J|A|C|S
ARTICLES

Published on Web 02/23/2009

Hydration of Watson−Crick Base Pairs and Dehydration of Hoogsteen Base Pairs Inducing Structural Polymorphism under Molecular Crowding Conditions

Daisuke Miyoshi,[†] Kaori Nakamura,[‡] Hisae Tateishi-Karimata,[†,‡] Tatsuo Ohmichi,[†] and Naoki Sugimoto*[,†,‡]

Frontier Institute for Biomolecular Engineering Research (FIBER) and Department of Chemistry, Faculty of Science and Engineering, Konan University, 8-9-1 Okamoto, Higashinada-ku, Kobe 658-8501, Japan

Received July 30, 2008; E-mail: sugimoto@konan-u.ac.jp

Abstract: It has been revealed recently that molecular crowding, which is one of the largest differences between in vivo and in vitro conditions, is a critical factor determining the structure, stability, and function of nucleic acids. However, the effects of molecular crowding on Watson−Crick and Hoogsteen base pairs remain unclear. In order to investigate directly and quantitatively the molecular crowding effects on base pair types in nucleic acids, we designed intramolecular parallel- and antiparallel-stranded DNA duplexes consisting of Hoogsteen and Watson−Crick base pairs, respectively, as well as an intramolecular parallel-stranded triplex containing both types of base pairs. Thermodynamic analyses demonstrated that the values of free energy change at 25 °C for Hoogsteen base-pair formations decreased from $+1.45 \pm 0.15$ to $+1.09 \pm 0.13$ kcal mol^{-1}, and from -1.89 ± 0.13 to -2.71 ± 0.11 kcal mol^{-1} in the intramolecular duplex and triplex, respectively, when the concentration of PEG 200 (polyethylene glycol with average molecular weight 200) increased from 0 to 20 wt %. However, corresponding values for Watson−Crick formation in the duplex and triplex increased from -10.2 ± 0.2 to -8.7 ± 0.1 kcal mol^{-1}, and from -10.8 ± 0.2 to -9.2 ± 0.2 kcal mol^{-1}, respectively. Furthermore, it was revealed that the opposing effects of molecular crowding on the Hoogsteen and Watson−Crick base pairs were due to different behaviors of water molecules binding to the DNA strands.

Introduction

The canonical structure of DNA is the B-form duplex with Watson−Crick base pairs.[1] However, some DNAs are prone to structural polymorphism, forming triplexes and quadruplexes with not only Watson−Crick but also non-Watson−Crick base pairs such as Hoogsteen base pairs.[2] The structural polymorphism of nucleic acids, which can be specific and important signals in nucleic acid−nucleic acid, nucleic acid−protein, and nucleic acid−drug interactions, is influenced not only by their sequence but also by their surroundings.[3] One of the most drastic differences between the surrounding conditions in vivo and in vitro is the concentration of biomacromolecules, which can occupy a significant fraction (20−40%) of the cellular volume.[4−6] Thus, biochemical reactions in vivo and in vitro progress under molecularly crowded and dilute conditions, respectively. It is well-known that molecular crowding is a critical factor determining the structure, stability, and function of a variety of proteins.[4−7] In addition, molecular crowding is recognized as one of the most important factors, physical or chemical, contributing to the compartmentation of the earliest eukaryote cells[8] and to responses to transient stretch in living cells.[9] Moreover, the effects of molecular crowding on the structure and stability of DNA is currently of great interest[10−14] in the quest to understand physiology and metabolism in vivo. We reported previously that a short antiparallel-stranded duplex formation via Watson−Crick base pairs was destabilized under molecular crowding conditions induced by high concentrations of co-solutes, whereas an antiparallel G-quadruplex formation with Hoogsteen base pairs was stabilized.[15] We also found that

[†] Frontier Institute for Biomolecular Engineering Research (FIBER).
[‡] Department of Chemistry, Faculty of Science and Engineering.

