



Conformational Study of Decamer DNA Duplex d(ACGTATACGT)₂ by NMR Spectroscopy

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Abstract: The conformation of the self-complementary decamer duplex, d(ACGTATACGT)₂ (TATA-duplex) has been studied by proton NMR spectroscopy. The duplex is essentially B-type, with distortions apparent at the TATA steps. These conformational distortion which may be preferable to occur in the thymine residue on the 5'-side, has been investigated by unusual NOE crosspeaks.

Understanding the biological function of biomolecules is one of the main reasons for studying their biophysical properties. The complete understanding of the biological function requires the detailed knowledge of the three-dimensional structure of DNA and its potential in adopting various conformations, some of which are yet to be uncovered. Of the currently available techniques for structural elucidation, NMR and X-ray crystallography allow one to observe the often subtle variation in DNA topology. Many reviews of the NMR method provide specific details and historical perspective.¹⁻⁴ The NMR method relies upon the use of the two-dimensional nuclear Overhauser effect (NOESY) to produce a set of interproton distance constraints that are used to refine model geometries.^{1,5}

DNA templates contain regions called promoter sites that specifically bind RNA polymerase and determine where transcription begins.⁶ In most eukaryotic system of protein transcription, the TATA box is positioned upstream of the transcription initiation site.⁷ Half of its sequence, 5'-TATA-3', is conserved well, while the other half is less so but generally has many adenine bases. After RNA polymerase binds to the promoter site, it

unwinds DNA beginning in the -10 region. It may be significant that the -10 region is rich in A•T base pairs, because these come apart or melt much more easily than G•C pairs.

Therefore, the conformational study of DNA duplex containing internal TATA sequences are essential for understanding of the transcriptional mechanism. In this paper, the conformational study of the DNA duplex, d(ACGTATACGT)₂ (TATA-duplex) are carried out by NMR spectroscopy.

MATERIALS AND METHODS

Sample preparation

The DNA oligonucleotides of d(ACGTATACGT)₂ were synthesized on an Applied Biosystems 391A synthesizer using β -cyanoethyl phosphoramidite chemistry on the 2 μ mol scales. They were deprotected and cleaved from the control pore glass, solid support by keeping in 4 ml of fresh concentrated ammonia overnight at 55 °C. After filtration and evaporation, the oligomers were separated by reverse phase HPLC C18-column in 100 mM TEAA buffer, pH 7, with acetonitrile gradient from 5 % to 35 %. The highest peak was collected and the solvent was evaporated. The collected oligonucleotides were kept in 1 ml of 80% acetic acid for 20 minutes at room temperature to deprotect 5'-DMT group. The sample containing the oligonucleotides was evaporated and coevaporated with 1 ml of ethanol/H₂O (1:1 in volume). After evaporation, the sample was dissolved in 3 ml and then the impurities were extracted with ether. The sample solution was freeze-dried, and then purified by Sephadex G-25 fine gel filtration column chromatography.

NMR experiments

The NMR samples of them were prepared by dissolving them to the 100 mM NaCl, pH 6.8 D₂O (99.96 % in glass ampoule purchased from Aldrich Co.) solution. After making the solutions, pH was readjusted to 6.8. A small amount of partially deuterated sodium 3-(trimethylsilyl)-1-propane-sulfonate (TSP) was added to the samples as internal proton chemical shift references.

All NMR experiments were done on a Bruker DMX 600 spectrometer at 298 K. NOESY spectra of 250 ms mixing time were taken for the purpose of spectral assignments and DQF-COSY and TOCSY were used to confirm the assignments. The NOESY spectra of 80 ms mixing time were recorded under identical spectrometer conditions for quantitative analysis of NOE constraints. The relaxation delay between acquisitions was 2 sec for NOE build-ups.

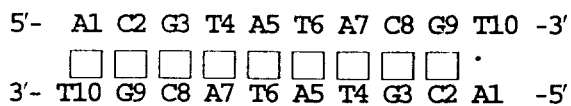


Fig. 1. DNA sequence contexts of the TATA-duplex.

