Sequence-dependent Conformation of DNA Duplexes

THE AATT SEGMENT OF THE d(G-G-A-A-T-T-C-C) DUPLEX IN AQUEOUS SOLUTION

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The nonexchangeable base and sugar protons of the octanucleotide d(G-G-A-A-T-T-C-C) have been assigned by two-dimensional correlated (COSY) and nuclear Overhauser effect (NOESY) methods in aqueous solution. The assignments are based on distance connectivities within the protons on each sugar ring. We observe the NOEs to exhibit directionality and are consistent with the d(G-G-A-A-T-T-C-C) duplex adopting a right-handed helix in solution. The relative magnitude of the NOEs between base and sugar H2' protons of the same and 5'-adjacent sugars characterizes the AATT segment to the B-helix type in solution.

There has been a great deal of recent emphasis on the conformation of DNA in the solid and solution states (1). There are three major families of DNA conformations designated right-handed A, right-handed B, and left-handed Z that have been observed in single crystals, in fibers, and in aqueous solution (1). There is much conformational flexibility in right-handed DNA structures with variations in the roll, twist, and pucker geometries, and between A and B families of nucleic acid helices (2-5). Recent advances in two-dimensional NMR (6-9) have permitted the development of accurate assignment procedures for base and sugar protons in right-handed duplexes (10-17). The inverse sixth power distance dependence of the magnitude of the nuclear Overhauser effect (NOE') between proton spins permits a qualitative differentiation between interproton distances in the range 2.5 to 4.5 Å (18-20). This procedure has been applied to evaluate glycosidic torsion angles, sugar puckering geometries, and between A and B families of nucleic acid helices (21-23). The methods require further quantitation to estimate accurate distances from the initial buildup of the NOEs and the development of efficient distance-geometry algorithms to generate conformation(s) consistent with the large set of interproton distance constraints of <4.5 Å.

Recent measurements of hydrogen exchange kinetics in DNA helices (24-26) have demonstrated that the thymidine imino protons in d(TATA) (27, 28) and d(TGTG) segments (29) exchange more rapidly than their nonalternating pyrimidine-purine counterparts. Our research will focus on the two DNA duplexes d(G-G-A-A-T-T-C-C) (designated AATT 8-mer) and d(G-G-T-A-T-A-C-C) (designated TATA 8-mer) and is directed towards an understanding of the conformation in the central AATT and TATA segments. These sequences form stable duplexes at room temperature and a crystal structure has been reported for the TATA 8-mer (30).

This paper reports on the two-dimensional NMR parameters for the AATT 8-mer and a later paper on the corresponding parameters for the TATA 8-mer in aqueous solution. Several qualitative conclusions are deduced at short mixing times from the two-dimensional NOESY experiments. This experimental data set will provide the basis for a future detailed analysis by distance geometry methods once development of the algorithms are complete.

One-dimensional proton NMR studies of the helix-coil transition of the d(G-G-A-A-T-T-C-C) duplex have been reported previously (31). This paper extends on these investigations by completely assigning all the base and sugar protons (except the super-positioned 5',5"-sugar protons) by two-dimensional correlated (COSY) and Overhauser (NOESY) methods and analyses for relevant interproton distances that differentiate between the A and B family of conformations. The resolved phosphorus spectrum of the AATT 8-mer has been recently assigned from selective '31P-labeling experiments (32). Brodo et al. (33) have recently reported on a two-dimensional NMR assignment study of the AATT 8-mer and their results will be compared with our conclusions under "Discussion."

EXPERIMENTAL PROCEDURES

The d(G-G-A-A-T-T-C-C) was custom synthesized by Pharmacia P-L Biochemicals and its sequence checked by Maxam-Gilbert sequencing gels. The sample was >97% pure as judged from its proton NMR spectrum in H2O and D2O solution. The AATT 8-mer was dissolved to a concentration of 20 mg in 0.4 ml in 0.1 M NaCl, 10 mM phosphate, 1 mM EDTA buffer for recording of NMR spectra.

