Communication

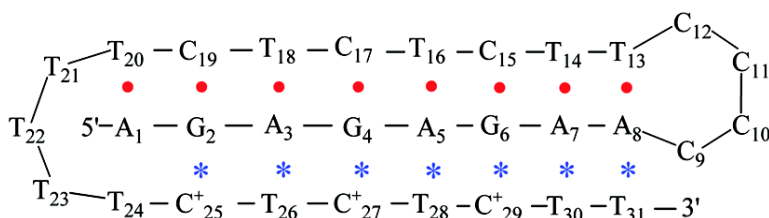# Probing Hydrogen Bonding in a DNA Triple Helix Using Protium–Deuterium Fractionation Factors

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Probing Hydrogen Bonding in a DNA Triple Helix Using Protium−Deuterium Fractionation Factors

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Protium−deuterium fractionation factors (ϕ) have been extensively used to examine hydrogen bonding in proteins. The interest in these studies has been the information on strength and geometry of individual hydrogen bonds, potentially available from ϕ values. In proteins, as in small molecules, correlations have been observed between ϕ values and hydrogen bond length.[1,2] Generally, stronger hydrogen bonds are associated with fractionation factors lower than unity. Unusually low ϕ values have been observed in several enzymes, suggesting that the corresponding short, strong hydrogen bonds play mechanistic roles in enzyme catalysis.[2−4] Fractionation factors have also been used to probe the role of hydrogen bonds in protein stability, folding, and function.[2,4−10] Similar characterizations of hydrogen bonds in nucleic acids have not yet been obtained.

In the present work we report protium−deuterium fractionation factors for the intramolecular DNA triple helix shown in Figure 1.

This molecule was chosen for two reasons. First, the DNA triple-helical structure allows comparison of Watson−Crick hydrogen bonds and Hoogsteen hydrogen bonds. Second, in the DNA triple helix investigated, the short three-base loop T_{21} to T_{23}, which connects the two pyrimidine "strands", imposes conformational strain on the structure. As a result, the first base in the third "strand" (i.e., T_{24}) does not form a triad with the Watson−Crick base pair A_1T_{20}.[12,13] Moreover, previous investigations from this laboratory using hydrogen exchange have shown that the presence of the short loop affects the free energy of stabilization of triads located at the 5'-end of the structure (e.g., $C_{25}^+ \cdot G_2C_{19}$ and $T_{26} \cdot A_3T_{18}$).[11,14]

The fractionation factors were measured for the imino protons that participate in Watson−Crick and Hoogsteen hydrogen bonds. The resonances of these protons are shown in Figure 2. Except for the resonances of the three guanines, all the other imino proton resonances are well resolved, thus allowing determination of the fractionation factor for each proton.

The protium−deuterium fractionation factor, ϕ, for an imino proton NH is defined as the equilibrium constant for the reaction:

$$NH + D \rightleftharpoons ND + H$$
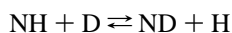
where D and H represent bulk deuterium and protium, respectively, in the solvent. Accordingly, the dependence of the intensity of an imino proton resonance (I) on the concentrations of D and H in solvents of various H_2O/D_2O compositions is:[16,17]

$$\frac{1}{I} = \frac{1}{I_0} + \frac{\phi}{I_0} \cdot \frac{[D]_{solvent}}{[H]_{solvent}} \qquad (1)$$

where I_0 is the intensity of the resonance in a solvent containing 100% H_2O. Representative examples of this dependence are shown in Figure 3.

The fractionation factors for the imino protons in the DNA triple helix are summarized in Figure 4. The values are equal to or lower than unity, and they range from 0.52 ± 0.02 to 1.04 ± 0.04.
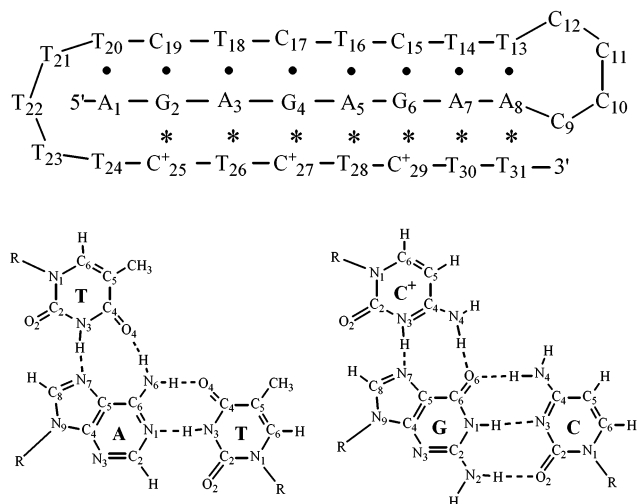

Figure 1. DNA triple helix investigated and the structures of its canonical T·AT and C$^+$·GC triads. In the triple helix structure, dots and asterisks indicate Watson−Crick and Hoogsteen hydrogen bonds, respectively. The DNA oligonucleotide was synthesized using phosphoramidite chemistry on an automated DNA synthesizer and was purified by reverse-phase HPLC as previously described.[11]
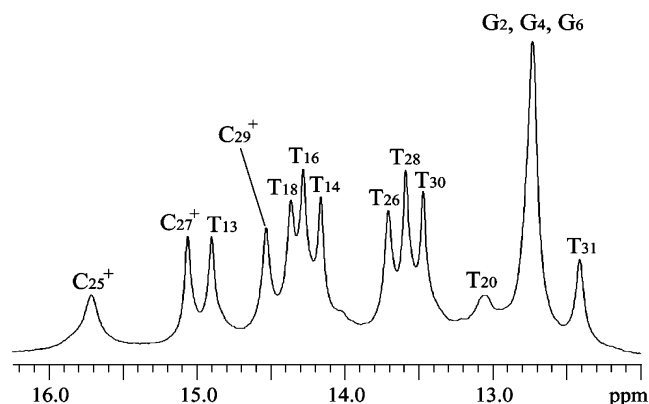

