Characterization by ^1H NMR of glycosidic conformations in the tetramolecular complex formed by d(GGTTTTTGG)

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ABSTRACT

We have conducted two dimensional NOESY studies on the molecule d(G₂T₅G₂) to characterize the structure of the tetramolecular complex previously identified by calorimetric and spectroscopic studies (1). Analysis of the NOE and exchange cross peaks observed in the NOESY spectra establishes the formation of structured conformations at low temperature (5°C). Significantly, within each strand of these structured conformations, the G1 and G8 residues adopt *syn* glycosidic torsion angles, while the G2 and G9 residues adopt *anti* glycosidic torsion angles. Consequently, any structure proposed for the tetramolecular complex of d(G₂T₅G₂) must have alternating G(*syn*) and G(*anti*) glycosidic torsion angles within each strand. The implications of this observation for potential structures of the tetramolecular complex of d(G₂T₅G₂) are discussed.

INTRODUCTION

Guanosine 5'-monophosphate is known to self-associate in the presence of monovalent cations (2–6). This self-association can result in a planar G-tetrad alignment stabilized by a full complement of hydrogen bonds as shown in Figure 1. Indeed, x-ray diffraction and electron microscopy studies of d(GpG) and fiber diffraction analysis of poly rG have been interpreted in terms of G-tetrad structures (7–9).

In general, the pairing alignment(s) of d(G)_n segments are of current interest since d(T₂G₄)_n, d(T₄G₄)_n, and d(T₂AG₃)_n single-stranded overhangs constitute the telomeric ends of chromosomes (10). Consequently, much effort has been focused on understanding the pairing alignment(s) and the overall folding patterns of d(G)_n segments (11–18). Available chemical protection, interference, and cross-linking data, in conjunction with the results of gel electrophoretic studies under non-denaturing conditions, establish that single strands with d(G)_n segments form ordered structures in the presence of sodium and potassium cations (11–18). In principle, these structures could form by alignment of four separate strands (13), by alignment of two hairpins (15, 16), or unimolecularly (15, 17). Structures

formed intramolecularly are *per force* antiparallel, while structures formed intermolecularly have no such constraint (13). In this connection, an intramolecular antiparallel alignment with alternating *anti* and *syn* glycosidic bonds around the G-tetrad has been proposed for several DNA oligomers (15, 17). By contrast, an intermolecular all parallel alignment with *anti* glycosidic bonds at all deoxyguanosine residues around the G-tetrad has been proposed for RNA polymers (7, 8).

High resolution NMR is well suited to distinguish between *syn* and *anti* glycosidic torsion angles since the *syn* orientation is characterized by a strong NOE between the base and its own sugar H1' proton, as demonstrated initially in Z-DNA oligomers (19). Indeed, NMR already has been used to demonstrate that several deoxyguanosine residues adopt a *syn* orientation in the 24-mer d(T₄G₄)₄ (11). However, the complexity of the associated proton spectrum prevented the authors from making the sequence specific assignments needed to derive detailed structural information. Detailed NMR analysis of a G-tetrad alignment may require a shorter molecule such as d(G₂T₅G₂), which has been shown to form a tetramolecular complex in 1M NaCl (1). Furthermore, between 0° and 30°C, narrow exchangeable proton resonances were detected for d(G₂T₅G₂) at ~12 ppm, which only broadened out on raising the temperature to 50°C. Thus, this molecule is well suited for structural studies of tetraplexes by NMR.

We report below on two dimensional NMR NOESY studies of d(G₂T₅G₂) in 0.1 M NaCl, 10 mM Na-phosphate, in D₂O, as a function of temperature. These studies reveal an equilibrium between low temperature ('structured') and high temperature ('melted') conformations of d(G₂T₅G₂). More importantly, the NMR data allow us to define the glycosidic torsion angles of G1, G2, G8, and G9 in the structured conformations, thereby providing insights into possible pairing alignments within a G-tetrad and the relationship among adjacent G-tetrads within this tetramolecular complex.

EXPERIMENTAL PROCEDURES

The molecule d(G₂T₅G₂) was synthesized on a 32 μmole scale using H-phosphonate chemistry on a Biosearch 8750 synthesizer.

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The general procedures for synthesis, deprotection, and HPLC purification have been reported previously (20, 21). The sample concentration was 400 A₂₆₀ units of d(G₂T₅G₂) in a volume of 0.4 mL containing 0.1M NaCl and 10 mM sodium phosphate at pH 6.8. This solution corresponds to a strand concentration of 12.6 mM.