One-dimensional proton NMR spectra of the AATT 8-mer in H2O were recorded on the JEOL GX-500 using a time-shared Redfield long pulse to null the solvent water signal (34,35). Two-dimensional correlated (COSY) (6, 7) and nuclear Overhauser effect (NOESY) (8, 9) data sets were recorded with quadrature detection for the AATT 8-mer in D2O solution on the Yale WM-500 spectrometer with saturation of the residual HOD resonance. We collected 512 t1 increments over a sweep range of 4400 Hz using 1024 complex points in the t2 dimension. The data sets were transferred to magnetic tape and processed on the Columbia VAX 11-780 using the Hare two-dimensional processing software. The magnitude COSY data were collected with a repetition delay of 1.5 s. They were apodized in t1 and t2 with an unshifted sine bell function which was zeroed at the 60th point and then Fourier transformed in both dimensions. The phase-sensitive NOESY data (36) were collected for 150- and 250-ms mixing times.
times with a repetition delay of 1.5 s. The data sets were Fourier transformed in both dimensions without application of window functions. Symmetrized contour plots were recorded on a 22-inch Zeta 822 plotter for analysis and assignment of resonances, and on a HP 7475A plotter for publication figures both of which were interfaced to a VAX 11-780.

RESULTS

The bases in the self-complementary d(G1-G2-A3-A4-T5-T6-C7-C8) duplex are numbered 1 to 8 (Scheme 1). The helix-coil transition has a midpoint of ~42.5 °C in 0.1 M NaCl solution (31).

One-dimensional Spectra in H2O Solution

The one-dimensional spectrum of the self-complementary AATT 8-mer duplex in 0.1 M NaCl, 10 mM phosphate, H2O, pH 7, at 20 °C exhibit four imino protons which resonate between 12.5 and 13.8 ppm while the cytidine amino and nonexchangeable base protons resonate between 6.6 and 8.4 ppm.

The imino proton assignments have been made by recording one-dimensional NOEs between imino protons on adjacent base pairs and are tabulated in the accompanying paper (40). The narrow adenosine H2 and broad cytidine hydrogen-bonded (8.1 to 8.4 ppm) and exposed (6.8 ppm) amino protons can be similarly assigned from intra-base pair NOEs to the known imino proton resonances. These are standard procedures (19, 37) and we move on next to the nonexchangeable proton assignments.

Two-dimensional Spectra in D2O

The 500 MHz proton NMR spectrum of the AATT 8-mer in 0.1 M NaCl, 10 mM phosphate, D2O at 25 °C exhibits sufficiently resolved resonances which can be classified by type into the spectral ranges 7.0 to 8.2 ppm (pyrimidine base H6 and purine base H8 and H2), 5.4 to 6.2 ppm (sugar H1’ and pyrimidine base H5), 4.5 to 5.1 ppm (sugar H3’), 4.0 to 4.5 ppm (sugar H4’ and H5’-5”), 2.0 to 3.0 ppm (sugar H2’-2”), and 1.2 to 1.6 ppm (thymidine CH3).

We have recorded two-dimensional spectra of the AATT 8-mer in 0.1 M NaCl, 10 mM phosphate at 25 °C with the contour plot of a magnitude COSY shown in Fig. 18 (see Miniprint Section) and a phase-sensitive NOESY with a 250-ms mixing time shown in Fig. 28 (see Miniprint Section). The details of data processing conditions are outlined under "Experimental Procedures."

The resolved cross-peaks in the COSY spectrum (Fig. 18) reflect two bond (H2’-H2”), three bond (H1’-H2’, H1’-H2”, H2’-H3’, H3’-H4’ and base H5-H6), and four bond (thymidine H6-CH3) coupling constants. The resolved cross-peaks in the 250-ms mixing time NOESY spectrum (Fig. 28) correspond to NOE effects between proton pairs separated by <4.5 Å. Some of the cross-peaks may not reflect direct interactions at this mixing time so that an NOE between a base proton and a sugar H1’ may, in part, be mediated through the sugar H2’ on the same residue.