Figure 2. Imino proton resonance region of the NMR spectrum of the DNA triple helix investigated in 100 mM NaCl, 5 mM $MgCl_2$, and 100 mM acetate buffer, at pH 5.0 and at 10 °C. The spectrum was recorded on a Varian NMR spectrometer operating at 9.4 T using the Jump-and-Return pulse sequence.[15] The relaxation delay between successive scans was 8 s. The assignments of resonances to individual imino protons have been obtained by Feigon and co-workers.[12]

Therefore, relative to the isotopic distribution in the solvent, the imino hydrogen bonds in the triple helix have a preference for protium over deuterium. This differs from patterns normally found in proteins where fractionation factors of amide protons span wider ranges, extending to values greater than unity. For example, ϕ values range from 0.3 to 1.5 in staphylococcal nuclease[4] and from 0.29 to 1.52 in ubiquitin[8] and are all greater than 1 in *Gallus gallus* src
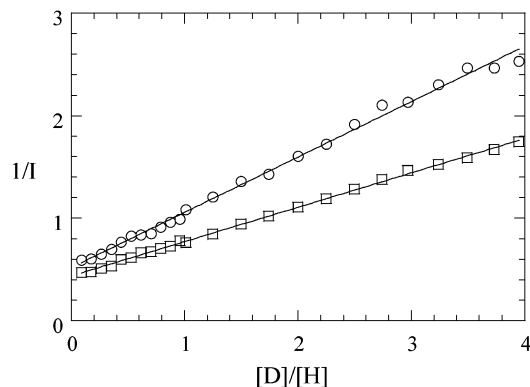
Figure 3. Selected examples of the determination of fractionation factors from the dependence of the intensity of imino proton resonances on [D]/[H] ratio. Squares: T_{16} imino proton ($\phi = 0.77 \pm 0.01$). Circles: T_{26} imino proton ($\phi = 1.04 \pm 0.04$). The lines correspond to linear least-squares fits to eq 1. Various [D]/[H] ratios were obtained by mixing appropriate amounts of two samples in the same buffer and of the same DNA concentration (1.45 mM), one in H_2O and the other in D_2O. The H concentration in the D_2O sample was determined in a separate experiment from the intensity of the residual water proton resonance. The rates of solvent exchange of imino protons in the DNA triple helix have been measured previously by this laboratory, and they range from 1×10^{-4} to 42 s^{-1}, at 10 °C.[11] Accordingly, an interval of at least 7 h was allowed for H/D equilibration following mixing of the two DNA samples. The intensities of imino proton resonances in each spectrum were normalized to the intensity of the thymine methyl resonance at 1.44 ppm (not shown).

SH3 domain.[8] In the DNA triple helix investigated most fractionation factors are between 0.6 and 0.8, and within this range, no significant differences are observed between Hoogsteen and Watson−Crick hydrogen bonds (Figure 4). The exceptions to this trend are the bases located in close proximity to the three-thymine loop connecting the two pyrimidine "strands" (Figure 1). The fractionation factor for the hydrogen bond in A_1T_{20} ($\phi = 0.52 \pm 0.02$) is the lowest among Watson−Crick base pairs. As explained above, this base pair differs from the other Watson−Crick AT base pairs in that it is not part of a T·AT triad. The implication of these results is that binding of a third strand to double-helical DNA perturbs the geometry of the imino Watson−Crick hydrogen bond in the AT base pair (e.g., increases the N···N distance) such that the fractionation factor is increased. Fractionation factors higher than the average ($\phi > 0.8$) are observed for the Hoogsteen hydrogen bonds in the two triads adjacent to the A_1T_{20} base pair (namely, $\phi = 0.88 \pm 0.02$ for C_{25}^+ and $\phi = 1.04 \pm 0.04$ for T_{26}). These higher ϕ values most likely reflect the weakening of the corresponding Hoogsteen hydrogen bonds in the absence of the first T·AT triad.
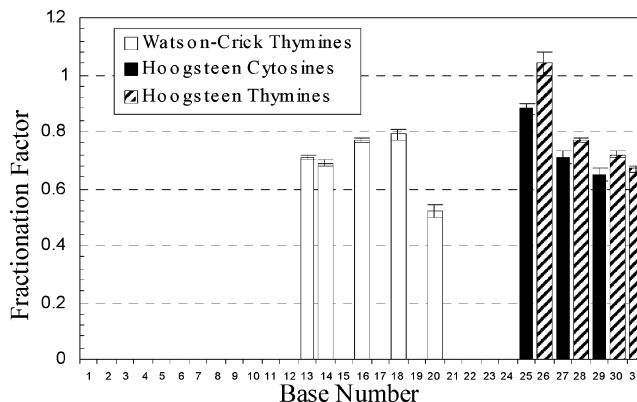

Figure 4. Protium−deuterium fractionation factors for the imino protons in the DNA triple helix investigated in 100 mM NaCl, 5 mM $MgCl_2$, 100 mM acetate buffer at pH 5.0 and at 10 °C. For the imino protons of the three guanines, a fractionation factor of 0.79 ± 0.01 was obtained from their overlapped resonances.

The experimental observations reported in this communication indicate that fractionation factors of imino protons in triple-helical DNA can identify regions of the molecule in which individual hydrogen bonds are perturbed. Correlation between this information and the energetics of the structure awaits future investigations of fractionation factors in other nucleic acid molecules.

References

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