Nitrogen-15-Labeled Oligodeoxynucleotides. 8. Use of ^{15}N NMR To Probe Hoogsteen Hydrogen Bonding at Guanine and Adenine N7 Atoms of a DNA Triplex

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Abstract: We have used [7-^{15}N]-labeled deoxyguanosine and deoxyadenosine to probe the Hoogsteen hydrogen bonding in C^+·GC and T·AT DNA triplexes. The triplex used in this work consists of a 21 base pair duplex with a 15 base third strand. Use of a triplex that is formed intermolecularly, rather than one formed intramolecularly, enabled us to examine separately the single strand, the duplex, and the triplex under the same conditions of temperature and pH. The single strands and the duplexes each show only a single resonance, while the triplex samples show two resonances at each intermediate temperature, one identical to that of the duplex and another 6 to 9 ppm upfield, which we assign to the triplex. At lower temperature or lower pH the intensity of the upfield (triplex) resonance is increased, while at higher temperature or higher pH it is decreased. These results provide the first direct evidence for the Hoogsteen hydrogen bonding to the purine N7 atoms postulated for C^+·GC and T·AT triplets. Moreover, the magnitude of the chemical shift changes seen for this DNA triplex suggests that ^{15}N NMR of appropriately labeled DNA and RNA molecules may be able to identify the presence of Hoogsteen hydrogen bonding to a purine N7 in less well defined systems. This may be of particular importance for RNA structures, where a wider variety of tertiary interactions is present.

Nitrogen-15 NMR of molecules containing [7-^{15}N]-labeled purines has the potential to provide model-independent, local information about Hoogsteen pairing like that present in triplexes.[1-3] Triple-stranded DNA[1-3] has been investigated extensively in recent years, primarily because of its potential therapeutic applications in transcription inhibition,[4-9] but also for its use in chromosome mapping[10,11] and its possible regulatory role in natural systems.[12,13] Although there are as yet no X-ray structures, much progress has been made in elucidating the structure of triplexes by ^1H NMR.[2,14-17] Two types of DNA triplexes can be formed, depending on whether the third strand is largely purine or largely pyrimidine in content. In each case the DNA duplex consists of one primarily purine strand and one primarily pyrimidine strand. The third strand binds to the purines of the duplex in the major groove. The two types of third strand differ in that a pyrimidine-rich third strand lies parallel to the duplex purine strand to which it forms Hoogsteen pairs, whereas a purine-rich third strand lies antiparallel to the duplex purine strand to which it forms reverse Hoogsteen pairs. In both cases the third strand binds with extremely high specificity, and in the pyrimidine case, C^+·GC and T·AT triplets are formed (Figure 1).

Our previous work with various site-specifically labeled DNA fragments has shown that the chemical shift of a ^{15}N atom can be a sensitive probe of base−base and of drug−base hydrogen bonding.[18-24] These examples include the Watson−Crick interaction between the adenine N1 and the thymine N3H of an A·T pair,[18] the bonding between the N1 of O^6-methylguanine and the cytosine amino or protonated N3 of an O^6MeG·C mispair,[24] and the interaction between an adenine N3 and the propylamidinium group of netropsin and distamycin.[23] In each case, hydrogen bonding to an sp^2 nitrogen resulted in an upfield change in chemical shift of 2−7.5 ppm relative to the hydrated, unbound state. We now report use of a 57-residue pyrimidine·purine·pyrimidine triplex to asses the ability of a purine [7-^{15}N]

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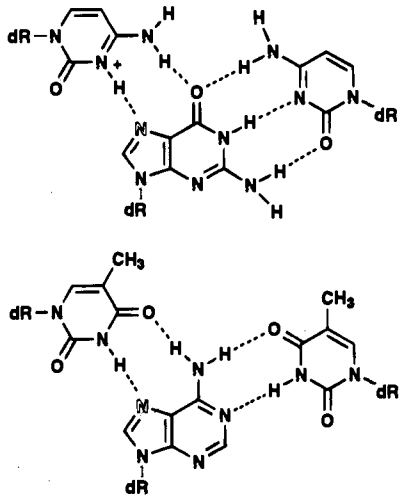