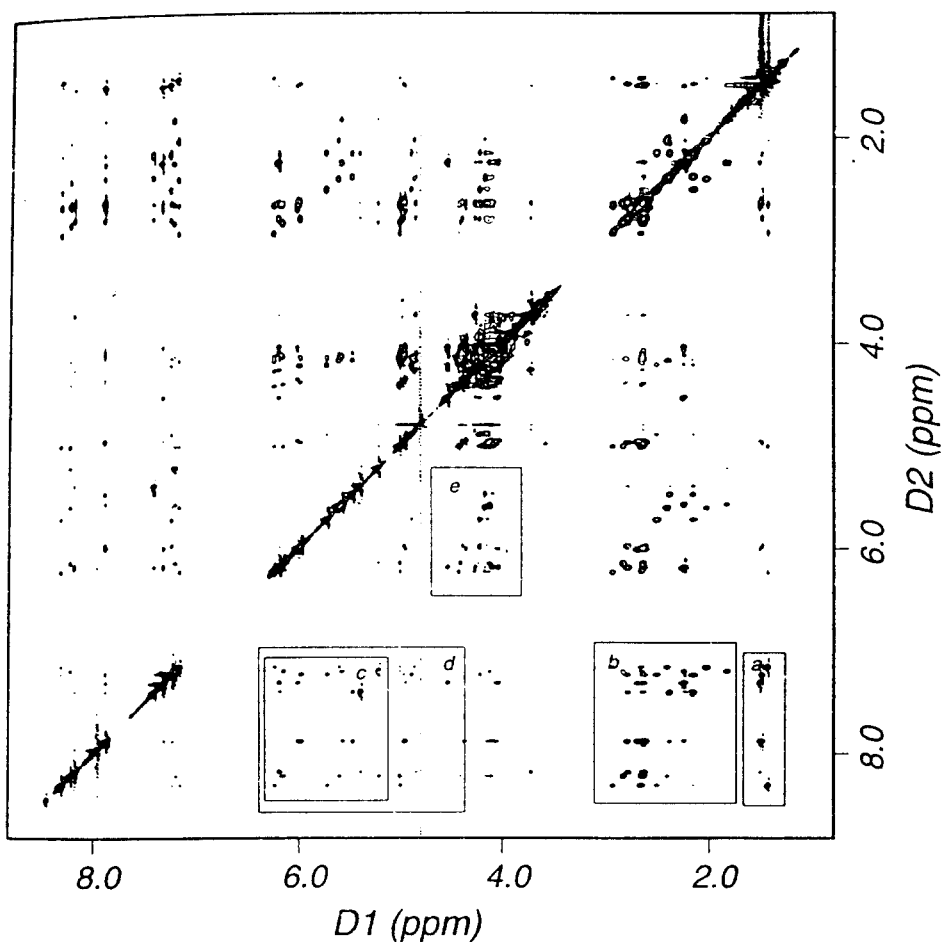


Fig. 2. NOESY (25 ms mixing time) contour plot of the TATA-duplex in D_2O solution containing 20 mM sodium phosphate buffer, 200 mM sodium chloride, pH 7.0, at 25 °C.

RESULTS AND DISCUSSION

The 250-ms NOESY spectrum of the TATA-duplexes in D₂O buffer solution, at 25 °C, is shown in Figure 2. The base H6/H8 proton resonances of all residues show strong NOE crosspeaks to the sugar H2'/H2'' resonances (boxed, *b* in Fig. 2) and weak NOEs to the H1' sugar resonances (boxed, *c* in Fig. 2). This indicates that all of the bases are in *anti* conformation, as expected for B-DNA.¹¹ Connectivities between the C-H5 and C-H6 protons and T-5Me and T-H6 protons are manifested by strong intrabase NOEs (Fig. 3(A) and 3(C)). Examination of B-DNA model indicates that the 5-positioned protons of pyrimidine residues are close to the base protons on its 5'-side but not on its 3'-side.¹² So, three NOE crosspeaks between the T-Me and base proton resonances on its 5'-side (T4-G3, T6-A5, and T10-G9) and two NOEs between C-H5 and H8 proton resonances of adenine residues on its 5'-side (C2-A1 and C8-A7) are observed (Fig. 3(A) and 3(C)).

Complete assignment of the base as well as all of the sugar H1' resonances is accomplished by inspection of small residual and sequential NOE crosspeaks appearing between the base and sugar H1' proton resonances as seen in Fig. 3. Also, the sugar H2'/H2'' and H3' proton resonances are assigned by the residual NOEs to the base proton resonances (Fig. 3(B) and 4(A)) and the H4' sugar resonances are assigned by the residual H1'↔H4' NOE crosspeaks (Fig. 4(B)). The assignments of nonexchangeable proton resonances of TATA-duplex are presented in Table 1.

Table 1. The chemical shifts of the proton resonances of the TATA-duplex.

