Nitrogen-15-Labeled Oligodeoxynucleotides. 5. Use of ^{15}N NMR To Probe H-Bonding in an O^{6}MeG·T Base Pair

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The O^6-alkylguanine lesions produced in DNA by alkylating agents play a primary role in the mutagenicity and carcinogenicity of these agents.[1-3] Studies of the mutagenic activity of O^{6}MeG both *in vitro* and *in vivo* have demonstrated that T (or U) is preferentially (but slowly) incorporated opposite O^{6}MeG lesions, leading to G→A transition mutations.[4] The structures of DNA fragments containing O^6-alkylguanine residues have been investigated by both ^{1}H NMR and X-ray techniques,[5-11] and the stability in DNA fragments of O^{6}MeG·N pairs has been determined by optical melting experiments.[9,12,13] Interestingly, the O^{6}MeG·N pairs differ little in stability, with the O^{6}MeG·T pair generally the least stable. It is significantly less stable than is a G·T mispair. However, the alignment of the bases in the O^{6}MeG·T pair may be more "Watson–Crick-like" than that of the other O^{6}MeG·N pairs, which may be the basis for the preferential incorporation of T.

It has been reported recently, on the basis of an X-ray study at 2-Å resolution, that in the self-complementary duplex {d[CGC-(O^{6}Me)GAATTTGCG]}$_2$, which contains two O^{6}MeG·T pairs, the spatial orientation of these bases was distinctly Watson–Crick.[9] To be specific, the O^{6}MeG methyl group was found to be *anti*, and the distances between the O^{6}MeG O6, N1, and N2 atoms, and the thymine O4, N3, and O2 atoms, respectively, were reported to be 2.9, 2.9, and 2.8 Å. These data are consistent with a fully H-bonded, Watson–Crick-like O^{6}MeG·T pair. In contrast to these solid-state data, in a ^{1}H and ^{31}P NMR study of the same molecule used in the present work, {d-[CGTGAATTC(O^{6}Me)GCG]}$_2$, the upfield shift to 9 ppm of the thymine H3 proton of the O^{6}MeG·T pair suggested that for this duplex, in solution, H-bonding between the O^{6}MeG N1 and the thymine H3 atoms was either longer than normal or absent.[6] In order to probe further the solution structure of this O^{6}MeG·T pair, we have synthesized the corresponding ^{15}N-labeled duplexes containing either [1-^{15}N]- or [2-^{15}N]-O^6-methyl-2′-deoxyguanosine and monitored the ^{15}N chemical shifts through the duplex-to-coil transition.[14] The syntheses were carried out using an H-phosphonate method, and the products were purified and characterized as reported previously.[12,15]

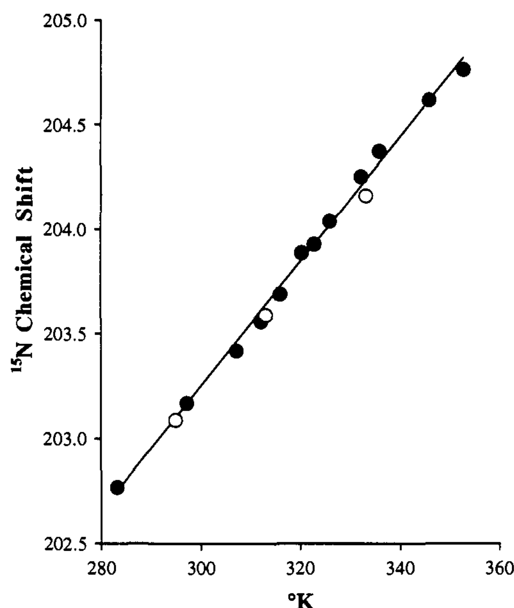

Figure 1. Plot of ^{15}N chemical shift vs temperature for: ●, d-[CGTGAATTC(^{15}N^{1}O^{6}Me)GCG] (**1**), 11.2 mM, in 80% H_2O/20% D_2O, 0.1 M NaCl, 10 mM phosphate, 1 mM EDTA, pH 6.8; and ○, [1-^{15}N]-O^6-methyl-2′-deoxyguanosine, 12 mM, in 90% H_2O/10% D_2O, 0.1 M NaCl, 10 mM phosphate, 1 mM EDTA, pH 7.3. A single-pulse ^{15}N experiment with a flip angle of ~90° and an experimental recycle delay of 8.8 s was used. Chemical shifts are reported relative to NH_3, using external 1 M [^{15}N]HNO_3 in 90% D_2O at 25 °C at 375.80 ppm as a reference.[20] A least-squares fit to the ^{15}N chemicals shifts to **1** gives the line shown; $y = 202.45 + 0.029\,695x$, $R = 0.997\,87$.