(1) Watson, J. D.; Crick, F. H. C. *Nature* **1953**, *171*, 737–738.
(2) Turner, D. H. *Conformational Changes*; Bloomfield, V. A., Crothers, D. M., Tinoco, I., Jr., Eds.; University Science Books: Mill Valley, CA, 2000; pp 259–334.
(3) Miyoshi, D.; Sugimoto, N. *Recent Dev. Nucleic Acids Res.* **2006**, *2*, 49–70.
(4) Zimmerman, S. B.; Minton, A. P. *Annu. Rev. Biophys. Biomol. Struct.* **1993**, *22*, 27–65.
(5) Minton, A. P. *J. Biol. Chem.* **2001**, *276*, 10577–10580.
(6) Ellis, R. J.; Minton, A. P. *Nature* **2003**, *425*, 27–28.
(7) Sasaki, Y.; Miyoshi, D.; Sugimoto, N. *Nucleic Acids Res.* **2007**, *35*, 4086–4093.
(8) Kurland, C. G.; Collins, L. J.; Penny, D. *Science* **2006**, *312*, 1011–1014.
(9) Trepat, X.; Deng, L.; An, S. S.; Navajas, D.; Tschumperlin, D. J.; Gerthoffer, W. T.; Butler, J. P.; Fredberg, J. J. *Nature* **2007**, *447*, 592–595.
(10) Goobes, R.; Kahana, N.; Cohen, O.; Minsky, A. *Biochemistry* **2003**, *42*, 2431–2440.
(11) Miyoshi, D.; Matsumura, S.; Nakano, S.; Sugimoto, N. *J. Am. Chem. Soc.* **2004**, *126*, 165–169.
(12) Li, J.; Correia, J. J.; Wang, L.; Trent, J. O.; Chaires, J. B. *Nucleic Acids Res.* **2005**, *33*, 4649–4659.
(13) Miyoshi, D.; Karimata, H.; Sugimoto, N. *Angew. Chem., Int. Ed.* **2005**, *24*, 3740–3744.
(14) Kan, Z. Y.; Lin, Y.; Wang, F.; Zhuang, X. Y.; Zhao, Y.; Pang, D. W.; Hao, Y. H.; Tan, Z. *Nucleic Acids Res.* **2007**, *35*, 3646–3653.

