational properties of the −35 region of the trp promoter in solution: comparison of the wild-type sequence with an AT transversion

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Abstract. The majority of the ^{1}H NMR resonances of the protons in a tetradecamer containing the −35 region of the trp promoter d(GCTGTTGACAATTA): d(TAATTGTCAACAGC) and in the **TA** transversion have been assigned. The conformational properties of the nucleotides have been determined and compared in the two duplexes. Analysis of spin-spin coupling and NOEs shows that all sugar puckers are in the south domain (i.e. near C2′ endo) and the glycosidic torsion angles are *anti* ($\chi \approx 110°$). The NMR data are consistent with the duplex being in the B family of conformations. Significant differences in chemical shifts between the two molecules were observed only for nearest neighbours to the transversion site, suggesting the absence of long range conformational effects. This was confirmed by the similarity of coupling constants and NOEs. Other properties are also not greatly affected at positions more than two base pairs from the mutation site. These results are consistent with the hypothesis that unconstrained oligonucleotides are highly flexible, and can readily accommodate significant perturbations of the local structure, such as a transversion.

Key words: Trp promoter – Solution conformation – Transversion mutation

Introduction

Bacterial promoters typically span about 40 base-pairs upstream of the transcription start site, and consist of three regions (Hawley and McClure 1983; Harley and Reynolds 1987). These are the −35 region, having the consensus sequence TTGACA, a spacer of 17 ± 2 base pairs, and the −10 region, or Pribnow box, which has the consensus sequence TATAAT (Harley and Reynolds 1987). In some promoters, there is a fourth AT-rich stretch downstream of the −35 region which may be involved in bending the promoter around RNA polymerase (Bertrand-Burggraff et al. 1990), or other sites which are recognised by transcriptional activators such as the catabolite activator protein (de Crombrugghe et al. 1984).

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Initiation of transcription involves recognition of the promoter by RNA polymerase to form the so-called closed complex, followed by isomerisation to the open complex, which is associated with opening of base-pairs downstream of the −10 region to the start site. The apparent second order rate constant for the formation of the open complex is a measure of the promoter strength (Mulligan et al. 1984), which is found to vary over several orders of magnitude for different promoters (Mulligan et al. 1984; Buc and McClure, 1985). Because the RNA polymerase is the same in each case, the variation in promoter strength must in part reflect the properties of the different promoters themselves. It has been shown that mutations that make the sequence more similar to this consensus sequence tend to make the promoter stronger, whereas mutations that decrease homology with the consensus sequence diminish the promoter strength (Hawley and McClure 1983; Szoke et al. 1987).

One proposal to account for this observation is that specific hydrogen bonding between RNA polymerase and groups in the major grooves of the DNA is sufficient to provide the binding energy both for recognition and isomerisation. However, this proposal takes no account of the conformation of the DNA sequences. DNA is known to vary considerably in its local structure, depending on the base sequence (Fratini et al. 1982). Further, DNA is a relatively flexible molecule, and its conformational flexibility is also sequence-dependent, which may be related to the ease with which the promoter can wrap around RNA polymerase to optimise essential enzyme-DNA contacts (Travers 1989). Hence, while specific hydrogen bonding is likely to be an important determinant of specificity in the initial recognition of the promoter, it is not the only factor involved. It is important to determine the local structure and conformational flexibility of promoters, and relate them to the observed promoter strengths.

The properties of the consensus Pribnow box (Patel et al. 1983; Wemmer et al. 1984; Patel et al. 1985), of the lac P and lac UV5 (Nerdal et al. 1988), and of the trp promoters have been examined in solution using NMR methods (Lefèvre et al. 1987, 1988; Lane 1989). Although

the overall conformation is on average B-like, there are sequence-dependent variations in the local conformations. Further, the AT rich regions in the Pribnow boxes were shown to be conformationally flexible. However, the conformational properties of the −35 region have not been reported, nor is it known what are the sequence requirements for intrinsic flexibility of DNA segments.

We are using high-resolution NMR to investigate the solution conformations of the trp promoter in solution. We report here the ^{1}H NMR assignments of the trp −35 region, which contains the consensus sequence TTGACA (Bennett et al. 1978), and its solution properties. This complements the results obtained for the −10 region, and a promoter down mutation of −10 (Lefèvre et al. 1987, 1988, Lane 1989). In addition, we address the influence of the transversion at TTGACA → TAGACA on the local conformation of the promoter, which corresponds to a known promoter down mutation (Hawley and McClure 1983; Szoke et al. 1987).

Experimental

Materials

Four 14-base oligodeoxynucleotides were synthesised on a Beckman synthesiser using phosphoramidite chemistry. The sequences are:

5′ GCTGTTGACAATTA 3′
5′ TAATTGTCAACAGC 3′

In the third and fourth sequences, the **A** and **T** residues were exchanged, to produce the A6T transversion.

The DNA strands were purified by FPLC using an ion-exchange column (mono Q, Pharmacia), and a gradient of 0 to 1 M ammonium bicarbonate. Fractions of the 14 base oligomers were pooled, and lyophilised to remove the ammonium bicarbonate. The dry powders were dissolved in 0.2 ml buffer containing 100 mM KCl, 10 mM sodium phosphate, pH 7.0, and run on a 20% polyacrylamide gel to check the purity. Equal amounts of the two strands were mixed, heated to 90 °C for 10 min, and allowed to cool slowly. The annealed DNA was passed over a Sephadex G75 column equilibrated in the same buffer. Fractions from the first peak eluting from the column (duplex DNA) were collected, lyophilised and finally dialysed against 100 mM KCl, 10 mM Na phosphate and 0.2 mM EDTA, pH 7.0. The dialysed sample was then lyophilised, and redissolved in 99.96% D_2O (Aldrich) containing 0.1 mM DSS as an internal chemical shift reference.