The temperature dependence of the ^{15}N chemical shifts of d[CGTGAATTC(^{15}N^{1}O^{6}Me)GCG] (**1**), of d[CGTGAATTC-(^{15}N^{2}O^{6}Me)GCG] (**2**), and of [1-^{15}N]- and [2-^{15}N]-O^6-methyl-2′-deoxyguanosine is shown in Figures 1 and 2. The sigmoidal plot of the 2-amino chemical shift of **2** (Figure 2) is very similar to that observed for the adenine 6-amino chemical shift (^{15}N-labeled) in a Watson–Crick A·T pair.[16] In both cases the duplex chemical shift is downfield of the single-strand chemical shift, as expected for a hydrogen-bond donor.[17] In the present case the total chemical shift change is 1.1 ppm, while for the A·T pair it was 2.7 ppm. The thermodynamic values shown in Table I for this duplex-to-coil transition were obtained from a nonlinear least-squares fit[18] to the data for **2**, with the assumption that the transition is two-state. The same values were also obtained by calculating the fraction of single strands in the duplex form, α, and the equilibrium constant, K, and plotting ln K vs T^{-1}.[16] The fact that the values we obtained from this local monitor agree well with the global values obtained for this same molecule by optical techniques[12] both demonstrates that this local probe is sensitive to the melting transition and confirms the two-state nature of the transition. From these results it is clear that there is H-bonding between the O^{6}MeG N2 and, presumably, the thymine O2.

The plot of the N1 chemical shift of **1** (Figure 1), in contrast to that of the N2, does not reflect the melting transition of this duplex. Instead of a sigmoidal plot, the O^{6}MeG N1 chemical shift shows only a linear downfield drift, identical to that seen for the [1-^{15}N]-labeled monomer. The overall chemical shift change for **1** is 2.0 ppm, somewhat larger than the 1.1-ppm change

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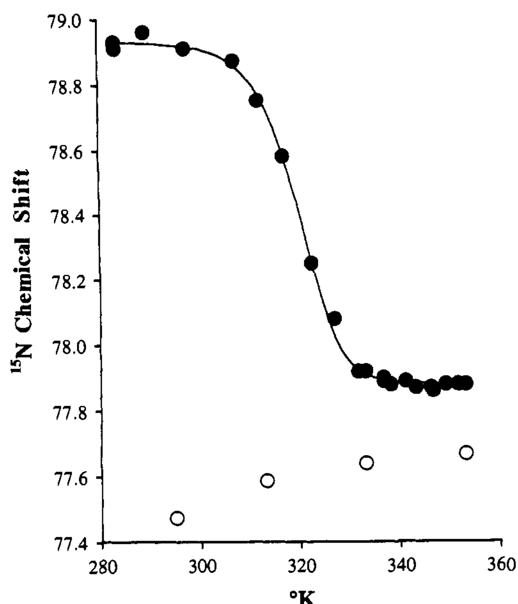


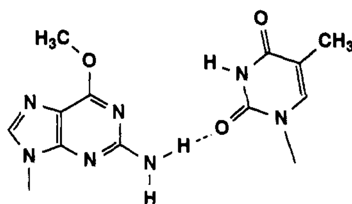
Figure 2. Plot of ^{15}N chemical shift vs temperature for: ●, d-[CGTGAATTC($^{15}N^2O^6$Me)GCG] (**2**), 10.7 mM, in 80% H_2O/20% D_2O, 0.1 M NaCl, 10 mM phosphate, 1 mM EDTA, pH 6.8; and ○, [2-^{15}N]-O^6-methyl-2′-deoxyguanosine, 12 mM, in 90% H_2O/10% D_2O, 0.1 M NaCl, 10 mM phosphate, 1 mM EDTA, pH 7.3. A non-linear least-squares fit to the ^{15}N chemical shifts of **2** gives the curve shown, from which the thermodynamic values listed in Table I were obtained.[18] The relevant equations are included in the supplementary material.