One and two dimensional proton NMR experiments in D₂O solution were recorded on Bruker AM400 and AM500 spectrometers and on a Varian VXR 500 spectrometer. Phase sensitive two dimensional nuclear Overhauser effect (NOESY) spectra of d(G₂T₅G₂) were collected with a repetition delay of 1.5 sec, a sweep width of 10 ppm, and mixing times of 250 and 50 msec. The carrier frequency was positioned on the residual HOD resonance, which was irradiated with the decoupler channel. The data sets were collected with 512 t₁ experiments and 64 scans per t₁ increment. The NOESY data sets were Fourier transformed in both dimensions with a 900 shifted sine bell function using the Hare Research FTNMR software.

Figure 1. Putative hydrogen bonding pattern for a planar G-tetrad.

Figure 2. The nonexchangeable proton NMR spectra (5.0 to 8.2 ppm) of d(G₂T₅G₂) in 0.3M NaCl, 10 mM Na-phosphate, in D₂O, pH 6.8 at (A) 5°C, (B) 25°C and (C) 40°C. The H8 protons of G residues are labelled by G1, G2, G8, and G9, and subscripted L and S for the major structured and minor structured conformations in (A) and subscripted U for the 'melted' conformation in (C).

RESULTS AND DISCUSSION

We report below on two dimensional NOE and exchange studies on the molecule d(G₂T₅G₂) which has been shown to form a tetramolecular complex in 1M NaCl (1).

Temperature and sodium dependence

The proton NMR spectra of the nonexchangeable base and sugar H1' protons (5.2 to 8.4 ppm) for d(G₂T₅G₂) in 0.3 M NaCl buffer in D₂O at 5°, 25°, and 40°C are plotted in Figure 2. We detect proton resonances from a major structured conformation (designated L) along with resonances from a minor structured conformation (designated S) at low temperature (Figure 2A). On gradually raising the temperature to 40°C, the resonances from the major structured conformation decrease in intensity and new resonances from a 'melted' conformation (designated U) increase in intensity (Figure 2C). Spectra of the nonexchangeable base protons recorded at elevated temperatures reveal that increasing the sodium concentration simply shifts the equilibrium towards the structured conformation, with no other detectable differences. Consequently, we have recorded the two dimensional NMR data sets at moderate sodium concentration (0.1 M).

NOE cross peaks

We have recorded NOESY spectra of d(G₂T₅G₂) as a function of temperature to characterize structural features of both the major

Figure 3. Expanded 250 msec mixing time NOESY contour (A) and stacked (B) plots establishing distance connectivities between base protons (7.2 to 6.4 ppm) and sugar H1' protons (5.3 to 6.4 ppm) for d(G₂T₅G₂) in 0.1M NaCl, 10mM Na-phosphate, in D₂O, pH 6.8 at 5°C. The NOEs between guanine H8 protons and their own sugar H1' protons are labelled G1, G2, G8, and G9, and subscripted by L and S for the major structured and minor structured conformations. The tracing in (A) follows NOE connectivities between base protons and their own and their 5'-flanking sugar H1' protons in the major structured conformation. Cross peak A corresponds to the NOE between H8 of G8 and H1' of G9.

and minor conformations detected at low temperature, and the 'melted' conformation detected at high temperature. A detailed analysis has been undertaken on the 250 msec NOESY spectrum at 5°C. An expanded contour plot, establishing distance connectivities between the base protons (7.2 to 8.2 ppm) and the sugar H1' protons (5.1 to 6.4 ppm), is presented in Figure 3A. The corresponding stacked plot is shown in Figure 3B. The relevant proton chemical shift data are listed in Tables I and II.

Major structured conformation. As shown in Figure 3A, the distance connectivities in the major conformation can be established by tracing the NOEs between the base protons (deoxyguanosine H8 and deoxythymidine H6) and their own as well as their 5'-flanking sugar H1' protons. NOEs involving H8 protons of G residues in the molecule can be identified since they disappear upon heating in D₂O solution at basic pH. Furthermore, the assignment for the H8 proton of G2 has been independently confirmed by ¹H detected ¹⁵N NMR studies on d[G(¹⁵N⁷)GT₅G₂], in which the [7-¹⁵N] atom of G2 is coupled to the H8 (22). We note strong NOEs from the H8 proton to its own H1' proton for G1 and G8 (designated G1_L and G8_L in Figure 3B) for the major structured conformation. This observation also holds for data collected at shorter mixing times (50 msec). It has been established previously that *anti* glycosidic torsion angles (interproton separation 3.7 Å) are characterized by weak H8 to H1' NOEs while *syn* glycosidic torsion angles (interproton separation 2.5 Å) are characterized by strong H8 to H1' NOEs (19). The NOE patterns we observe (Figure 3) establish that G1 and G8 are *syn* while G2 and G9 are *anti* in the major structured conformation at low temperature. Similarly,