Residue	H5/Me	H6/H8	H1'	H2'	H2''	H3'	H4'
A1		8.18	6.19	2.63	2.78	4.85	4.26
C2	5.40	7.40	5.47	2.15	2.38	4.84	4.17
G3		7.88	5.97	2.62	2.79	5.03	4.38
T4	1.47	7.23	5.72	2.15	2.50	4.89	4.22
A5		8.31	6.23	2.65	2.92	5.00	4.42
T6	1.42	7.15	5.60	2.02	2.40	4.86	4.17
A7		8.22	6.15	2.63	2.82	5.00	4.40
C8	5.22	7.19	5.57	1.82	2.24	5.00	4.12
G9		7.88	6.01	2.62	2.68	5.03	4.35
T10	1.50	7.31	6.18	2.24	2.24	4.53	4.03

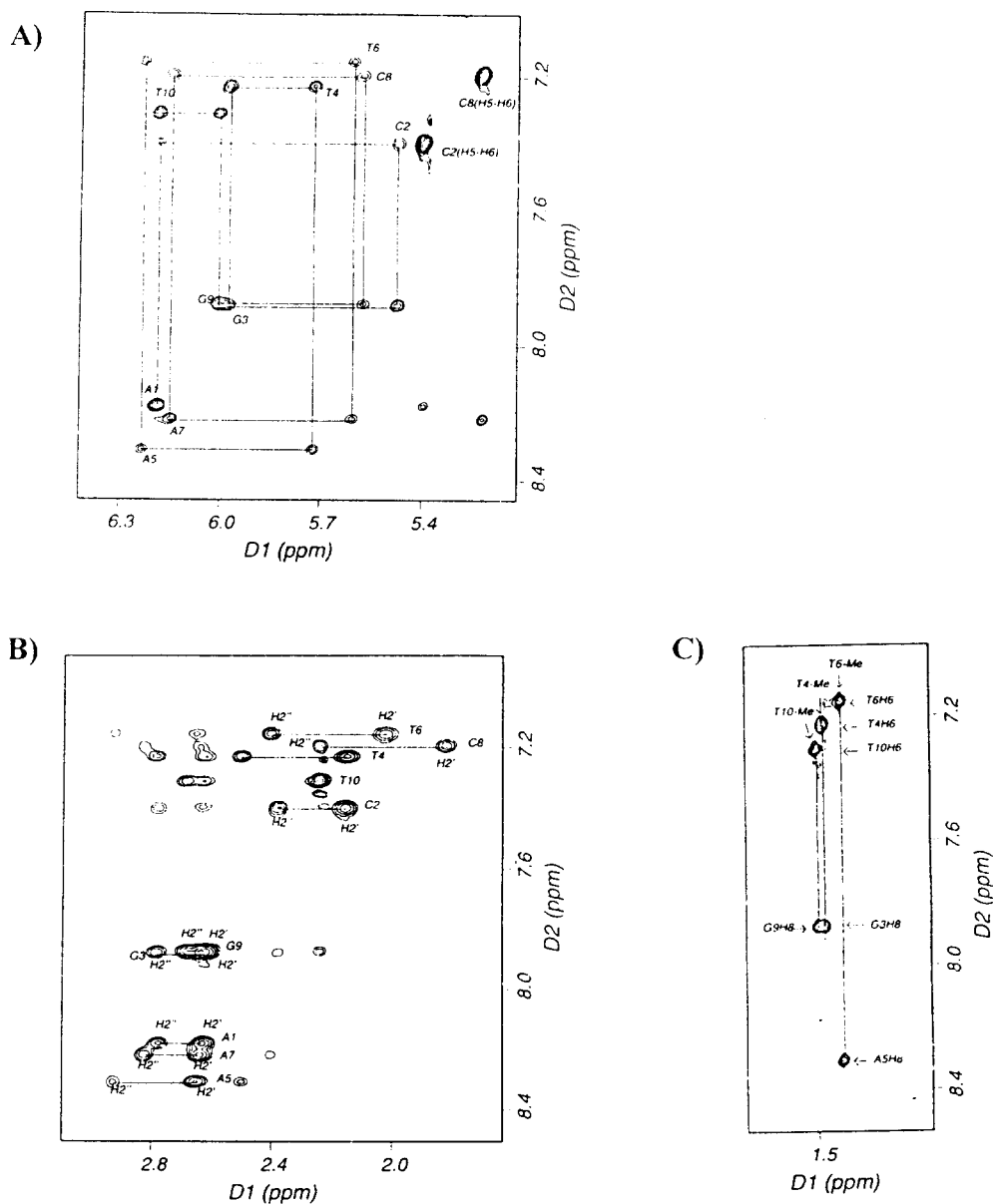
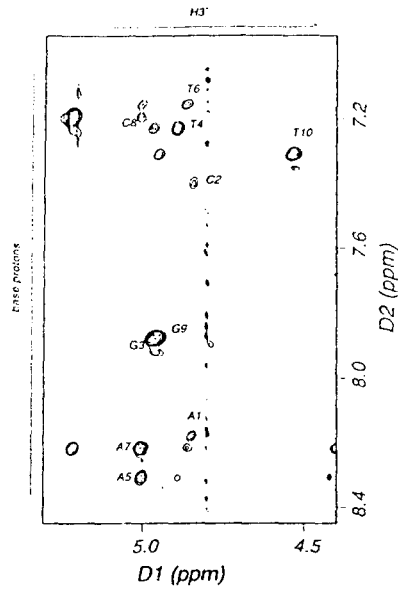