Figure 1. Base pairing alignments for the C^+·G·C and T·A·T triplets, with the [7-^{15}N] label shown in outline.

label to probe Hoogsteen hydrogen bonding. We find that triplex formation gives rise to large upfield shifts of 6 and 9 ppm for the [7-^{15}N]-labeled adenine and guanine residues, respectively. This is a direct observation of Hoogsteen hydrogen bonding in these triplets that demonstrates the ability of specific ^{15}N labeling to probe Hoogsteen bonding, even in a complex of this size.

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          5'-d[T T T T T C T C T C T C T C T]-3'          (1)
5'-d[G C T A A A A A G A G A G A G A G A T C G]-3'  (2)
3'-d[C G A T T T T T C T C T C T C T C T A G C]-5'  (3)
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The triplex used in this work consists of a 21 base pair duplex (**2**·**3**) with a 15 base third strand (**1**). This complex has been characterized by optical and calorimetric measurements.[25] With the labels centrally placed, it is long enough to avoid the end effects which may be present in shorter complexes. We chose to use a triplex that is formed intermolecularly, rather than one formed intramolecularly, so that it would be possible to examine separately the single strand, duplex, and triplex under the same conditions of temperature and pH. The 7-^{15}N-labeled deoxyguanosine and deoxyadenosine were prepared as previously reported,[26] and were incorporated at the indicated sites in two separate preparations of the purine strand (**2**). All strands were synthesized using H-phosphonate chemistry, at a 35 μmol scale, as described elsewhere.[27]

The temperature dependence of the [7-^{15}N] chemical shifts of the adenine- and guanine-labeled single strands, duplexes, and triplexes in 0.1 M NaCl at pH 7 is shown in Figure 2. The single strands and the duplexes each show only a single resonance. The triplex samples, in contrast, show two resonances at each intermediate temperature, one identical to that of the duplex and another significantly upfield, which we assign to the triplex. The duplex−single strand equilibrium generally does not evidence slow exchange, while slow exchange is common for triplexes.[17] At lower temperature or lower pH the intensity of the upfield (triplex) resonance is increased, while at higher temperature or higher pH it is decreased (Tables 1 and 2). This behavior, together with the upfield chemical shift of these purine [7-^{15}N] labels, is consistent with triplex formation involving the C^+·GC and T·AT triplets shown in Figure 1.

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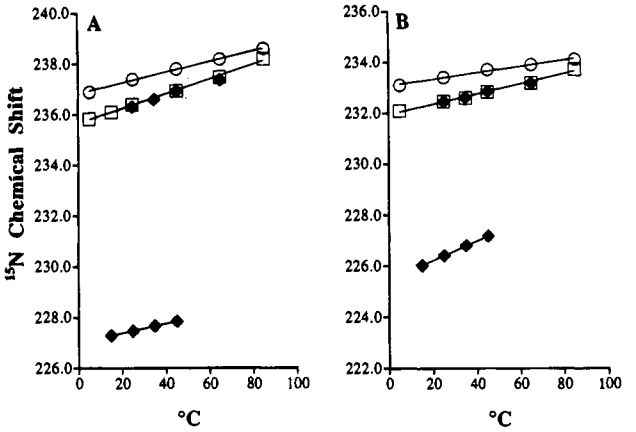

Figure 2. Plots of the guanine (A) and adenine (B) ^{15}N7 resonances as a function of temperature, where ○ represents the single strand samples, □ represents the duplex samples, and ♦ represents the triplex samples. Chemical shifts are reported relative to NH_3, using external 1 M [^{15}N]HNO_3 in 90% D_2O at 25 °C at 375.8 ppm as a reference.[30] The guanine-labeled sample was 4.0 mM in each strand and the adenine-labeled sample was 3.0 mM, both in 0.1 M NaCl, 10 mM phosphate, and 1 mM EDTA in D_2O at pH 6, 7, or 8. The spectra were acquired at 40.5 MHz on a Varian XL400 using 1D experiments with delays of 1 or 2 s, or (single strand only) using an HSQC experiment at a ^{1}H frequency of 500 MHz with a resolution of 0.6 ppm/point for the ^{15}N dimension as reported previously.[23]

Table 1. Temperature Dependence at pH 7

triplex[a]	1	3/4	1/2	1/4	0
temp, °C	15	25	35	45	65

[a] Approximate fraction of triplex present estimated from the peak integrals. The fraction is the same for each label.

