Chemical Probe for Glycosidic Conformation in Telomeric DNAs

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Received December 8, 1993

Chemical modifications are powerful tools to probe nucleic acid structure and protein–nucleic acid interactions. Nucleic acids can be modified either randomly by a variety of chemical reagents[1] or site-specifically with synthetic base analog substitutions.[2-4] We report here the use of 8-bromodeoxyguanosine (8-Br-dG) as a modification that is directly sensitive to a dihedral angle. 8-Br-dG should prove useful as a chemical probe for the conformation about glycosidic bonds in unusual nucleic acid structures.

Telomeres, which are the ends of linear chromosomes, contain tandem repeats of G-rich DNA sequences that form structures based on the G-quartet, a cyclic hydrogen-bonded array of four coplanar guanine bases,[5] shown in Figure 1a. High-resolution structures of several telomeric DNAs[6,7] and of the thrombin binding aptamer[8,9] show that the glycosidic conformation of the guanine residues alternates between *syn* and *anti* along the polynucleotide chain.

Bulky substituents on purine nucleosides at the 8-position shift the equilibrium conformation about the glycosidic bond to favor the *syn* conformation by at least 1–2 kcal/mol[10-12] (see Figure 1a). Thus, bromination of G residues in poly(d(GC)) reduces the salt requirements for conversion to Z-DNA,[13] in which the guanine nucleotides adopt the *syn* conformation. We reasoned that substitutions of 8-bromodeoxyguanosine (8-Br-dG) at positions that show *syn* conformations in G-quartet structures might stabilize the structure, while substitution at positions required to be *anti* might destabilize the structure.

To test 8-Br-dG as a possible chemical probe of glycosidic torsion, we have prepared a set of oligonucleotides containing site-specific 8-Br substitutions. A reagent suitable for use in conventional DNA synthesis was prepared by direct bromination of DMT-*N*-isobutyryldeoxyguanosine (DMT-*N*-iBu-dG),[14] followed by conversion to the (β-cyanoethyl)phosphoramidite.[15] Eight single 8-Br-dG substitutions were introduced into the sequence d(TTTGGTTTGGTTTGGTTTGG) = $d(T_3G_2)_4$,[16] which forms a G-quartet structure (Figure 1b) in the presence of KCl (data not shown).

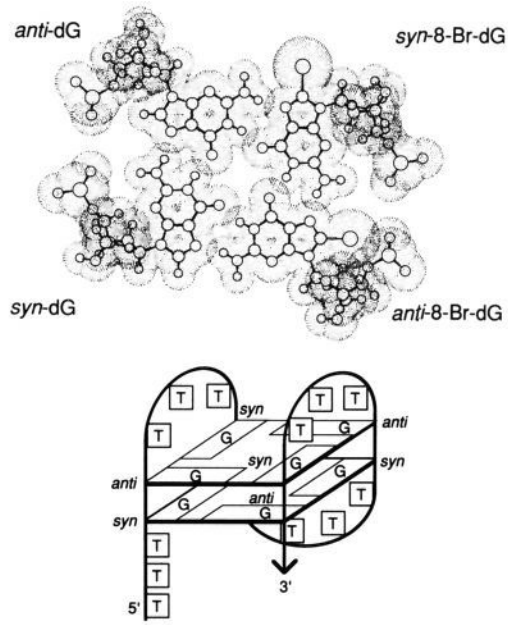

Figure 1. (a, top) A G-quartet composed of two *anti* G residues and two *syn* residues. Two of the guanines have been substituted with Br at the 8-position to illustrate the effect of the substitution in the context of a quadruple helix. Substitution at a *syn* position places the Br atom in the groove, while substitution at an *anti* position introduces crowding with the backbone. (b, bottom) Schematic representation of the G-quartet structure adopted by $d(T_3G_2)_4$. Two stacked G-quartets are capped by loops of thymidine residues. The glycosidic conformation alternates *syn–anti* for each pair of adjacent G's.