10.1021/ja805972a CCC: $40.75

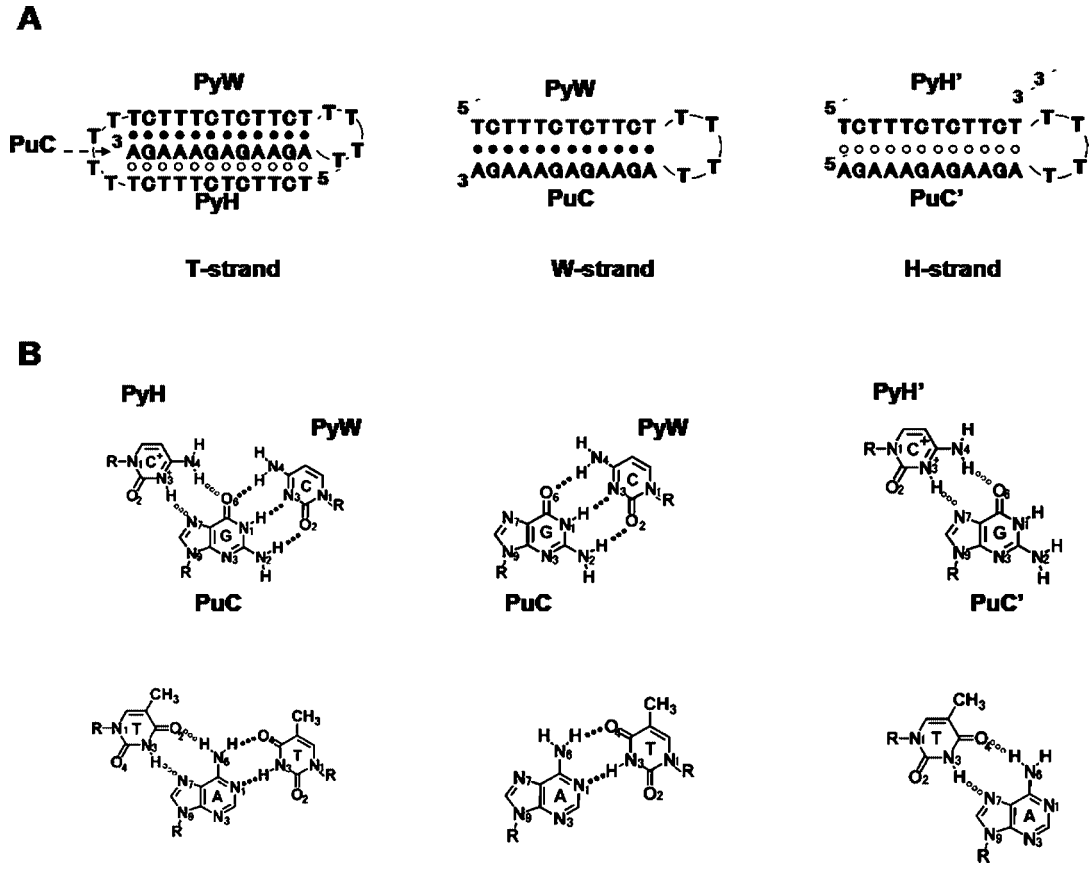

Figure 1. (A) Sequences and schematic structures of T-strand, W-strand, and H-strand. (B) Chemical structures of base pairs formed in the parallel-stranded triplex, antiparallel-stranded duplex, and parallel-stranded duplex. Filled and open circles indicate Watson−Crick and Hoogsteen base pairs, respectively.

molecular crowding of co-solutes affected the thermodynamics of DNA structure formation by altering the DNA hydration.[15,16] These results are consistent with the molecular crowding effects on DNA triplexes reported by Spink and Chaires and by Goobes and Minsky.[17,18] These groups also demonstrated that DNA triplexes consisting of Hoogsteen base pairs were stabilized under molecular crowding conditions, and that the thermodynamics of formation of DNA duplexes and triplexes were regulated by DNA hydration. These and our previous studies suggested that noncanonical DNA structures consisting of Hoogsteen base pairs were more favorable than the canonical DNA duplex under molecular crowding conditions. However, the structures and sequences used in these studies were different from each other, making direct comparison of results difficult. Therefore, the effects of molecular crowding on Watson−Crick and Hoogsteen base pairs remain unclear.

In order to investigate directly and quantitatively the effects of molecular crowding on Watson−Crick and Hoogsteen base pairs, we designed and synthesized intramolecular antiparallel-stranded and parallel-stranded hairpin loop DNAs composed of Watson−Crick base pairs and Hoogsteen base pairs, respectively, as shown in Figure 1. The sequences of these two DNAs are identical, although the parallel structure was made by conjugating the 3′ ends of two DNA strands. As a reference, we also designed an intramolecular parallel-stranded triplex involving the same sets of Watson−Crick base pairs as the antiparallel-stranded hairpin loop DNA and the Hoogsteen base pairs of the parallel-stranded hairpin loop DNA (Figure 1). Structural and thermodynamic analyses demonstrated that Hoogsteen and Watson−Crick formations in the DNA structures were stabilized and destabilized, respectively, by molecular crowding with PEG 200 [polyethylene glycol with average molecular weight (MW) 200]. Moreover, the relationship between an observed equilibrium constant for the DNA structure formation and water activity revealed dehydration of the Hoogsteen base-pair formation (releasing water molecules upon structure formation) and hydration of the Watson−Crick base formation (taking up water molecules upon structure formation) in both triplex and duplex structures. These behaviors were also confirmed by molecular dynamics studies of these DNA structures with water molecules.