Table 2. pH Dependence at 35 °C

triplex[a]	1	1/2	0
pH	6	7	8

[a] Approximate fraction of triplex present estimated from the peak integrals. The fraction is the same for each label.

The upfield shift of the guanine [7-^{15}N] in this protonated C^+·GC triplet is 9 ppm, while that of the adenine [7-^{15}N] in the neutral T·AT triplet is 6 ppm. The former is the largest chemical shift change that we have seen for any "pairing" interaction. Perhaps the best comparison for the C^+·GC triplet is with the O^6MeG [1-^{15}N] atom in a protonated O^6MeG·C pair (Figure 3).[24] At low pH the O^6MeG·C pair has the O^6MeG N1 hydrogen bonded to a protonated cytosine N3, like the guanine N7 in the C^+·GC triplet, and shows a 7.5-ppm shift. This shift is second only to the 9-ppm shift seen for the C^+·GC example. At neutral pH the O^6MeG N1 is hydrogen bonded to the neutral cytosine amino group, and the shift is only 3 ppm. The 6-ppm shift of the adenine [1-^{15}N] in the T·AT triplet can be compared to this 3-ppm shift for the O^6MeG N1 or to the 3-ppm shift for the adenine N1 in an A·T pair, where the hydrogen donor is also the thymine N3H.[18] The larger shifts seen for these triplets may reflect stacking differences, since these chemical shift changes include contributions from stacking as well as hydrogen bonding.

The relative intensities of the triplex and duplex resonances in the triplex samples as a function of temperature, listed in Table 1, indicate that the melting temperature is in the area of 35 °C. Extrapolation of the T_m using the calorimetrically measured transition enthalpy[25] of this triplex−duplex equilibrium predicts a T_m of 56−58 °C at the high concentrations used in the work described here. The large difference between predicted and observed T_m is consistent with the conclusion that

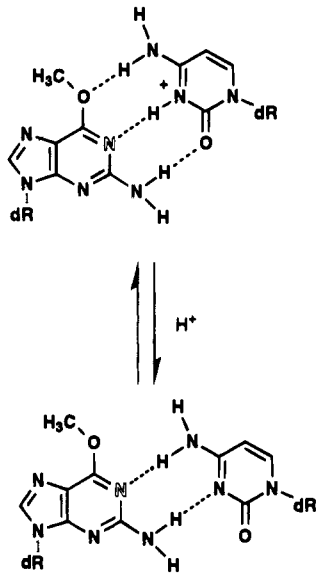

Figure 3. A rotamer of an O⁶MeG·C wobble pair (bottom) and a resonance form of a protonated O⁶MeG•C pair (top), with the [1-^{15}N] label shown in outline.

triplex formation for this system approaches pseudomonomolecular behavior.[25] The relative intensities listed in Table 2 as a function of pH show an apparent pK_a in the area of 7. This value is similar to that found in ^{1}H NMR studies of other triplexes,[14,17] and is significantly higher than the 2′-deoxycytidine monomer pK_a of 4.3.[28]

The results reported above demonstrate that [7-^{15}N] labels are useful probes of Hoogsteen hydrogen bonding, and further demonstrate that the sizes of the chemical shift changes are correlated, at least to a first approximation, with the strengths of the hydrogen bonds formed between the donor and acceptor groups involved.[29] This approach should be particularly valuable with molecules or complexes that are too large for other methods.

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Supporting Information Available: Tables of the ^{15}N chemical shifts of [7-^{15}N]-dA and dG in **2** for the single strand, duplex, and triplex (1 page). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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