We analyze below individual regions at the AATT 8-mer COSY (Fig. 18) and NOESY (Fig. 28) contour plots which yield the detailed spectral assignments.

NOESY Contour Plots

Base to H1’—An expanded region of the 250-ms mixing time NOESY contour plot relating the base protons (7.1 to 8.2 ppm) with the sugar H1’ and cytidine H5 protons (5.2 to 6.3 ppm) is presented in Fig. 1. For a right-handed duplex it can be shown that the purine H8 and pyrimidine H6 will be <4.5 Å from its own sugar H1’ and the sugar H1’ in the 5’-direction but >4.5 Å from the sugar H1’ in the 3’ direction. Thus, each of these base protons will exhibit an NOE to its own and 5’-flanking sugar H1’ except for G1, the terminal nucleotide which lacks a 5’-flanking sugar. Thus it is possible to walk between adjacent bases on the same strand of the helix using the sugars as a stepping stone. This procedure (10-17) permits the assignment of the base H8 and H6 protons and sugar H1’ protons and these are listed in Table I.

Fig. 1. An expanded contour plot of the phase-sensitive NOESY spectrum (mixing time 250 ms) of the AATT 8-mer at 25 °C correlating the base protons (7.1 to 8.2 ppm) with the sugar H1’ protons (5.2 to 6.3 ppm). The sugar H1’ assignments are depicted next to the contour peaks while the additional cross-peaks A to F are discussed and assigned in the text. The lines follow the connectivities between adjacent base protons through their intervening sugar H1’ protons.

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Figs. 18–4S are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 85M-2051, cite the authors, and include a check or money order for $2.40 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
Several additional cross-peaks are observed in the expanded contour plot in Fig. 1. These include the strong cross-peaks between the pyrimidine H6 and H5 protons on the same cytidine and a cross-peak (peak D, Fig. 1) which represents an NOE between the adjacent base H6 proton of T6 and the H5 proton of C7 in the T6-C7 step in the d(G-G-A-T-T-C-C) sequence.

We also detect cross-peaks between the H2 proton of A4 and the H1' of its own A4 sugar (peak C, Fig. 1), its 3'-flanking T6 sugar on the same strand (peak A, Fig. 1) and the T6 sugar on the partner strand in the opposite direction (peak B, Fig. 1). Similarly, the H2 proton of A3 exhibits NOEs to the H1' protons of residues A3 and C7 (peak E, Fig. 1) and A4 (peak F, Fig. 1). The adenosine H2 protons bridge sugar H1' protons on both strands thus extending assignments from one strand to the other as noted previously.

**Base to Base**—We also detect NOEs between adjacent base protons on the same strand in the contour sections covering the symmetrical region 7.0–8.4 ppm (Fig. 2A). Thus, we observe NOEs between adjacent purine H8 protons in the G2-A3 step (peak A, Fig. 2A), between adjacent adenosine H2 protons in the A3-A4 step (peak D, Fig. 2A), between adjacent pyrimidine H6 protons in the T5-T6 step (peak F, Fig. 2A) and the T6-T7 step (peak E, Fig. 2A), and between the purine H8 of A4 and adjacent pyrimidine H6 of T5 in the A4-T5 step (peak C, Fig. 2A). Thus, we have been able to assign the base protons from G2 to C7 in the same strand without recourse to the other strand.

The NOEs in the CH3 region of the AATT 8-mer (Fig. 2B) exhibit directionality reflecting the handedness of the helix.

Thus, the CH3 of T6 exhibits a strong NOE with the H6 of T5 (peak I, Fig. 2B) in the T5-T6 step but a weak NOE with the H6 of C7 (peak H, Fig. 2B) in the T6-C7 step. Similarly, the CH3 of T5 exhibits a strong NOE to the H8 of A4 (peak G, Fig. 2B) in the A4-T5 step but a weak NOE to the H6 of T6 (peak J, Fig. 2B) in the T5-T6 step.