The effects of molecular crowding with various co-solutes such as ethylene glycol (MW 62), 1,3-propanediol (MW 76), 2-methoxyethanol (MW 76), 1,2-dimethoxyethane (MW 90), and glycerol (MW 92), as well as PEG 200, indicated that the stabilization and destabilization of the DNA structures were largely affected by the nature of the co-solutes. Therefore, it appears that molecular crowding conditions, wherein water activity decreases and hydration is unfavorable, stabilize DNA structures formed by Hoogsteen base pairs and destabilize those formed by Watson−Crick base pairs (depending on the co-solute properties) leading to structural polymorphism of various DNA

(15) Miyoshi, D.; Karimata, H.; Sugimoto, N. *J. Am. Chem. Soc.* **2006**, *128*, 7957–7963.
(16) Nakano, S.; Karimata, H.; Ohmichi, T.; Kawakami, J.; Sugimoto, N. *J. Am. Chem. Soc.* **2004**, *126*, 14330–14331.
(17) Spink, C. H.; Chaires, J. B. *J. Am. Chem. Soc.* **1995**, *117*, 12887–12888.
(18) Goobes, R.; Minsky, A. *J. Am. Chem. Soc.* **2001**, *123*, 12692–12693.

sequences under the molecular crowding conditions that mimic conditions found in the cell.

Materials and Methods

Preparation of Oligonucleotides and Buffer Solutions. The high performance liquid chromatography (HPLC) purification grade DNA oligonucleotides (Figure 1), W-strand [5′-TCTTTCTCT-TCTTTTTAGAAGAGAAAGA-3′ (loop region underlined)] and T-strand [5′-TCTTCTCTTTCTTTTTCTTTCTCTTCTTTTTAG-AAGAGAAAGA-3′ (loop region underlined)] were purchased from Hokkaido System Science (Sapporo, Japan). The HPLC purification grade DNA oligonucleotide, H-strand [5′-TCTTTCTCTTCT-3′/3′-TTTTAGAAGAGAAAGA-5′ (loop region underlined)] was purchased from Tsukuba Oligo service (Tsukuba, Japan). Single-strand concentrations of the DNA oligonucleotides were determined by measuring the absorbance at 260 nm at a high temperature using a Shimadzu 1700 spectrophotometer (Shimadzu, Kyoto, Japan) connected to a thermoprogrammer. Single-strand extinction coefficients were calculated from mononucleotide and dinucleotide data using the nearest-neighbor approximation.[19]

Water Activity Measurements. The water activity was determined by the osmotic stressing method via vapor phase osmometry using a model 5520XR pressure osmometer (Wescor, Utah, U,S,A,) or by freezing point depression osmometry using a Typ Dig L osmometer (KNAIER, Berlin, Germany). In order to calculate the water activity with various concentrations of co-solutes, we assumed that co-solutes did not interact directly with DNA.[15,16]

Circular Dichroism (CD) Measurements. CD experiments utilizing a JASCO J-820 spectropolarimeter (JASCO, Hachioji, Japan) were done at 4 °C in a 1.0-cm path length cuvette for 5 μM total strand concentration of DNA in buffer [NaCl (100 mM), Na_2EDTA (1 mM) and Na_2HPO_4 (10 mM; pH 7.0) or Tris-acetate (50 mM; pH 5.0)] containing various concentrations of co-solutes. The CD spectrum was the average of at least three scans from 200 to 350 nm. The temperature of the cell holder was regulated by a JASCO PTC-348 temperature controller, and the cuvette-holding chamber was flushed with a constant stream of dry N_2 gas to avoid condensation of water on the cuvette exterior. Before measurement, the sample was heated to 80 °C, cooled at a rate of 1 °C min^{-1}, and incubated at 4 °C for 1 h.

Gel Electrophoresis. Nondenaturing gel electrophoresis was carried out with 20% polyacrylamide. Ice-cold loading buffer (5 μL) was mixed with DNA sample (3 μL, 5 μM) in a buffer [NaCl (100 mM), Na_2EDTA (1 mM), and Na_2HPO_4 (10 mM; pH 7.0)] containing various concentrations of co-solutes. A 3 μL aliquot of the mixed solution was loaded and analyzed by electrophoresis at 5 V cm^{-1} for 5 h at 4 °C. The gel was stained using GelStar and imaged using an FLA-5100 (Fujifilm Co., Ltd., Tokyo, Japan). Before measurement, the sample was heated to 80 °C, cooled at a rate of 1 °C min^{-1}, and incubated at 4 °C for 1 h.