Table I. Nonexchangeable base and sugar proton chemical shifts for the major structured conformation of d(G₂T₅G₂) at 5°C^a

Base	H8	H6	Chemical Shifts, ppm				
			CH ₃	H1'	H2'	H3''	H3'
G1	7.41			5.89	2.59	2.78	4.97
G2	8.17			5.99	2.75	2.54	5.04
T3		7.41	1.74	6.14	2.24	2.47	4.78
T4		7.33	1.70	5.41	1.95	2.33	4.57
T5		7.44	1.70	5.80	2.06	2.16	4.61
T6		7.28	1.63	5.68	1.85	2.39	4.69
T7		7.53	1.74	6.10	2.06	2.42	4.61
G8	7.42			6.12	3.57	2.98	4.96
G9	8.20			6.36	2.68	2.53	4.81

^a0.1M NaCl, 10mM Na-phosphate, D₂O, pH 6.8.

Table II. Base H8 and sugar H1' proton chemical shifts of the structured and 'melted' conformations of d(G₂T₅G₂) at 25°C^a

Base	Major Structured		Chemical Shifts, ppm			
	H8	H1'	Minor Structured H8	Minor Structured H1'	'Melted' H8	'Melted' H1'
G1	7.40	5.88	7.26	6.00	7.76	5.99
G2	8.13	6.00	8.05		7.98	
T3	7.38	6.09	7.43	6.03	7.53	5.92
T4	7.36	5.55	7.33	5.71	7.60	6.18
T5	7.44	5.85	7.51	6.01	7.63	6.21
T6	7.28	5.71	7.48	5.82	7.61	6.19
T7	7.50	6.06	7.33	6.04	7.43	6.01
G8	7.41	6.11	7.58	6.05	7.83	5.92
G9	8.14	6.36	8.15		7.97	6.14

^a0.1M NaCl, 10 mM phosphate, D₂O pH 6.8.

all five deoxythymidines are found to be *anti*. Table I lists the chemical shifts of selected base and sugar protons for the major structured conformation at 5°C based on an analysis of the entire NOESY spectrum.

Minor structured conformation. We detect two NOE cross peaks, designated G1_S and G8_S, which originate from protons in the minor structured conformation of d(G₂T₅G₂) at 5°C (Figure 3B). They disappear on deuterium exchange of the deoxyguanosine H8 protons and hence are assigned to NOEs between the H8 and H1' protons from two of the four deoxyguanosines. The fraction of the minor structured conformation is 10 to 20% of the major structured conformation at 5°C, so that the observed moderate cross peak intensities of G1_S and G8_S (as assigned below) require that these two deoxyguanosines adopt *syn* glycosidic torsion angles (Figure 3). The remaining NOEs between base and sugar H1' protons for the minor structured conformation are either much weaker or not detectable so that the other two deoxyguanosines and all five deoxythymidines again adopt *anti* glycosidic torsion angles.

Figure 4. Expanded 250 msec mixing time NOESY contour plots of d(G₂T₅G₂) in 0.1M NaCl, 10 mM Na-phosphate, in D₂O, pH 6.8, at 25°C. Exchange cross peaks are detected for specific protons between the major structured (subscripted L), minor structured (subscripted S), and 'melted' (subscripted U) conformers of d(G₂T₅G₂) in slow equilibrium. (A) Cross peaks establish exchange and distance connectivities in the symmetrical 7.2 to 8.3 ppm base proton region. The exchange cross peaks A to H are assigned as follows: A: G1_S(H8)–G1_U(H8); B: G1_L(H8)–G1_U(H8); C: G1_S(H8)–G1_L(H8); D: G8_L(H8)–G8_U(H8); E: G8_S(H8)–G8_U(H8); F: G8_S(H8)–G8_L(H8); G: G9_L(H8)–G9_U(H8); H: G2_L(H8)–G2_U(H8). (B) Cross peaks establish exchange and distance connectivities in the symmetrical 5.4 to 6.5 ppm sugar H1' proton region. The exchange cross peaks A to F are assigned as follows: A: T4_L(H1')–T4_U(H1'); B: T4_S(H1')–T4_U(H1'); C: T5_L(H1')–T5_U(H1'); D: T5_S(H1')–T5_U(H1'); E: T6_L(H1')–T6_U(H1'); F: T6_S(H1')–T6_U(H1').

