Hydrogen Exchange Rates of the DNA Oligomer Duplex, d[CGCGCGCCATAAC]/d[GTTATGGCGCGCG] Determined by NMR Spectroscopy

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The measurement of hydrogen exchange rates has been an important tool for studying the macromolecular conformation or dynamics.[1,2] The imino proton resonances of nucleic acids are good probes of the hydrogen exchange study for studying the dynamics of each base pair.[1,2] The exchange rates of imino protons depend on not only the base pair stability but also solvent accessibility.[2] Hydrogen-deuterium (H/D) exchange method is widely used to measure the exchange rates of slowly exchanging imino protons, whereas the exchange rates of fast-exchanging imino protons can be determined by water magnetization transfer method of NMR.[1,2]

NMR hydrogen exchange experiment can provide the information on the thermodynamics and kinetics for base-pair opening in the nucleic acids.[2] Lots of results of these experiments determined in various DNAs,[3-6] RNAs[6,7] and DNA-protein complexes[8,9] were reported. Analysis of the hydrogen exchange of imino protons generally was based in the two-state (open/closed) model for the base pair where hydrogen exchange only occurs from the open state.[1,2,10] The opening/closing rate constants and/or equilibrium constant for base-pair opening can often be determined by measuring the exchange as a function of the concentration of external catalysts such as ammonia or TRIS.

Left-handed Z-DNA is a higher-energy form of the double helix produced at an alternating CG sequence region and is stabilized by high salt concentration or negative super-coiling.[11] Recently, X-ray crystal structure of the B-Z junction induced by the Z-DNA binding protein, where the alternating CGCGCG exhibited left-handed Z-DNA whereas a non-alternating AT-rich region maintained B-form helix, was reported.[12] They also found the continuous stacking of bases between B-DNA and Z-DNA segments with the breaking of one base pair at the junction.[12] This result indicates that the one base pair at the B-Z junction should be destabilized and then its bases might be extruded to allow the B-Z conformational change at the CGCGCG region. Here, we measured the imino proton exchange rates for a bzDNA-13 DNA duplex formed by an 15-nt bzA-15mer, d[GTCGCGCGCCATAAC] and a 13-nt bzB-13mer, d[GTTATGGCGCGCG] (Fig. 1A) by water magnetization transfer method to understand the dynamic character of base pairs.

Experimental Section

DNA oligonucleotides, bzA-15mer and bzB-13mer (Figure 1A) were obtained from Genotech (Daejeon, Korea). Each oligonucleotide was purified and desalted by Sephadex G-25 column. DNA duplex, bzDNA-13, was prepared by dissolving the bzA-15mer and bzB-13mer strands at a 1:1 stoichiometric ratio in an NMR buffer (10 mM sodium phosphate (pH 8.0), 100 mM NaCl in 90%H_2O/10%D_2O). NMR experiments were carried out on a Varian Inova 600-MHz spectrometer (KAIST, Daejeon) equipped with x,y,z-axis pulsed-field gradient triple resonance probe. 1D NMR data were processed and analyzed with the program FELIX (Accelrys) or VNMR J as previously described.[13] 2D NMR data were processed with the program NMRPIPE and analyzed with the program Sparky as previously described.[13,14] The exchange rates of the imino protons were determined as previously described.[15] The apparent longitudinal relaxation rate constants, R_{1a}, of the imino protons were determined by semi-selective inversion recovery 1D NMR experiments.[15] The hydrogen exchange rates of the imino protons were measured by water magnetization transfer experiments.[15] The R_{1w} was determined by water selective inversion recovery experiment.[15]

The imino hydrogen exchange rate constants (k_{ex}) were determined by fitting the data to Eq. (1):

$$\frac{I_o - I(t)}{I_0} = 2\frac{k_{ex}}{(R_{1w} - R_{1a})}(e^{-R_{1a}t} - e^{-R_{1w}t}) \quad (1)$$

where R_{1a} and R_{1w} were the independently measured and are the apparent longitudinal relaxation rates of the imino proton and water, respectively, and I_0 and $I(t)$ are the peak intensities of the imino proton in the water magnetization transfer experiments at times zero and t, respectively.[10]