Table I. Thermodynamic Properties of {d[CGTGAATTC(O^{6}Me)GCG]}$_2$

source	$-\Delta H°$ (kcal/mol)	$-T\Delta S°$ 25 °C (kcal/mol)	$-\Delta G°$ 25 °C[c] (kcal/mol)	T_m (°C) (0.0107 M)[d]
UV[a]	76.9	66.5	10.4	58
^{15}N NMR[b]	73.4	65.7	7.7	47

[a] Determined as reported previously in 1 M NaCl, estimated accuracy ±10%.[12] [b] Calculated from a non-linear least-squares fit to the ^{15}N data plotted in Figure 2, estimated accuracy ±10%.[18] [c] Calculated from $\Delta G° = \Delta H° - T\Delta S°$. [d] Calculated using the equation $T_m^{-1} = [(R \ln C_T)/\Delta H°] + (\Delta S°/\Delta H°)$.

for **2** but still smaller than the 5.2-ppm change for the adenine N1 in the A·T pair that we studied, which displayed a clearly sigmoidal plot from which we obtained correct thermodynamic values.[16] The insensitivity of this O^{6}MeG N1 chemical shift to the melting transition of this duplex confirms the ^{1}H NMR conclusion[6] that this O^{6}MeG N1 is not directly H-bonded to the thymine H3 proton in this duplex in solution.



Comparison of the temperature dependence of the N1 chemical shift of **1** with that of the guanine N7 in the two non-self-complementary duplexes d[G($^{15}N^7$)GTTTTTGG]·d[CCAAAAACC] (**3**) and d[T($^{15}N^7$)GGGT]·d[ACCCA] (**4**)[15] reveals that the O^{6}MeG N1 in **1** either is fully accessible to hydration in the duplex, perhaps due to a bridging water molecule, or is opening and closing rapidly. Duplexes **3** and **4** differ in that, while **3** has a relatively strong C·G pair to the 5′ side of the label, **4** has a relatively weak A·T pair. Thus, duplex **4** is likely to be substantially frayed to the 5′ side of the labeled base, and the N7 should be relatively accessible to solvent, while in duplex **3** the N7 should be less accessible. Duplex **4**, like **1**, shows a linear downfield chemical shift change with increasing temperature, with no detectable sensitivity to the melting transition. Duplex **3**, in contrast, shows an initial downfield shift, below the T_m, followed by an *upfield* shift as the duplex melts. This discontinuous behavior presumably reflects the significantly greater accessibility to hydration of the N7 in the single strand in this case. Further, at low temperature, there is no difference between the duplex and single-strand chemical shifts for **4**, while for **3** the single-strand chemical shift is upfield of the shift in the duplex by about 2 ppm. Thus, the behavior of the N1 label in **1** is similar to that of the N7 label in **4**, which is fully hydrated, but is different from that of the N7 label in **3**, where hydration is restricted.

It should be noted that this analysis assumes that it is H-bonding, not ring anisotropy, that is primarily responsible for the ^{15}N chemical shift changes observed. In general, ^{15}N chemical shifts are expected to be more influenced by the paramagnetic term, σ_p, than by the anisotropic term, σ_a.[19,20] The chemical shift differences we observe, however, are small enough that the possibility of significant anisotropic effects cannot be dismissed. Nevertheless, to date the direction of the ^{15}N chemical shift changes with increasing temperature that we and others have observed in both monomers and short DNA fragments are uniformly downfield for an H-bond acceptor nitrogen and upfield for an H-bond donor nitrogen.[15,16,21-25] This is the directionality to be expected for the effect of H-bonding on σ_p[17] but which anisotropy would not have to follow. The only exception to this directionality is the discontinuous behavior of duplex **3**, discussed above, which still is consistent with H-bonding as the predominant influence on chemical shift.

The results presented above demonstrate that in the O^{6}MeG·T pair studied, there is direct base·base H-bonding at the O^{6}MeG N2, but not at the N1, and that the N1 is instead H-bonded to solvent H_2O. While these findings are consistent with earlier ^{1}H NMR studies of the same molecule,[6] they differ from those of an X-ray study of a different duplex containing an O^{6}MeG·T pair.[9] The origin of these differences may be in the sequence context or may reflect solution properties of the O^{6}MeG·T pair which are constrained in the crystal.

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Supplementary Material Available: Tables of ^{15}N chemical shifts of **1**, **2**, [1-^{15}N]-and [2-^{15}N]-O^6-methyl-2′-deoxyguanosine, the equations used for curve-fitting, and HPLC characterization of **1** and **2** (5 pages). Ordering information is given on any current masthead page.

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