NMR spectroscopy

^{1}H NMR spectra were recorded at 500 MHz and 400 MHz on Bruker AM spectrometers. Both spectrometers were equipped with an ASPECT 3 000 computer and a 16 bit digitiser. One-dimensional spectra were recorded using a 90° pulse and an acquisition time of 1.64 s. For spectra in ^1H_2O, the $1\bar{3}3\bar{1}$ pulse sequence (Hore 1983) was used to suppress the intense solvent signal. Phase-sensitive two-dimensional NMR spectra were recorded using the TPPI scheme (Marion and Wüthrich 1983), using a spectral width of 10 ppm. NOESY spectra were recorded at several mixing times, with 2 048 real points in F2 and 512 t_1 increments. The data matrices were filled with zeros to 4 096 by 2 048 real points before Fourier transformation. Phase-sensitive proton INADEQUATE (Mareci and Freeman 1983) spectra were recorded using a mixing pulse pulse of 135°. HOHAHA spectra were recorded using MLEV-17 isotropic mixing (Bax and Davis 1985) at a spin-lock field of 8 kHz, and mixing times of 27, 40, 60 and 90 ms. 768 t_1 increments of 4 096 complex points were recorded. These data matrices were zero-filled to 8 192 and 1 024 points prior to Fourier transformation, giving a final digital resolution of 0.98 Hz per point in F2. The residual HDO signal was suppressed during the relaxation delays using a DANTE (Morris and Freeman 1978) sequence. Spin-spin relaxation rate constants were obtained for singlet base protons using the 90-τ−180-τ spin-echo experiment. One-dimensional NOEs were obtained using the method of Wagner and Wüthrich (1979). For slowly relaxing protons, the relaxation delays were increased up to 10 s to allow essentially complete relaxation between exciting pulses. Apparent distances between C2H resonances of neighbouring adenine residues (both intra- and interstrand) were determined from the cross-relaxation rate constant, σ, and the equation:

$$r = \{\alpha\,[6J(2\omega) - J(0)]/\sigma\}^{1/6} \quad (1)$$

where r is the distance, α is a constant = 56.92 Å^6 ns^{-2} and $J(\omega)$ is the spectral density function defined as:

$$J(\omega) = \tau/(1 + \omega^2\,\tau^2)\,. \quad (2)$$

The H2-H2 vector is nearly parallel to the helix axis, so that the appropriate correlation time to use in (2) is that for end-over-end tumbling, τ_L. τ_L was calculated from the correlation time of the Cyt H6-H5 vectors according to the relationship (Birchall and Lane 1990):

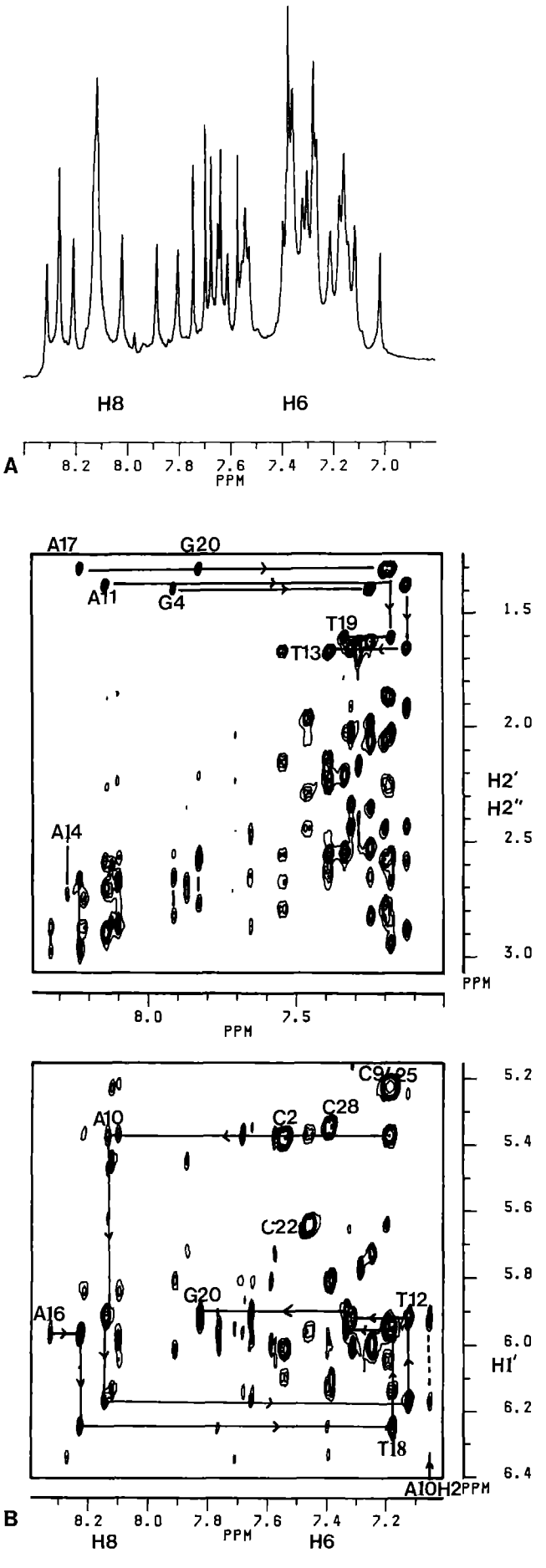
$$\tau_L = 2\,\tau_{cyt}(2\gamma + 1)/(\gamma + 5) \quad (3)$$

where $\gamma = \tau_L/\tau_S$, and τ_{cyt} is the measured correlation time for the Cyt H6-H5 vector. γ was calculated using the Perrin equations (Woessner 1962) assuming an axial ratio of 2.0, which is the expected value for a 14 base-pair fragment of DNA in the B conformation. τ_{cyt} was determined by measuring the cross relaxation rate constant (σ) for the H6-H5 vectors of the 5 cytosine residues as a function of temperature (Lane et al. 1986). Nucleotide conformations were determined from time-dependent NOEs using the combined conformational search and least-squares optimising program NUCFIT as previously described (Lane 1990).