Fig. 3. Expanded region of NOESY spectrum of the TATA-duplex, (A) showing the NOE crosspeaks between the base protons and the sugar H1' proton resonances (c in Fig. 2), (B) showing the NOE crosspeaks between the base protons and the sugar H2'/H2'' proton resonances (b in Fig. 2) and (C) showing the NOE crosspeaks between the base protons and methyl proton resonances of the thymine residues (a in Fig. 2).

A)



B)

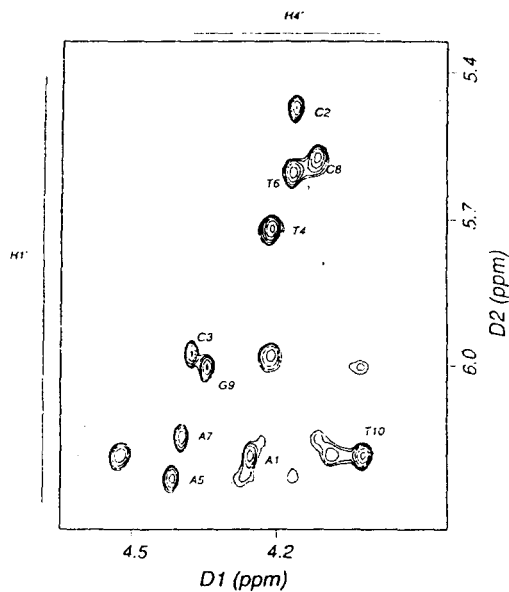


Fig. 4. Expanded region of NOESY spectrum of the TATA-duplex, (A) showing the NOE crosspeaks between the base protons and the sugar H3' proton resonances (d in Fig. 2) and (B) showing the NOE crosspeaks between the sugar H1' and H4' proton resonances (e in Fig. 2).

The distance between the sugar H1' and H4' protons and the sugar H2'' and H4' protons do vary sufficiently to convey information concerning the sugar conformation.¹² The residual H1'↔H4' NOE crosspeaks could be used for determining the sugar conformation. This NOE crosspeaks of the T4 residue is stronger than that of the T6 residue (Fig. 4(B)). This suggests that the T4 residue has *C1'-exo* sugar puckering, when the sugar puckering of the T6 residues remains in *C2'-endo* range. When the glycosyl bond shows the *anti* conformation, the distance between the base proton and sugar H3' proton also give the information about the sugar puckering.¹² The H6↔H3' NOE crosspeak of the T4 residue appears stronger than those of other pyrimidine residues except the terminal residues (Fig. 4(A)). This suggests that the sugar puckering of all residues shows S-type, but the T4 residue has *C3'-exo*-type sugar puckering, whereas those of other pyrimidines remain in *C2'-endo* range. The unusual sugar conformation of T4 residue makes the glycosyl bond torsional angle differ from those of the normal B-DNA. This is consistent with the fact that the residual and sequential H6↔H1' NOE crosspeaks of T4 residue is slightly stronger than those of the T6 residue (Fig. 3(A)). The unusual sequential G3-H1'↔T4-H6 NOE crosspeak of the T4 residue means disrupting of the base-stacking in the G3/T4 step. The unusual conformation of the T4 residue also perturbs the T4•A7 base-pair. This is consistent with the stronger residual H8↔H2'' and H8↔H3' NOEs of the A7 residue than those of the A5 residue. This conformational perturbation is caused by the interstrand base-base interaction between the A5 and A7 residue on the complementary strand.

The interstrand hydrophobic interaction between the adenine residues which have the bulky bases causes distortion in the outer base-pair not in the inner base-pair, and this distortion appears in the thymine, not adenine, residue of the outer A•T base-pair. So far, the 5'-side thymine, T4 residue, is the most perturbed nucleotide in TATA step.

Acknowledgements

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