Thermodynamic Analysis. UV absorbance was measured with a Shimadzu 1700 spectrophotometer (Shimadzu) equipped with a temperature controller. Melting curves of DNA structures were obtained by measuring the UV absorbance at 260 or 295 nm in a buffer [NaCl (100 mM), Na_2EDTA (1 mM), and Na_2HPO_4 (10 mM; pH 7.0)] containing various concentrations of co-solutes. The T_m values for 5 μM DNA structures were obtained from the UV melting curves as described previously.[15] The heating rate was 0.2 °C min^{-1} for the T- and H-strands, and 0.5 °C min^{-1} for the W-strand. The thermodynamic parameters (enthalpy change $\Delta H°$, entropy change $\Delta S°$, and free energy change $\Delta G°$) were calculated from the fit of the melting curves to a theoretical equation for an intramolecular association as described previously.[15] Before measurement, the sample was heated to 80 °C, cooled at a rate of 1 °C min^{-1}, and incubated at 0 °C for 1 h.

Molecular Dynamics. The Insight II software package (BIOSYM/Molecular Simulations, San Diego, CA, USA) was used for model building, energy minimization, and molecular dynamics of DNA structures. Energy minimization and molecular dynamics were performed with the program CHARMM with the force fields CHARMM27 (for the antiparallel-stranded duplex of W-strand) and CHARMM (for the triplex of T-strand and parallel-stranded duplex of H-strand). The antiparallel-stranded duplex of W-strand and the triplex of T-strand were built up using a triplex structure in BIOPOLYMER module. The parallel-stranded duplex of H-strand was built by removing the third (PyW) strand from the triplex structure (see Figure 1 for the sequence and schematic structure). The atomic charges were 1.00 on H4 of the cytosine of the triplex and parallel-stranded duplex. The structure configurations were solvated in a water box of size 60 Å × 60 Å × 60 $Å^3$, and the transferable intermolecular potentials with three point charges (TIP3P) water method was used for the simulation. For the van der Waals interactions, a 10 Å cutoff was used. The solved DNA systems were prepared for molecular dynamics simulations by applying 10000 steps of energy minimization using the steepest descent method. The simulation used the NVT ensemble (i.e., the number of particles N, the volume V, and the temperature T of the system are kept constant) at 300 K. A time step of 1.0 fs was used for all simulations. The dielectric constant was set to unity as is usually dome in conventional molecular dynamics simulation of biomolecular system.[20] We did not add ions in the system. The estimated numbers of water molecules shown in this manuscript are the numbers of water molecules directly binding to the DNA strands via hydrogen bond(s). The numbers of water molecules directly binding to the DNA strands were counted manually.

Results

Design of Intramolecular Duplex and Triplex DNAs with Watson−Crick and Hoogsteen Base Pairs. The main purpose of this study was to investigate directly and quantitatively the effects of molecular crowding on both Watson−Crick and Hoogsteen base pairs. In order to allow direct comparisons, the sequence and structure of the oligonucleotides with Watson−Crick or Hoogsteen base pairs should be designed to be the same. We found previously that a 1:1:1 mixture of three oligonucleotides, 5′-TCTTTCTCTTCT-3′ (PyW), 5′-AGAAGAGAAAGA-3′ (PuC), and 5′-TCTTCTCTTTCT-3′ (PyH), folded into a parallel-stranded triplex at pH 7.0.[21] We designed an intramolecular parallel-stranded triplex containing both Watson−Crick and Hoogsteen base pairs: three strands were connected by two loops (see Figure 1A for the sequence and structure). This oligonucleotide (T-strand) triplex folds with Watson−Crick base pairs between PyW and PuC and with Hoogsteen base pairs between PuC and PyH (see Figure 1B for the base-pair formation) An oligonucleotide that can fold into an intramolecular antiparallel-stranded hairpin loop with Watson−Crick base pairs (W-strand) was designed using PuC and PyW (parts A and B of Figures 1). W-strand was made by conjugating the 3′ end of PyW and the 5′ end of PuC, which was the standard linkage of nucleotides. We already reported that W-strand folded into the intramolecular antiparallel-stranded hairpin loop with Watson−Crick base pairs under dilute and molecular crowding conditions, although the buffer and pH used here are different from those in the previous report.[15] Moreover, it was demonstrated that PuC and PyH were able to form a parallel-stranded duplex with fully matched Hoogsteen base pairs or an antiparallel-stranded duplex with two bulges, depending on pH.[21] On