Exchange Cross Peaks

We detect exchange cross peaks between the major structured, minor structured, and 'melted' conformations in the 250 msec NOESY spectra of $d(G_2T_5G_2)$ at 25°C. An expanded contour plot of the symmetrical 6.8 to 8.2 ppm region recorded at 25°C is shown in Figure 4A. A knowledge of the base and sugar H1' assignments of G1, G2, G8, and G9 for the major structured conformation (subscripted L) in turn yields the corresponding assignments for the minor structured (subscripted S) and 'melted' (subscripted U) conformations. Thus, the H8 proton of $G1_L$ at 7.40 ppm exhibits an exchange cross peak to the same proton of $G1_S$ at 7.26 ppm (peak C, Figure 4A) and of $G1_U$ at 7.76 ppm (peak B, Figure 4A). Similarly, the H8 protons of $G1_S$ and $G1_U$ also exhibit an exchange cross peak (peak A, Figure 4A). Such an analysis also extends to the exchange cross peaks for the H8 protons of G8, G2, and G9, and the assigned cross peaks (peaks D to H, Figure 4A) are tabulated in the figure caption. We note that the H8 chemical shifts of G1 and G8 in the 'melted' conformation are substantially downfield of their corresponding shifts in the major and minor structured conformations. It is therefore not surprising that the corresponding exchange cross peaks involving the structured and 'melted' conformations of G1 (peaks A and B, Figure 4A) and G8 (peaks D and E, Figure 4A) are furthest from the diagonal. The exchange cross peaks in the NOESY experiment correlate the cross peaks of $G1_L$ for the major structured conformation with $G1_S$ for the minor structured conformation and similarly correlates $G8_L$ with $G8_S$ in the stacked NOESY plot drawn in Figure 3B. Thus, the NOE and exchange cross peak data establish that G1 and G8 are *syn* while G2 and G9 are *anti* for the minor structured conformation as they are for the major structured conformation. The deduced chemical shifts of the H8 and H1' protons of G1, G2, G8, and G9 for the major structured, minor structured, and 'melted' conformations of $d(G_2T_5G_2)$ are listed in Table II.

We also detect a pattern of exchange cross peaks in the expanded 250 msec mixing time NOESY contour plot of the symmetrical 5.4 to 6.5 ppm sugar H1' proton region of $d(G_2T_5G_2)$ at 25°C (Figure 4B). The exchange cross peaks detected between the major and minor structured conformations and the 'melted' conformation for T4, T5, and T6 are labelled in Figure 4B and assigned in the Figure caption. The deduced chemical shifts for the H6 and H1' protons of the T residues for the major structured, minor structured, and melted conformations of $d(G_2T_5G_2)$ are listed in Table I.

We also have measured the exchangeable proton spectrum (6.5 to 14 ppm) of $d(G_2T_5G_2)$. Consistent with our previously reported data (1), four partially resolved narrow N1 protons are detected at ~12 ppm. We now tentatively assign these narrow exchangeable resonances to the hydrogen-bonded deoxyguanosine N1 protons, while the broad resonances between 10 and 11 ppm can be assigned to deoxythymidine N3 protons. These data are consistent with tetraplex formation through G-tetrad alignment.

G-tetrad structures

In principle, G-tetrad containing structures generated from alignment of four separate strands can have either a parallel or an anti-parallel orientation. Modelling suggests that parallel alignment of four $d(G_2T_5G_2)$ strands would result in formation of all *syn* or all *anti* glycosidic bonds within each G-tetrad. By contrast, modelling suggests that antiparallel alignment of four $d(G_2T_5G_2)$ strands would result in formation of alternating *syn/anti* glycosidic bonds around the G-tetrad.

In summary, our NMR data establish that within each strand, the glycosidic bonds in each of the two $d(G-G)$ segments alternate between *syn* and *anti* conformations. Thus, any proposed structure for the tetramolecular complex of $d(G_2T_5G_2)$ must be consistent with this feature.

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