Results and Discussion

All imino proton resonances from Watson-Crick base pairs of bzDNA-13 were assigned by the strong G-imino to C-amino or T-imino to A-H2 NOE cross peaks in the water-gate NOESY (mixing time: 200 ms) acquired at 15 and 35 °C. Figure 1B shows the temperature dependence of the

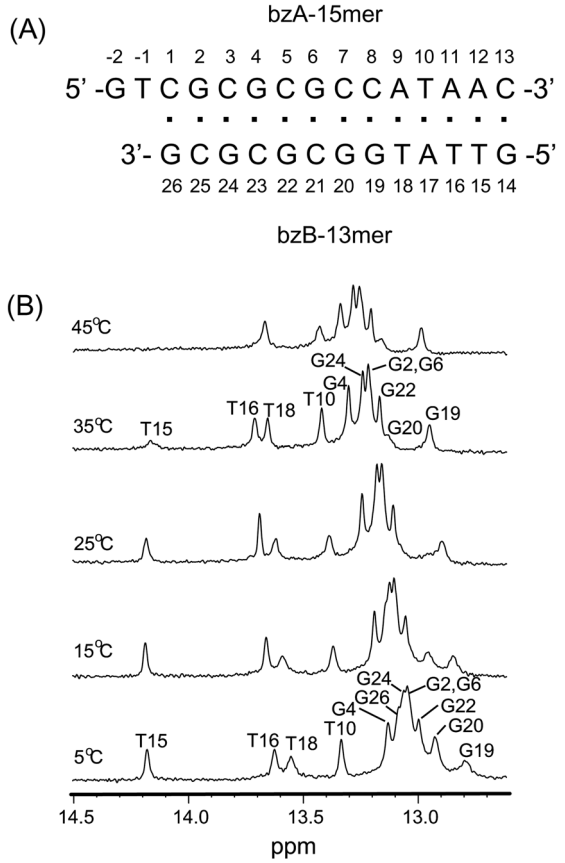

Figure 1. (A) DNA sequence context of the bZDNA-13 duplex. (B) Temperature dependence of the imino proton resonances of the ^{1}H-NMR spectra for the bzDNA-13 duplex in a NMR buffer containing 10 mM sodium phosphate (pH 8.0) and 100 mM NaCl. The experimental temperatures are shown on the left.

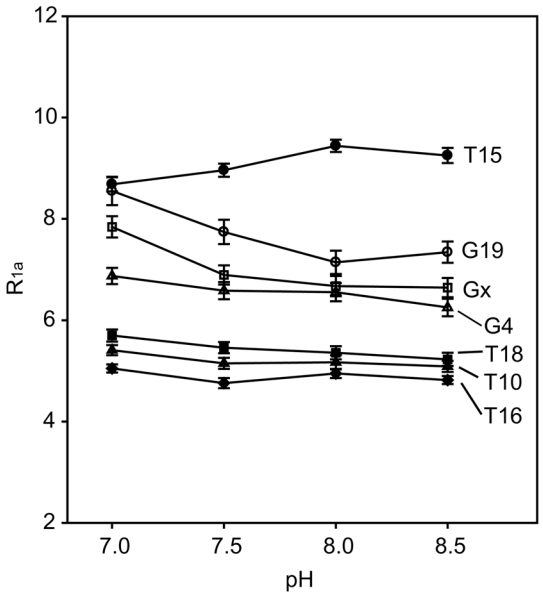

Figure 2. The apparent relaxation rate constants (R1a) (s-1) of the imino proton resonances for the bzDNA-13 duplex as function of pH of the NMR buffer. The pH of all NMR buffer containing 10 mM sodium phosphate, 100 mM NaCl is adjusted by addition of NaOH or HCl.