(19) Richards, E. G. Use of tables in calculation of absorption, optical rotatory dispersion and circular dichroism of polyribonucleotides. Fasman, G. D., Ed.; *Handbook of Biochemistry and Molecular Biology*, 3rd ed.; CRC Press: Cleveland, OH, 1975; Vol. 1, pp 596−603.

(20) Ishikata, H.; Knapp, E. *J. Am. Chem. Soc.* **2004**, *126*, 8059–8064.

(21) Sugimoto, N.; Wu, P.; Hara, H.; Kawamoto, Y. *Biochemistry* **2001**, *40*, 9396–9405.

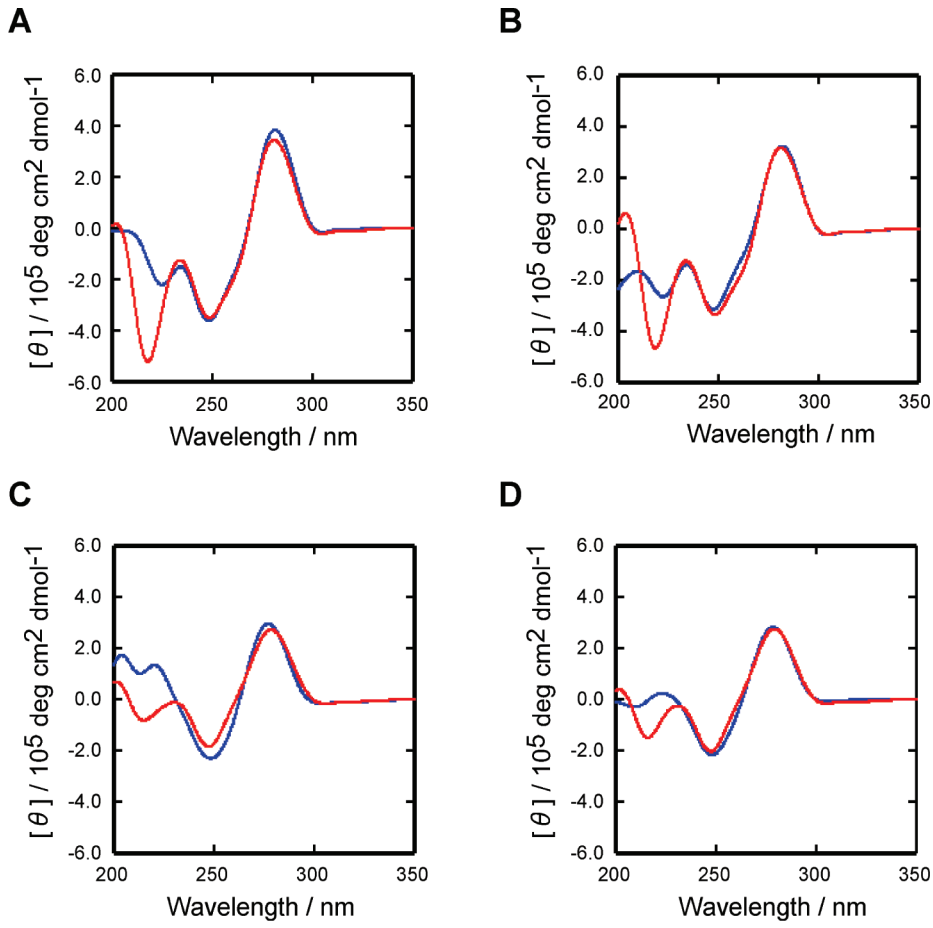

Figure 2. CD spectra in buffers containing NaCl (100 mM), Na_2EDTA (1 mM), and Na_2HPO_4 (10 mM; pH 7.0) (blue) or NaCl (100 mM), Na_2EDTA (1 mM) and Tris-acetate (50 mM; pH 5.0) (red) at 4 °C. (A) 5 μM T-strand without PEG 200; (B) 5 μM T-strand with 20 wt % PEG 200; (C) 5 μM H-strand without PEG 200; (D) 5 μM H-strand with 20 wt % PEG 200.

the basis of these results, we designed an oligonucleotide that formed an intramolecular parallel-stranded hairpin loop with the sequence identical to that of the W-strand but with the different base pair type, that is Hoogsteen base pair (A and B of Figures 1). This oligonucleotide, H-strand, was made by conjugating the 3′ end of PuC′ and the 3′ end of PyH′ sequences which are in the direction opposite that of PuC and PyH, respectively. These sequences are useful to compare the effects of molecular crowding on Watson−Crick and Hoogsteen base pairs in duplex and triplex.

The Molecular Crowding Effects on Structures of T-, W-, and H-Strands. First, we studied the structure of the T-strand using CD spectroscopy. Figure 2A shows CD spectra of 5 μM T-strand at pH 5.0 and pH 7.0. The spectrum at pH 5.0 had a negative peak around 218 nm, which is typical for a parallel-stranded duplex containing Hoogsteen base pairs.[21,22] This negative peak was not observed at pH 7.0, indicating that the Hoogsteen base pairs between PuC and PyH were destabilized at the higher pH. These results are consistent with the CD spectra of an intermolecular triplex DNA formed by the 1:1:1 mixture of PyW, PuC, and PyH.