Results

^{1}H NMR assignments of the trp promoter

Because the 14-base pair duplex is not self-complementary, and is also AT rich, parts of the ^{1}H NMR spectra



overlap significantly. We have therefore used a wide variety of two-dimensional NMR methods to assign most the protons (Chazin et al. 1986). Figure 1 A shows a typical one-dimensional spectrum of the wild-type sequence that demonstrates the considerable overlap in the aromatic region. However, there are resolved resonances corresponding to the H8 resonances of purine residues, H6 resonances of cytosines, and C2H resonances of Adenines.

Figure 1 B shows a NOESY spectrum recorded with a mixing time of 300 ms, in which there is a large number of cross-peaks. It was relatively simple to follow the sequential connectivities for the H8/6-H1′-H8/6 resonances. However, the overlap in the H2′/H2″ region precluded complete assignment of the sugar protons using NOESY alone. We have therefore used both HOHAHA spectra recorded with high digital resolution and different mixing times, and the proton INADEQUATE experiment. The latter was particularly useful to connect the H2′ and H2″ resonances to the H1′ resonances (not shown); all 28 H1′ resonances are resolved in this experiment, and the absence of a diagonal allowed the frequencies of both H2′ and H2″ to be read off with ease. These assignments were confirmed by comparison with the NOESY and the HOHAHA experiment connectivities (Fig. 2). The relayed connectivities that were observed in HOHAHA spectra recorded with a mixing time of 50 ms or longer were particularly helpful in assigning the H3′ and H4′ resonances. These spectra allowed us to assign most of the non-exchangeable protons with the exception of the H5′/H5″ resonances. The resulting assignments are given in Table 1.

The assignments of the non-exchangeable protons in the A6T transversion mutant were also made using NOESY and HOHAHA spectroscopy. Figure 3 A shows a portion of the NOESY spectrum showing the base to H1′ connectivities. In addition to correlating sugar protons, the HOHAHA experiment is also helpful for finding the H6 frequencies of thymidine nucleotides, through the four-bond coupling to the methyl group (Fig. 3 B). The Thy H6 resonances are useful in the sequential assignment because of the relatively strong NOE from the H8/H6 to the methyl groups in XT steps (c.f. Fig. 2 B). The chemical shifts of the T6A transvertant are given in Table 2.

Figure 4 shows the variation of the difference chemical shifts for the base and H1′ resonances along the sequence (except T/A6, which will differ by virtue of the different base). Shift differences larger than 0.05 ppm are observed only for nearest and next nearest neighbours. Indeed, the pattern of shift differences is very similar for both strands, in which only the nearest neighbour 5′ to the mutation site is significantly affected by, whereas both the nearest and next nearest neighbours 3′ to the

Fig. 1 A, B. 500 MHz ^{1}H NMR spectra of the trp −35 region wild-type sequence at 303 K. **A** One-dimensional spectrum showing the base proton region. **B** 300 ms NOESY showing H1′ to H8/6 connectivities and H2′ to H8/H6. The data were apodised using a 60°-shifted sine-squared function in both dimensions

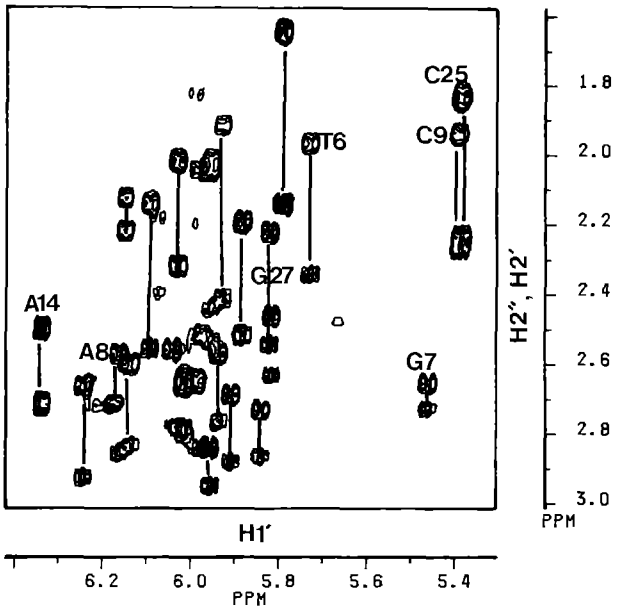


Fig. 2. Scalar correlated spectra of the trp −35 region. The HOHAHA spectrum of the wild-type sequence was recorded at 303 K with a mixing time of 50 ms as described in the methods

Table 1. Assignments of the non-exchangeable protons of the −35 region of the trp promoter. Assignments were made using NOESY, HOHAHA and ^{1}H INADEQUATE at 30 °C as described in the text