The effect of the 8-Br-dG substitution on the stability of the structure was determined by measuring the melting temperature of each oligonucleotide by UV thermal denaturation. Representative melting curves for the parent sequence and for the Br-9 and Br-10 substitutions are shown in Figure 2. The melting curves were analyzed by least-squares fitting to $\Delta H°$ and T_m for a two-state transition,[17] and the results are summarized in Table 1. The T_m of the parent oligonucleotide is 45 °C, while the T_m's of four of the 8-Br-dG substitutions were depressed by 3–6 °C, and the T_m's of the other four substitutions were increased by 5–6 °C. Substitution at the first dG of each GG step results in an increase in stability, while substitution at the second dG of each GG step results in a decrease in stability. On the basis of the predicted

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(14) 8-Br-DMT-*N*-iBu-dG was prepared by treatment of 500 mg (0.78 mmol) of DMT-*N*-iBu-dG (Peninsula Labs) in 20 mL of dioxane and 15 mL of 0.5 M NaOAc pH 5.4, with 2 equiv of Br_2 (~80 μL), added in four equal portions at 15-min intervals. The reaction was quenched with 10% sodium thiosulfate, followed by standard workup. Flash chromatography purification yielded 380 mg of 8-Br-DMT-*N*-iBu-dG (0.52 mmol, 67%). ^1H NMR ($CDCl_3$): δ 6.7–7.7 (mm, 13H, DMT), 6.28 (t, H1′), 5.02 (m, H4′), 4.08 (m, H3′), 3.70/3.78 (s, OMe), 3.47/3.22 (m, H5′/H5″), 2.35 (m, 1H), 1.95 (m, 1H), 1.77 (m, 1H), 0.94 (m, CH_3), 0.74 (m, CH_3). The identity of the product was verified by degradation of the protected nucleoside to 8-bromoguanine, which coeluted with authentic 8-bromoguanine (Sigma) on reverse phase HPLC.

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(16) Oligonucleotides were synthesized on an Applied Biosystems Model 381 DNA synthesizer. Detritylation with 0.2 M dichloroacetic acid in dichloromethane minimized depurination of the 8-Br-dG residues. Oligonucleotides were purified on 20% (19:1) polyacrylamide gels containing 7 M urea. Laser desorption/ionization time-of-flight mass spectral analysis verified the presence of bromine in two of the derivatives. The $(M-H)^-$ for the parent oligonucleotide $d(T_3G_2)_4$ was m/z 6220 (calculated m/z 6217), for Br-4 was m/z 6299 (calculated m/z 6296), and for Br-20 was m/z 6300 (calculated m/z 6296).

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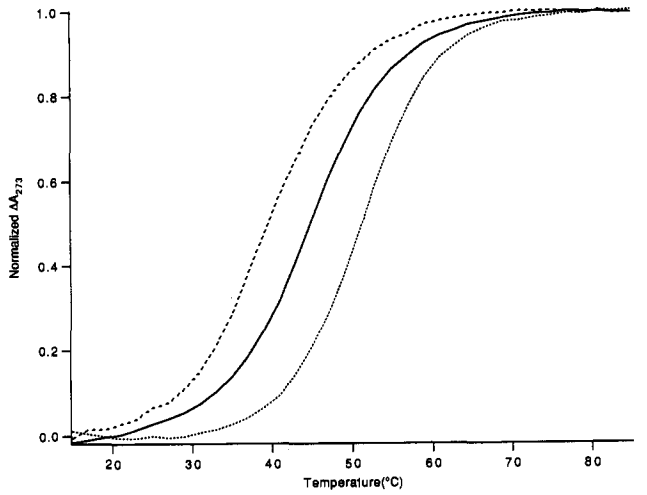

Figure 2. Representative thermal denaturation curves for oligonucleotides. The curves have been normalized after subtraction of upper and lower base lines derived from the curve fitting from the raw data: (—) $d(T_3G_2)_4$, (- - -) Br-9, (– – –) Br-10.