[22] Figure 2B shows equivalent spectra of 5 μM T-strand in buffers containing 20 wt % PEG 200. The spectrum of T-strand at pH 7.0 with 20 wt % PEG 200 is almost identical to that without PEG 200 (Figure 2A), indicating that T-strand was not able to form the stable triplex structure at pH 7.0 in the presence of the PEG 200.

On the basis of the results for T-strand, the structures of H-strand and W-strand were investigated. Under dilute conditions, the CD spectrum of H-strand at pH 5.0 had a large negative peak around 218 nm, the intensity of which decreased (θ value increased) at pH 7.0 (Figure 2C). This phenomenon was also observed in the H-strand spectra under the molecular crowding condition (Figure 2D). These results indicate that H-strand folded into the intramolecular parallel-stranded duplex with Hoogsteen base pairs at pH 5.0, and the duplex was destabilized at pH 7.0, under both dilute and molecular crowding conditions. In addition, CD spectra of W-strand at pH 5.0 and pH 7.0 under both dilute and molecular crowding conditions were typical of a B-form duplex, with a positive peak at around 218 nm (Figure S1, Supporting Information), indicating that W-strand folded into the intramolecular antiparallel duplex with Watson−Crick base pairs.

In order to confirm the structures of H-strand and W-strand, we performed native gel electrophoresis in the presence of 0−20 wt % PEG 200 at pH 7.0 (Figure S2, Supporting Information). A single migration of H-strand and W-strand was observed, and the migration of H-strand was slower than that of W-strand. It has been shown previously that an antiparallel-stranded DNA duplex with Watson−Crick base pairs migrates faster than a

(22) Ohmichi, T.; Kawamoto, Y.; Wu, P.; Miyoshi, D.; Karimata, H.; Sugimoto, N. *Biochemistry* **2005**, *44*, 7125–7130.