It is possible to follow the T6-C7 step through the NOE between the CH3 of T6 and the H5 of C7 in the NOE spectrum of the AATT 8-mer.

**COSY Contour Plots**

**H1' to H2' and H2**—A cross-section of the magnitude COSY spectrum of the AATT 8-mer between the sugar H1' protons (5.2 to 6.3 ppm) and the sugar H2'-2" protons (1.9 to 3.0 ppm) is presented in Fig. 3. The sugar H1' protons have been assigned by analysis of the NOESY data in Fig. 1 so that it is straightforward to assign the sugar H2'-2" protons to individual residues in Fig. 3. The contour patterns are different for the H1'-H2' and H1'-H2" connectivities with the H2' resonance resonating at higher field in each case (Fig. 3). The H2'-2" resonances for each sugar are resolved from each other except for terminal sugar C8 where the H2'-2" resonances are superimposed on each other. The sugar H2'-2" chemical shifts in the AATT 8-mer at 25 °C are summarized in Table I.

**H2' to H3**—The cross-peaks corresponding to COSY cross-peaks between the sugar H2' protons (1.9 to 2.5 ppm) and sugar H3' protons (4.5 to 5.3 ppm) is shown in Fig. 4A. The cross-peaks between the H2' proton and the H3' proton show a distinct shape and the H3' protons can be assigned from the known H2' assignments. These values are listed in Table I. We do not detect cross-peaks between the H2' and H3' protons indicative of a small coupling constant between these protons for residues 1 to 7 in the AATT 8-mer duplex.

**H3' to H4**—The contour section corresponding to cross-peaks between the H3' protons (4.5 to 5.3 ppm) and the H4' protons (3.9 to 4.5 ppm) in the AATT 8-mer duplex is presented in Fig. 4B. The H4' proton assignments readily follow from the known H3' assignments and are listed in Table I.
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Fig. 4. An expanded contour plot of the magnitude COSY spectrum of the AATT 8-mer at 25 °C establishing coupling connectivities between (A) the sugar H2' protons (1.9 to 2.8 ppm) and sugar H3' protons (4.5 to 5.3 ppm) and (B) between the sugar H3' protons (4.5 to 5.3 ppm) and the sugar H4' protons (3.9 to 4.5 ppm).

We cannot definitively assign the H4' proton of G1 at this time.

H2'-2' Region—The sugar H2' and H2" protons have been assigned to each residue in the AATT 8-mer above. These assignments can be checked by monitoring the cross-peaks dispersed about the diagonal corresponding to the sugar H2'-2" region (1.9 to 3.0 ppm). Seven of these cross-peaks are off the diagonal while the eighth from the terminal C8 residue falls on the diagonal.

Base H6 to Base H5/CH3—The three-bond 8 Hz coupling between cytidine H6 (7.54 to 7.57 ppm) and cytidine H5 (5.55 to 5.70 ppm) protons gives strong cross-peaks in the COSY spectrum (Fig. 1S). The four-bond 1 Hz coupling between the thymidine H6 (7.10 to 7.40 ppm) and CH3 (1.23 to 1.54 ppm) protons gives weak cross-peaks in the COSY spectrum (Fig. 1S). Since the thymidine H6 and cytidine H6 protons have been identified we can assign the corresponding H5 and CH3 protons in the AATT 8-mer duplex. These are listed in Table I and completes the assignment of the base and sugar protons in the AATT 8-mer duplex at 25 °C.

NOESY Contour Plots

Base to H3' and H4'—An expanded region of the 250-ms mixing time NOESY contour plot relating the base protons (7.1 to 8.2 ppm) with the H3' protons (4.5 to 5.1 ppm) and the H4' protons (4.0 to 4.5 ppm) is presented in Fig. 3S (see Miniprint Section). Each pyrimidine H6 and purine H8 proton exhibits an NOE to its own sugar H3' and the H3' in the 5' direction. These connectivities are presented in Fig. 3S and it is possible to walk between adjacent bases on a given strand by stepping through the H3' protons.