imino proton spectra of the bzDNA-13. All imino proton resonances except the terminal G14 and G26 are still observed up to 35 °C (Fig. 1B). The T15 imino proton resonance became broadened as temperature was increased and then was disappeared at 45 °C, indicating instability of the A12·T15 base pair (Fig. 1B). Although the T18 imino proton resonance showed significant line-broadening at low temperature, it became sharpened at 35 °C (Fig. 1B). Unusually, the G20 imino proton from central G·C base pair

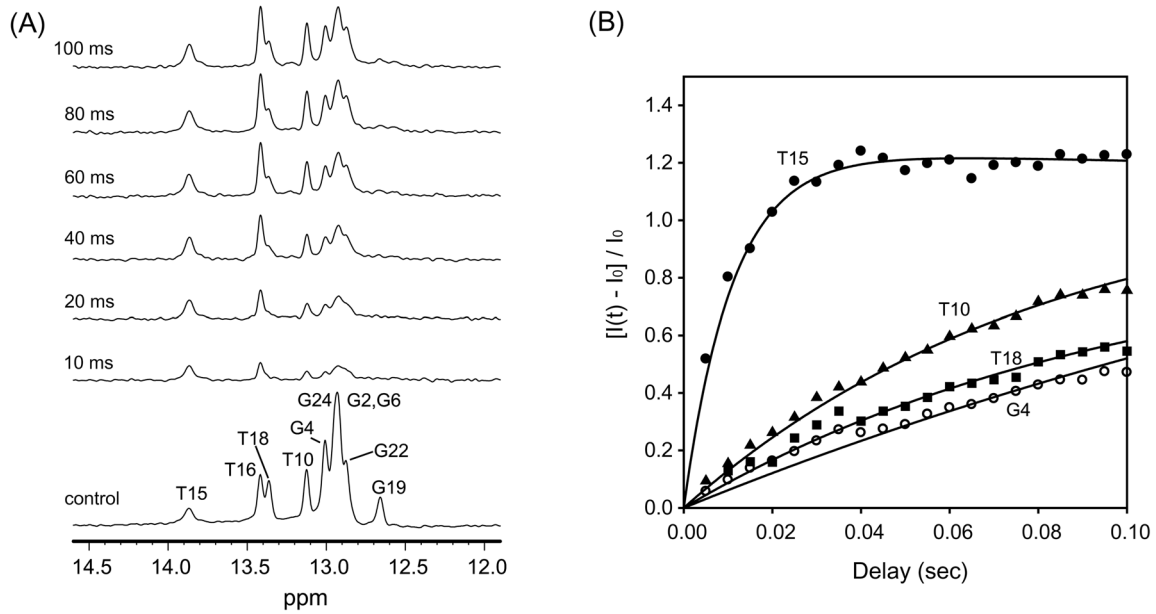

Figure 3. (A) Difference spectra between the control (no selective inversion for water) and exchange after selective inversion in the water magnetization transfer experiment of imino protons of the bzDNA-13 in an NMR buffer at 35 °C. The delay times after selective water inversion were shown on the left of each spectrum. (B) Relative peak intensities, $[I(t)–I_0]/I_0$, in the difference spectra for some imino protons as function of delay time. Solid lines indicate the best fitting of these data using Eq. (1).

had broadened resonance at high temperature compared to other G imino proton resonances. These results may demonstrate that the unusually unstable conformation is formed between the CG alternating region and AT rich region.