Base	Chemical shift (ppm)						
	H8/H6	H2/H5/ CH_3	H1′	H2′	H2″	H3′	H4′
G1	7.97	–	6.01	2.65	2.75	4.84	4.14
C2	7.54	5.38	6.09	2.14	2.55	4.79	4.21
T3	7.37	1.63	5.79	2.22	2.54	4.90	nd
G4	7.92	–	6.01	2.64	2.77	4.98	nd[a]
T5	7.25	1.36	6.00	2.04	2.48	4.85	4.22
T6	7.23	1.61	5.71	1.97	2.32	4.84	4.06
G7	7.89	–	5.36	2.65	2.71	4 98	4.39
A8	8.14	7.66	6.15	2.61	2.83	5.00	4.44/35
C9	7.19	5.23	5.37	1.93	2.24	4.82	4.14
A10	8.14	7.01	5.92	2.67	2.88	5.02	4.44/35
A11	8.13	7.62	6.16	2.58	2.88	5.01	4.50
T12	7.13	1.35	5.92	1.89	2.39	4.81	4.37
T13	7.30	1.63	5.97	1.98	2.31	4.85	nd
A14	8.26	7.74	6.34	2.48	2.72	4.72	4.21
T15	7.31	1.68	5.74	1.65	2.16	4.58	3.98
A16	8.33	7.32	5.97	2.85	2.95	5.04	4.35
A17	8.22	7.66	6.25	2.62	2.92	5.02	4.48
T18	7.18	1.29	5 96	2.01	2.50	4.99	nd
T19	7.32	1 59	5.91	2.20	2.52	4.89	4.15
G20	7.84	–	5.94	2.55	2.73	4.93	4.37
T21	7.19	1.29	5.96	2.03	2.38	4.81	4.18
C22	7.47	5.64	5.32	1.95	2 24	4.94	nd
A23	8.23	7.17	5.82	2.76	2.87	5.03	4.34
A24	8.11	7.56	6.04	2.65	2.76	4.98	4.41
C25	7.21	5.22	5.35	1 84	2.24	4.78	4.07
A26	8.10	7.56	5.97	2.64	2.84	4.98	4.35
G27	7.66	–	5.80	2.47	2.63	4.93	4.33
C28	7.37	5.35	6 13	2.12	2.20	4.43	4 05

[a] nd: not determined

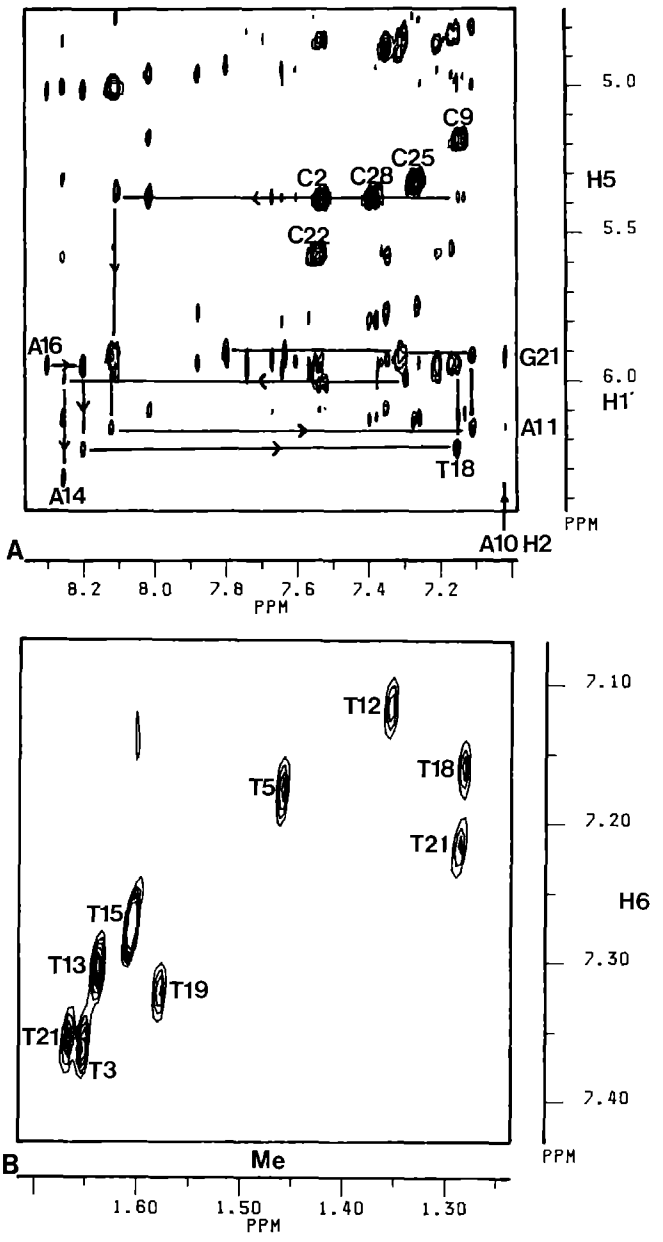


Fig. 3 A, B. Two-dimensional NMR spectra of the T6A transvertant. Spectra were recorded at 303 K as described in the methods. **A** NOESY spectrum with a mixing time of 200 ms. The data were processed by zero-filling to 4 096 by 2 048 points, and apodising with a 60°-shifted sine-squared function in both dimensions. **B** HOHAHA spectrum with a mixing time of 50 ms, and $B_1 = 8$ kHz. The data were processed by zero-filling to 8 192 by 1 024 complex points and apodising with 60°-shifted sine-squared functions in both dimensions

mutation are affected. Much of the change can be attributed to the difference in the ring current fields of A and T. According to Giessner-Prettre et al. (1976), the shielding in the neighbourhood of an adenine residue is larger than that from a thymidine residue. Hence, a transversion would be expected to produce opposite effects on the chemical shifts of the nearest neighbours in the two strands, as is observed (Fig. 4). Significant changes in shielding are also predicted for conformational differences involving changes in glycosidic torsion angles and