The H4' and H5'-5" protons are superimposed between 4.0 and 4.5 ppm in the contour plot in Fig. 3S. The H4' protons have been assigned from the COSY spectrum analysis and these assignments are depicted in Fig. 3S.

Base to H2'-2"—The cross-peaks in the 250-ms NOESY spectrum relating the base protons (7.1 to 8.2 ppm) with the H2'-2" protons (1.9 to 3.0 ppm) are depicted in Fig. 4S (see Miniprint Section). Cross-peaks are observed between the pyrimidine H6 and purine H8 protons and the H2'-2" protons of its own and the 5'-flanking sugar. The sugar H2'-2" assignments are consistent with those derived from the COSY analysis above.

We have taken one-dimensional slices through the H6 protons of T6 (7.38 ppm) and T5 (7.12 ppm) and are presented for the H2'-2" region extending between 1.8 and 3.0 ppm in Fig. 5, A and B, respectively. These results for a 150-ms mixing time NOESY of the AATT 8-mer show that the H6 proton of T6 exhibits a larger NOE to its own H2' proton at T6 compared to the H2' proton at T5 (Fig. 5A). Similarly, the H6 proton of T5 appears to exhibit a large NOE to its
own H2' proton at T5 compared to the H2' proton of A4 (Fig. 5B).

The lack of resolution of the H8 protons of A3 and A4, of the H8 protons of G1 and G2 and of the H6 protons of C7 and C8 (Fig. 4S) prevents an accurate comparison of the relative NOEs between the base and the H2' proton of its own and 3'-flanking sugar for these bases.

Other Regions—The NOESY data presented above has focused on the cross-peaks between the base protons and the H1', H2', H3', and H4' protons in the AATT 8-mer duplex. This analysis has also been extended to the NOE cross-peaks between various sets of sugar protons observed in Fig. 2S. Thus, the H1' protons exhibit NOEs to their own H2', H2", H3', and H4' protons but not those of the 5'- and 3'-flanking sugars (Fig. 2S).

We have also detected NOEs between the cytidine H5 and thymidine CH3 protons and the H1', H2', H2", H3', and H3' protons of the 5'-flanking sugar but not those of their own and 3'-flanking sugars. Thus, the H5 of C7 exhibits an NOE to the H1' of T6. Similarly, the CH3 of T5 exhibits an NOE to the H1' of A4 while the CH3 of T6 exhibits an NOE to the H1' of T5.

The above NOEs provide cross-checks for the assignments listed in Table I and their intensities will be useful in an eventual distance-geometry analysis of the quantitative NOESY data sets.

DISCUSSION

Assignments—We have assigned the nonexchangeable base and sugar protons of the d(G-G-A-A-T-T-C-C) duplex at room temperature (Table I) from an analysis of the two-dimensional COSY and NOESY spectra in D2O solution. The base protons were tentatively assigned previously based on a comparison of ring current shift estimated for duplex formation with those experimentally observed for the coil to helix transition (31). This study confirms the earlier assignments and further puts forward the complete H1', H2', H2", H3', and H4' assignments for all the sugars in the AATT 8-mer duplex.

This analysis validates the general approach put forward earlier for nucleic acid proton assignments based on COSY and NOESY two-dimensional analysis of DNA fragments (10-17). The excellent resolution of the AATT 8-mer phase-sensitive NOESY spectrum at 25 °C permits, in addition, correlations among the base protons and between the base protons and sugar protons on both strands not considered in detail before.

Right-handed Helix—We have probed for the handedness of the AATT 8-mer helix by monitoring the directionality of specific NOEs among the base protons and between the base proton and its neighboring sugar protons.

Thus for right-handed helices the pyrimidine H5, CH3 exhibits a strong NOE to the purine H8 or pyrimidine H6 of its adjacent 5' base and a weak NOE to the same protons of its 3' base. This pattern is indeed observed for all the base protons in the AATT 8-mer duplex and demonstrate unequivocally the handedness of the octanucleotide helix.