The apparent relaxation rate constants, R_{1a}, of the imino protons of bzDNA-13 were determined at 15 °C as function of pH (Fig. 2). The R_{1a} of the imino proton is actually the sum of the spin lattice relaxation rate constant R_1 and k_{ex}.[2] The R_{1a} for each imino proton was determined by curve fitting of the data from the semi-selective inversion recovery experiments on the imino protons to a single exponential function.[15] All imino protons showed no or little change of R_{1a} as the pH varied in the range of 7.5-8.5 (Fig. 2), indicating that the conformational or dynamics changes of the bzDNA-13 did not occurred in this pH range. As the pH increased from 7.5 to 8.5, the concentrations of two base catalysts, OH^- and HPO_4^{2-} are changed 0.32-to-3.2 μM and 6.7-to-9.5 mM, respectively. These results mean that the change of the base catalyst concentrations had little effect on the hydrogen exchange of imino protons under this experimental condition.

The exchange rates of the imino protons for the bzDNA-13 were determined by water magnetization transfer method at 15 °C and 35 °C. Figure 3A shows 1D difference spectra on bzDNA-13 at 35 °C for the water magnetization transfer experiments as a function of delay time between the selective water inversion and detection pulses. The relative peak intensities of the difference spectra for the imino proton resonances of the bzDNA-13 at 35 °C are plotted as a function of delay time in Figure 3B. The k_{ex} values of the imino protons were determined by the curve fitting of the data in Figure 3B to Eq. (1). The k_{ex} values for the bzDNA13 at 15 and 35 °C are given in Table 1. At 15 °C, most imino protons had the k_{ex} in the range of 1-1.6 s^{-1}, whereas the G20 and T15 imino protons were rapidly exchanged ($k_{ex} \geq 3$ s^{-1}). However, the significant difference was observed in the k_{ex} of the imino protons of the bzDNA-13 determined at 35 °C. The G imino protons except G20 and terminal G imino protons had smaller k_{ex} than the T imino protons, indicating a higher stability for the G·C base pairs. In the crystal structure of the complex of the Z-DNA binding protein with the B-Z junction, one A·T base pair was very unstable and thus both bases were flipped out.[12] Surprisingly, the T18 from the identical A·T base pair with above has the slowest exchanging imino proton with a factor of 2-14 smaller k_{ex} value than the other T imino protons. This means that this A9·T18 base pair is the most stable in the A·T base pair located at AT-rich region contrast to the complex with Z-DNA binding protein. The G20 imino proton from the C8·G20 base pair, which is located between CGCGCG alternating sequence and AT-rich region (see Fig. 1), showed very broadened peak at high temperature (Fig. 1B) and also had a larger k_{ex} than other G imino protons at 15 °C (Table 1). This result indicates the instability of the C8·G20 base pair which may allow the B-Z conformational change of the CGCGCG sequence by binding to protein with maintaining the B-form helix of the AT-rich region. However, the C8·G20 base pair might become stable after binding to proteins. Instead, two bases of the A9·T18 base pair were flipped out and then this unusual structure may allow forming the B-Z junction.

Table 1. Exchange rates (s^{-1}) of the imino protons for bzDNA-13 in 90%H_2O/10%D_2O NMR buffer containing 10 mM sodium phosphate (pH 8.0), 100 mM NaCl at 15 and 35 °C determined by water magnetization transfer method

Base pair	Imino proton	15 °C	35 °C
G2·C25	G2	1.6 ± 0.5[a]	3.3 ± 0.6[a]
C3·G24	G24		
C5·G22	G22		
G6·C21	G6		
G4·C23	G4	1.4 ± 0.5	3.2 ± 0.7
C7·G20	G20	3.0 ± 1.8	n.d.[b]
C8·G19	G19	1.0 ± 0.9	3.5 ± 1.4
A9·T18	T18	1.2 ± 0.7	4.6 ± 0.7
T10·A17	T10	1.2 ± 0.5	6.9 ± 0.5
A11·T16	T16	1.2 ± 0.6	15.3 ± 1.2
A12·T15	T15	4.9 ± 0.4	56.0 ± 3.0

[a]These four imino proton resonances were overlapped. [b]Not determined.

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