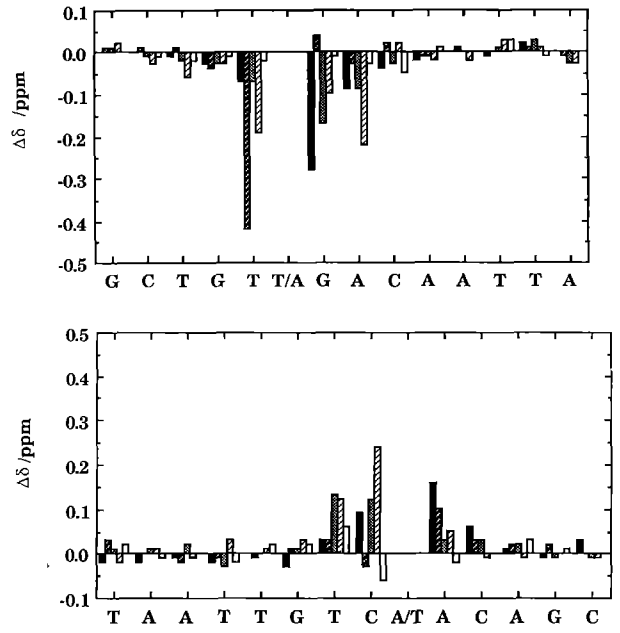


Fig. 4. Chemical shift differences between wild-type and the TA6 mutant. The chemical shifts at 303 K were determined as described in the text. The differences mutant minus wild type were calculated from Tables 1 and 2, ■, H8/6; ▨, H1′; □, H2′; ▨, H2″; □, H3′

sugar puckering (Giessner-Prettre and Pullman 1987). Some of the observed shift changes may therefore also be due to local conformational rearrangements. However, the localisation of significant changes in chemicals shifts indicates that any substantial conformational rearrangements arising from the transversion are not propagated far along the helix. This result is in agreement with an analogous study on the trp operator, where conformational changes arising from exchange of a purine for a purine were minor (Lane 1991).

Imino and Ade H2 protons were assigned by one and two-dimensional NOE experiments in 90% ^1H_2O:10% ^2H_2O. Figure 5 shows the iminoproton region of the spectra of the wild-type and T6A mutant. Although the spectra are crowded, there is sufficient resolution to assign most of the imino protons by one-dimensional NOE experiments. Also, the broad resonance at approximately 13.0 ppm disappears as the temperature is raised from 288 K to 298 K, while the resonance at 12.95 ppm broadens substantially over the temperature range 298 K to 313 K. This "fraying" behaviour suggest that the two resonances are from the imino protons of GC1 and CG2, respectively. The NOE experiments also connect NH to the Ade C2H resonances. There remained some ambiguity in the assignments, which was resolved by measuring NOEs between H2 resonances, which can only be observed when there are neighbouring adenines either on the same strand or on opposite strands of the duplex (see below). Iminoproton-aminoproton and iminoproton-Ade C2H connectivities are conveniently found in the NOESY experiment. In agreement with Rajagopal et al. (1988) we have found that presaturation of the H_2O resonances does not lead to complete disappearance of the imino proton resonances provided that the solution contains low concentrations of buffer salts and the spectra are recorded at relatively low temperature at pH < 7. Under these conditions, exchange with solvent protons must occur at rates slower than the spin-lattice relaxation rate constant. The assignments of the exchangeable protons are given in Table 3. The principal chemical shift differences are found for the imino proton of the base-pair transversion and its nearest neighbours.

Table 2. Proton assignments in the trp promoter T6A transversion at 303 K. Resonances were assigned using NOESY and HOHAHA spectra as described in the text

Base	H8/6	H2/5/Me	H1′	H2′	H2″	H3′	H4′
G1	7.97	–	6.02	2.66	2.77	4.84	nd
C2	7.54	5.41	6.10	2.13	2.52	4.78	nd
T3	7.36	1.65	5.80	2.20	2.48	4.88	4.18
G4	7.89	–	5.97	2.61	2.74	4.97	nd
T5	7.18	1.46	5.58	1.97	2.29	4.83	4.15
A6	8.12	7.7	5.97	2.67	2.86	5.02	nd
G7	7.61	–	5.40	2.48	2.61	4.97	nd
A8	8.03	7.68	6.12	2.52	2.80	4.98	nd
C9	7.15	5.20	5.39	1.90	2.26	4.77	4.13
A10	8.12	7.01	5.91	2.66	2.86	5.03	nd
A11	8.13	7.63	6.17	2.58	2.86	5.01	4.45
T12	7.12	1.35	5.92	1.90	2.42	4.80	nd
T13	7.32	1.63	5.98	2.01	2.32	4.84	nd
A14	8.26	7.74	6.33	2.45	2.69	4.72	4.21
T15	7.29	1.60	5.77	1.66	2.14	4.60	3.97
A16	8.30	7.32	5.97	2.86	2.96	5.03	4.35
A17	8.21	7.67	6.23	2.64	2.91	5.02	4.35
T18	7.16	1.28	5.95	1.98	2.53	4.97	4.15
T19	7.32	1.58	5.90	2.20	2.53	4.91	nd
G20	7.80	–	5.95	2.56	2.76	4.95	nd
T21	7.22	1.29	5.99	2.16	2.50	4.87	nd
C22	7.56	5.61	5.93	2.07	2.48	4.88	nd
T23	7.34	1.65	5.61	2.07	2.40	4.87	nd
A24	8.27	7.65	6.14	2.68	2.81	5.01	nd
C25	7.27	5.31	5.38	1.87	2.23	4.78	4.08
A26	8.11	7.57	5.99	2.66	2.83	5.01	nd
G27	7.65	–	5.82	2.46	2.63	4.94	nd
C28	7.42	5.42	6.13	2.11	2.19	4.43	4.05

Table 3. Assignments of exchangeable protons. Exchangeable protons were assigned from one dimensional NOE and NOESY spectroscopy in ^1H_2O as described under Experimental. The chemical shifts were determined at 283 K

Proton	Chemical shift/ppm		
	wt	T6A	$\Delta\delta$
GC1	13.0	13.03	0.03
CG2	12.94	12.94	0.0
AT3	13.84	13.86	0.02
CG4	12.58	12.59	0.01
AT5	13.82	13.61	−0.21
AT6	13.77	13.49	−0.28
CG7	12.52	12.54	0.02
AT8	13.54	13.57	0.03
GC9	12.36	12.32	−0.04
TA10	13.57	13.57	0.0
TA11	13.67	13.69	0.2
AT12	na	na	–
AT13/AT14	13.63	na	–