Table 1. Thermodynamic Parameters from UV Thermal Denaturation of 8-Br-dG Substituted into d(TTTGGTTTGGTTTGGTTTGG)[a]

oligonucleotide	T_m (K)	$\Delta H°$ (kcal/mol)	ΔT_m (°)	$\Delta\Delta G°_{298}$ (kcal/mol)
parent	318.4	40.7		
Br-4	324.5	43.7	6.1	1.0
Br-5	312.5	33.7	–5.9	–1.0
Br-9	324.2	45.2	5.8	0.8
Br-10	312.7	35.6	–5.7	–0.9
Br-14	323.7	43.7	5.3	0.9
Br-15	312.0	33.4	–6.4	–1.1
Br-19	324.5	44.0	6.1	1.0
Br-20	314.8	37.8	–3.6	–0.6

[a] Thermal denaturation profiles were recorded on an Aviv 14DS spectrophotometer using a thermoelectrically controlled sample holder. Sample conditions were 2–5 μM oligonucleotide, 100 mM KCl, 20 mM potassium phosphate, pH 7. Samples were heated to 95 °C for 2 min and then briefly degassed in vacuum. The absorbance of the sample was recorded at 2 °C intervals, after a 2-min equilibration period. Both heating and cooling curves were recorded to check for possible hysteresis. The values reported are from the heating curves. The T_m's for the heating and cooling curves and T_m's from independent runs generally agreed to within 1 °C, while $\Delta H°$ values were reproducible to within 10%.

effect of the 8-Br-dG substitution, these results would suggest that the first dG residue in each GG step adopts the *syn* conformation, while the second dG residue adopts the *anti* conformation.

In order to test if the predicted glycosidic torsions were correct, we examined the conformation of the parent sequence, $d(T_3G_2)_4$, using NMR spectroscopy.[18] Formation of the G-quartet structure shown in Figure 1b for $d(T_3G_2)_4$ was confirmed by the appearance of eight imino proton resonances, indicating formation of two G-quartets (data not shown). The *syn* or *anti* conformations are readily distinguished by the magnitude of the NOE between the guanine H8 proton and the deoxyribose H1′ proton; in the *anti* conformation these protons are nearly 4 Å apart, while in the *syn* conformation this distance is ~2.2 Å. Examination of a NOESY spectrum indicated the presence of a set of H8 protons, at ~7.5 ppm, that exhibited strong NOEs to H1′ protons, while another set of H8 protons, at ~8.2 ppm, exhibited weak NOEs to the H1′ protons (data not shown). In addition, each of the H8 protons at ~8.2 ppm exhibits a strong internucleotide NOE to the H2′/H2″ on the previous residue. This pattern of NOEs is consistent with a *syn*-G preceding an *anti*-G at each GG step.[6] The observed pattern of NOEs and the chemical shifts are very similar to those observed for the thrombin binding aptamer, which has a similar sequence and structure.[8,9]

These results correlate exactly with the predicted glycosidic torsins based on the change in melting temperature of the 8-Br-dG substitutions. The free energy differences between 8-Br-dG substitutions at *syn* versus *anti* positions in $d(T_3G_2)_4$, shown in Table 1, are comparable to the differences observed in nucleosides. A simple explanation for this observation might be that energy is required to convert normally *anti* nucleotides to the *syn* conformation upon folding. The 8-Br-dG substitution at *syn* positions in the G-quartet structure preorganizes that nucleotide and lessens the energetic penalty for folding. In the case of substitutions at *anti* positions, the situation is essentially reversed. The nucleotide must be flipped from *syn* to *anti* in the folded form, with an energetic penalty due to the steric crowding of the bulky bromine.

In summary, a method for preparing 8-Br-dG substituted oligonucleotides has been presented. The utility of this derivative for conformational analysis has been illustrated by prediction of glycosidic conformers in a G-quartet structure. The 8-Br substitutions should prove useful for probing glycosidic conformations in nucleic acids, including telomeric DNAs, Z-DNA, and RNA structures, where *syn* G residues are commonly found.

Acknowledgment. The authors wish to thank B. Wang for the mass spectral analysis, performed at the MIT Mass Spectrometry Resource, supported by the NIH (RR-00317, to K. Biemann), and Julius Rebek for comments on the manuscript. J.L.B. was supported by the NIH Interdepartmental Biotechnology Program (GM-08334). This work was supported by grants from the NIH (GM-46314), Pfizer, Inc., and the Searle Scholars Program of the Chicago Community Trust.

(18) NMR spectra of $d(T_3G_2)_4$ were recorded on a Varian VXR-500 spectrometer. Sample conditions were 4 mM DNA, 1 M KCl, 10 mM potassium phosphate pH 7, 25 °C.