B-DNA Helix—The difference in the A-DNA and B-DNA conformations is reflected in a few inter-proton distances relating the base and sugar rings. It has been pointed out that the purine H8 or pyrimidine H6 is close (~1.8 Å) to its own sugar H2' and far (~3.9 Å) from the adjacent sugar H2' in the 5' direction in B-DNA while the reverse is time for A-DNA (23, 39). Thus, for B-DNA the stronger NOE is directed between the base and its own sugar H2' proton while in A-DNA the stronger NOE is detected between the base and its 5'-flanking sugar H2' proton (23, 39).

The thymidine H6 protons of T5 and T6 exhibit larger NOEs to their own sugar H2' protons compared to the H2' proton of the 5'-flanking sugar in the AATT 8-mer 150-ms mixing time NOESY spectrum (Fig. 5). These data demonstrate that T5 and T6 in the AATT segment of the AATT 8-mer adopt a B-DNA conformation in solution.

The nucleic acid B to A transition is induced with increasing alcohol concentrations. We intend to investigate the AATT 8-mer in water-alcohol solutions in the future in an attempt to determine whether the base to H2' NOEs, characteristic of the A-DNA conformation, are observed on decreasing the water activity.

A Comparison with Previous NMR Studies—We have compared the base and sugar proton assignments in the d(G-G-A-A-T-T-C-C) duplex at 25 °C (Table I) reached in the two-dimensional NMR COSY and NOESY studies reported in this paper with those derived from a parallel NOESY study by Brocio et al. (33) on the same duplex at 20 °C. There is good agreement between the two investigations for the base, sugar H1', and sugar H3' assignments but there are discrepancies in the sugar H4' assignments. The sugar H4' assignments of the AATT 8-mer reported in our study follow directly from cross-peaks in the COSY spectrum linking the sugar H3' and H4' protons as shown in Fig. 4B. The studies of Brocio et al. (33) focused only on the NOESY spectrum of the AATT 8-mer which may account for the observed discrepancy between these two studies. Brocio et al. (33), were also unable to distinguish between the H2 protons of A3 and A4 in the AATT 8-mer duplex. This is quite straightforward since the H2 proton of adenosine exhibits NOEs to the thymidine imino proton in the same base pair and also to three sugar H1' protons in the minor groove as shown in this paper.

REFERENCES


Supplemental Material to Sequence Dependent Conformation of DNA Duplexes:
The AAT Segment of the 5'-AAT-AAT-3' DNA Duplex in Aqueous Solution
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Figure 1a A contour plot of the magnitude COSY spectrum of the AAT 8-mer at 25C. The cross peaks off the diagonal correspond to protons coupled to each other through two, three and four bonds.

Figure 1b A contour plot of the phase sensitive NOESY spectrum (mixing time 200 msec) of the AAT 8-mer at 25C. The cross peaks off the diagonal correspond to interproton spatial distances of ≈4-8Å. The magnitude of the cross peaks is inversely related to the sixth power of the interproton distance.
Figure 36. An expanded contour plot of the phase sensitive NOESY spectrum (mixing time 250 msec) of the AAAT T 8-mer at 25°C establishing distance connectivities between the base protons (7.1 to 8.2 ppm) and the H3' (4.5 to 5.1 ppm) and H4' (4.0 to 4.5 ppm) protons. The sugar H3' and H4' assignments are depicted next to the contour peaks.

Figure 48. An expanded contour plot of the phase sensitive NOESY spectrum (mixing time 250 msec) of the AAAT T 8-mer at 25°C establishing distance connectivities between the base protons (7.1 to 8.2 ppm) and the H2' and H2'' protons (1.9 to 3.0 ppm). The sugar H2', 2'' assignments are depicted next to the contour peaks with the H2' protons resonating upfield of the H2'' protons for each sugar.