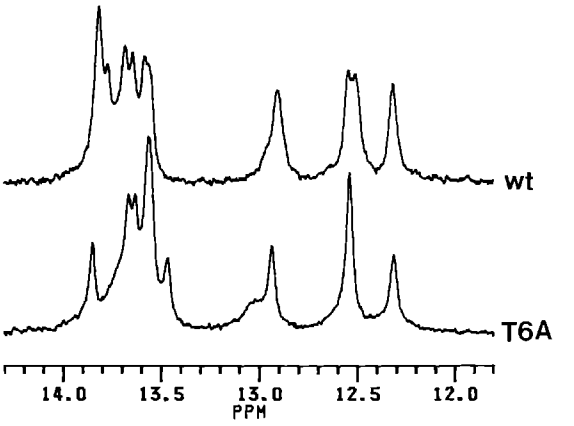


Fig. 5. Exchangeable protons of the trp promoter. The one-dimensional spectra were recorded at 288 K using the 1 $\bar{3}3\bar{1}$ pulse with 16 384 points zero-filled to 32 768 points over a spectral width of 12 kHz. The data were transformed using 2 Hz line-broadening

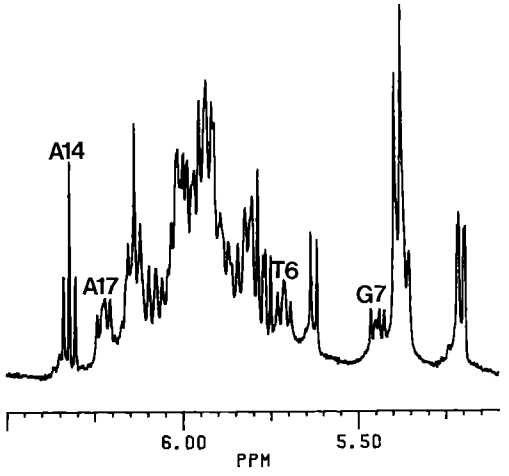


Fig. 6. H1′ region of the wild-type sequence. The spectrum was recorded at 303 K, and resolution enhanced using the Lorentz to Gauss transformation

Conformation

The relative intensities of the cross-peaks in the NOESY spectra are consistent with right-handed duplex DNA in which the gylcosidic torsion angles are all *anti*, and sugars are in the 'south' domain. Some of the H1′ resonances are resolved in one-dimensional NMR spectra (cf Fig. 6). Using mild resolution enhancement of the spectra, the coupling constants $^3J_{1'2'}$ and $^3J_{1'2''}$ can be measured (Table 4). For example, the H1′ resonance of A14 is a triplet for which the two coupling constants are equal (6.7 Hz). These values show that this terminal residue has a sugar pucker in the south domain, but may be a mixture of C2′ endo and C3′ endo state ($f_S \approx 0.65$) (Rinkel and Altona 1987; Rinkel et al. 1987). The other resolved H1′ resonances show coupling constants that are also typical of sugar puckers in the south domain. Further information on the sugar conformations was obtained from high resolution two-dimensional NMR experiments, both HOHAHA and NOESY. By taking rows parallel to F2 through the H1′ resonances, the sum of the coupling constants $^3J_{1'2'}$ and $^3J_{1'2''} = \Sigma_{1'}$ could be determined (cf Table 4). In all cases except for the 3′-termini, A14 and C28, $\Sigma_{1'}$ is larger than 14 Hz, indicating that all deoxyriboses are mainly in the south domain, with at most a 20% admixture of the C3′ endo conformation. Because both of these experiments give rise to in-phase cross-peak structures, derived coupling constants are lower limits to the true values, which therefore leads to an underestimate of the fraction of the south state. Only the terminal bases show evidence of large deviation from the typical range of puckers in B-like DNA. Table 4 also includes the phase angle, P, that is obtained assuming a single conformation (i.e. $f_S = 1$). The range of P that is consistent with the estimated error on the sums of the coupling constants (ca. ± 0.5 Hz) is around 40°. While discrimination between a unique conformation and a dynamic mixture is not possible on the basis of these data alone, it can be seen that the phase angles would be quite large for the smaller coupling constants. As sugar puckers in this range are energetically less stable than C2′-endo, it is probable that the mixture of conformations provides a better description of the conformation of the nucleotides. Further, as Table 5 shows, the coupling patterns in the T6A transvertant are very similar to those in the wild-type sequence, indicating that there are no substantial changes in sugar puck-

Table 4. Spin-spin coupling constants in the wild-type trp promoter. Coupling constants were determined either from resolution enhanced one-dimensional spectra or from rows in HOHAHA and NOESY spectra. $\Sigma_{1'} = {}^3J_{1'2'} + {}^3J_{1'2''}$. f_s is the fraction south calculated assuming a mixture of C2′ endo and C3′ endo states (Rinkel and Altona 1987)

Residue	$\Sigma_{1'}(HH)$ Hz	$\Sigma_{1'}(Ny)$ Hz	^3J	f_S deg	P
C2	14.0*			0.71	207
	13.5	14.5		0.71	207
T3	15.6			0.98	167
T6	15.1[a]		8.5, 6.6	0.89	184
	15.0	14.5		0.84	189
G7	15.3[a]		9.8, 5.5	0.92	171
	15.0	15.5		0.93	180
A8	14.0			0.71	207
C9	15.0	15.6		0.93	171
A10	nd	14.5		0.79	200
A11	15.0	14.8		0.95	189
T13	14.7	14.6		0.82	196
A14	13.4[a]		6.7, 6.7	0.60	216
	13.5	13.8		0.65	210
A16	14.7	14.9		0.81	194
A17	14.7[a]		8.6, 6.1	0.81	194
	14.6	nd		0.81	198
T19	14.7	nd		0.81	196
T21	nd	14.5		0.79	200
A23	14.0	14 7		0.77	202
A24	14.7			0.83	194
C25	15.0			0.88	189
G27	14.7[a]		8.6, 6.1	0.81	194
	15.0	14.5		0.84	189
C28	13.7			0.66	210

[a] Denotes value determined from one-dimensional spectra. P is the pseudorotation phase angle calculated assuming $f_S = 1$. The estimated error on the sums of coupling constants is ± 0.5 Hz, which leads to errors in $f_S \pm 0.08$ or in P of ca. $\pm 20°$

Table 5. Spin-spin coupling constants in the T6A transvertant. Coupling constants were determined either from resolution enhanced one-dimensional spectra or from rows in NOESY spectra. $\Sigma_{1'} = {}^3J_{1'2'} + {}^3J_{1'2''}$. f_S is the fraction south calculated assuming a mixture of C2′ endo and C3′ endo states (Rinkel and Altona 1987). P is the pseudorotation phase angle calculated assuming $f_S = 1$. Estimated errors are ± 0.08 for f_S and $\pm 20°$ for P (cf. Table 4)

Residue	$\Sigma_{1'}(Ny)$ Hz	$\Sigma_{1'}(1D)$ Hz	^3J	f_S	P deg
T3	14.9			0.86	192
T5	14.9			0.86	192
G7	15.3			0.93	172
T13	14.5			0.80	198
A14	13.7	13.8	6.8, 6.8	0.65	216
T15	14.5	14.9		0.80	198
A16	14.5		8.8, 5.7	0.80	192
A17	14.0	14.9		0.80	198
T18	13.5			0.63	216
T23	14.7			0.83	198
C25	14.5			0.80	198
G27	14 8		8.8, 6.0	0.84	192
C28	14.5			0.80	198

Table 6. Glycosidic torsion angles in the trp promoter at 303 K. Torsion angle were found from NOESY data at different mixing times using the sugar conformations given in Tables 3 and 4, and analysed with the program NUCFIT as described in the text. Typical errors are $\pm 4°$

Base	χ		$\Delta\chi$
	wt	T6A	
C2	−113	−107	6
T3	−112	−109	3
G4	−105	−104	1
G7	−107	nd	–
A8	nd	−104	–
C9	nd	−112	–
A10	− 99	nd	–
T12	−119	−120	−1
T13	−114	−112	2
A16	nd	−110	–
A17	nd	−106	–
T18	−112	nd	–
T19	−113	nd	–
G20	−109	−105	4
T21	nd	−114	–
C22	−110	−112	−2
T23	nd	−109	–
C25	nd	−111	–
G27	−110	nd	–

ers propagated through the DNA helix to nucleotides distant from the mutation site.

To analyse the nucleotide conformation further, we have recorded NOESY spectra at several mixing times (50, 100, 150, 200 and 250 ms). NOE intensities for the H8/H6-H1′, H2′, H2″ indicate that the nucleotides are all *anti*, with glycosidic torsion angles in the range −100 to −120°. Further, the relatively low intensity of the H8/H6-H3′ cross peaks also indicates that the sugar conformations are mainly near C2′-endo, in agreement with the conclusions based on the scalar couplings. The nucleotide conformations were further analysed using the program NUCFIT which treats directly the effects of spin-diffusion, conformational averaging in mixtures of sugar puckers, and the relatively small influence of rotational anisotropy (Lane 1990). The required rotational correlation times were obtained from the cross-relaxation rate constant for the cytosine H6-H5 vectors as previously described (Lane et al. 1986; Birchall and Lane 1990). As the relative intensities of the intraresidue H2′-H8/H6 NOEs vary by less than a factor of two along the sequence, it is clear that the nucleotide units all have a similar conformation. The combined conformational search with least-squares fitting of the time-dependent NOE intensities using NUCFIT confirmed that the glycosidic torsion angles lie in the *anti* range, i.e. −100 to −120° (Table 6). Conformations were determined only for those nucleotides where the H8/H6-H2′ NOE could be unambiguously measured. Where measurable, the glycosidic torsion angles are typical of B-like DNA, in which the mean torsion angle is −110°.

Table 6 also shows the derived glycosidic torsion angles for the T6A transvertant; the mean torsion angle is also −110°. In general glycosidic torsion angles are not significantly different between the two molecules.

The observation of weak NOEs between neighbouring iminoproton resonances indicates that the helical rise is on average neither very large or unusually small. Together with the relative internucleotide NOE intensities, and the conformations of the individual nulceotides, it is clear that the tetradecamer is a typical member of the B family of conformations. The observation of substantial NOEs from the adenine C2H to neighbouring H1′ (3′) and cross-strand NOEs to H1′ as well as NOEs between C2 protons of adjacent adenine residues, yielding apparent distances significantly shorter than expected for idealised B-DNA, is consistent with a substantial propellor twist for the AT base-pairs. Unfortunately, spectral overlap prevents us from quantifying a sufficient number of NOEs to determine the helical parameters in any greater detail.

Temperature-dependence of the NMR spectra

We have previously reported anomalous temperature-dependent behaviour of chemical shifts and line-widths of base protons in 5′-TAA trinucleotides (Lefèvre et al. 1988; Lane 1989, 1991). The trp −35 14-mer contains two similar sequences, viz. TAAT and 5′-CAAT. In addition, there is the CAAC subsequence that is potentially similar, as its general structure is YAAY. We have therefore measured the chemical shifts and spin-spin relaxation rate constants for base H8 and Ade C2 protons as a function of temperature. The majority of the base protons show a simple linear dependence of the chemical shift on temperature, with a slope for H8 resonances between −1.2 and −1.8 ppb/K and rather more variable slopes for the Ade H2 resonances (Table 7). Only A10 H8 shows any significant deviation from linearity, which is not as marked as seen previously in the related partial sequences TAAT and 5′-TAAC (Lefèvre et al. 1988; Lane 1989). A more sensitive test of the uniqueness of a conformation is the variation of the line-width (or R_2 value)

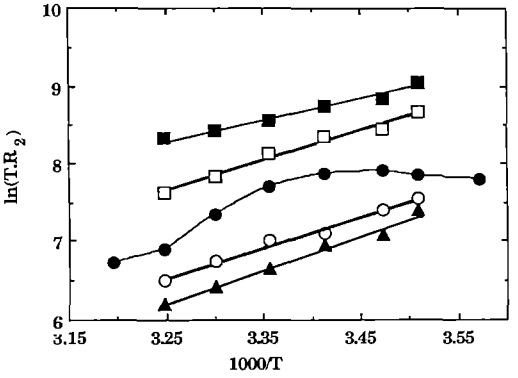


Fig. 7. Dependence of spin-spin relaxation rate constants on temperature. Spin-spin relaxation rate constants were measured as a function of temperature as described in the experimental section. The rate constants are presented as an Eyring plot. ■, G4 H8; □, A16 H8; ●, A10 H2; ○, A11 H2; ▲, A17 H2

Table 7. Properties of base protons. Chemical shifts were obtained from resolution enhanced spectra recorded at temperatures from 278 K to 313 K. Ade C2H shifts were determined from spin-echo spectra, making use of their much longer T_2 values compared with those of H6 and H8 protons. E_{app} is the apparent activation energy for the temperature variation of T_2

Proton	$d\delta/dT$ ppb K^{-1}		E_{app} kJ mol^{-1}
	wt	T6A	wt
G4 H8	−1.8	−2.1	22
A10 H8	nd	−0.6	nd
A10 H2	5.9	5.8	46[a]
A11 H2	2.2	2.1	22
A14 H8	0.4	0.2	nd
A16 H8	−1.9	−2.5	33
A16 H2	6.0	nd	22
A17 H8	−1.5	−1.9	nd
A17 H2	5.0	4.4	25
G20 H8	−0.8	−1.4	24
A24 H8	−1.0	−1.4	25
A24 H2	1.2	–	–
A26 H2	1.2	0.95	nd

[a] between 298 K and 313 K

with temperature. For a unique, temperature-independent conformation, R_2 should follow the viscosity law. We have already shown that the rotational correlation time of the molecule as a whole (determined from the cross-relaxation rate constants for the cytosine H6-H5 vectors) follows the viscosity law (Birchall and Lane 1990). The apparent activation energy for the rotation of the DNA molecules was determined to be 21 ± 3 kJ mol^{-1}. As shown in Fig. 7, the R_2 values for resolved base protons show a linear dependence on reciprocal temperature, with the marked exception of A10 H2, which shows a maximum at $1/T \approx 0.00345\ K^{-1}$ (i.e. at $T \approx 290$ K). This latter behaviour is clear evidence of conformational averaging that occurs on a time scale on the order of the chemical shift difference between the states being averaged. We note that this residue is in the segment 5′-CAAT. Further, with the exception of A10 C2H and A16 H8, the temperature-dependence of the linewidths gives an apparent activation energy similar to that of overall tumbling (i.e. 22 kJ mol^{-1}). A16 is found in the segment TAAT. A more detailed characterisation of the conformational transition in this molecule has recently been presented (Lane et al. 1992).

Discusssion

We have shown that the −35 region of the trp promoter, which contains the consensus −35 sequence of bacterial promoters TTGACA, is in the B family of conformations in solution. In idealised B-DNA (which has an unfavourable potential energy), the glycosidic torsion angles are about −100° which leads to intranucleotide NOE intensities for H6-H2′ four to five times as intense as the Cyt H6-H5 cross-peaks. The observed glycosidic torsion angles, however, are mainly in the range −110° to −120°, which is closer to the values obtained from energy minimised B-DNA (Table 6). In energy-minimised DNA, the H2″(i) to H8/6($i+1$) NOEs are predicted to be of very similar intensity to the H8/6(i)−H2′(i) NOEs. However, the experimental H2″(i) to H8/6($i+1$) NOEs are substantially less intense than the H8/6(i)−H2′(i) NOEs (cf Fig. 1 B), which suggests that the helical conformation is somewhat different from energy-minimised B-DNA. In this respect the promoter fragment is not markedly different from other DNA duplexes of mixed sequence that have been studied by NMR (Lefèvre et al. 1987; Nilges et al. 1987; Zhou et al. 1988; Baleja et al. 1990). The similarity of the NMR spectra of the wild-type and transvertant, apart from the base-pair itself and the nearest neighbours, suggests that the conformations of the two oligonucleotides are very similar. The similarity of the coupling patterns and intranucleotide NOEs in the two molecules is further evidence that locally at least, the conformation of the molecule is not strongly affected by the transversion. Further, the conformational properties of the molecules are similar, including the rotational behaviour and the temperature-dependence of the conformation.

This AT transversion, which corresponds to a promoter down mutation (Hawley and McClure 1983; Szoke 1987) is a relatively large local structural perturbation (exchange of a purine for a pyrimidine) that has rather small effects on the local conformation and properties of the oligonucleotide. This result is similar to a milder operator-constitutive mutation in the trp operator/Pribnow box region, in which adjacent purines are exchanged (Lane 1991) with little effect on conformation and solution properties. In the considerably greater local structural perturbation of an $A \cdot G$ mismatch, the conformation of residues other than the nearest neighbours were also not significantly affected (Lane et al. 1991).

These findings are consistent with the view that unconstrained DNA is highly flexible, such that even relatively large local structural perturbations have only small local effects that are not propagated along the helix. This is readily understandable in terms of a linear polymer whose units are joined together by easily deformable links that have many degrees of freedom to accommodate structural perturbations. Thus, although the muta-

tion has a measurable functional consequence, the similarity of the local conformation to the wild-type sequence suggest that recognition is not primarily dependent on the conformation, but on the precise sequence which determines the order of functional groups accessible in the major grooves, rather than the small sequence-dependent conformational features. However, sequence-dependent flexibility of DNA could have an important role in promoter function.

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