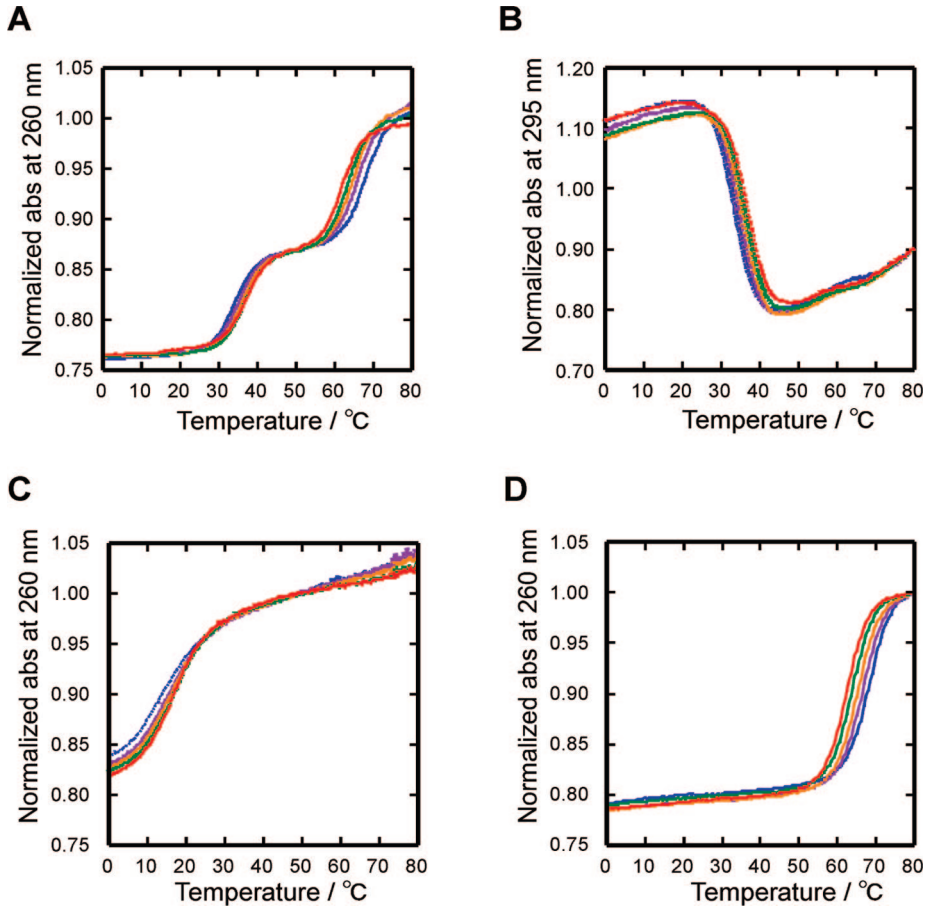

Figure 3. Normalized UV melting curves in buffers containing NaCl (100 mM), Na_2EDTA (1 mM) and Na_2HPO_4 (10 mM; pH 7.0) with 0 (blue), 5 (purple), 10 (orange), 15 (green), and 20 wt % PEG 200 (red). (A) 5 μM T-strand at 260 nm, (B) 5 μM T-strand at 295 nm, (C) 5 μM H-strand at 260 nm, and (D) 5 μM W-strand at 260 nm.

parallel-stranded DNA duplex with Hoogsteen base pairs.[23] Thus, the native gel electrophoresis confirmed that H- and W-strands formed parallel- and antiparallel-stranded duplexes, respectively. Therefore, these H- and W-strands are useful alongside a T-strand to investigate the quantitative effects of molecular crowding with PEG 200 on the thermodynamics of Hoogsteen and Watson−Crick base pairs in the intramolecular duplex and triplex structures.

Stability of Watson−Crick and Hoogsteen Base Pairs under Dilute and Molecular Crowding Conditions. T-strand was utilized to investigate the effects of molecular crowding on the stability of both Watson−Crick and Hoogsteen base pairs in the intramolecular triplex. Figure 3A shows normalized UV melting curves of 5 μM T-strand traced at 260 nm in buffers containing 0, 5, 10, 15, and 20 wt % PEG 200. Two melting transitions were obvious: the melting temperatures (T_m's) of the transitions in the absence of PEG 200 were estimated to be 33.9 $\pm$ 0.2 and 67.7 $\pm$ 0.4 °C, while T_m's in the presence of 20 wt % PEG 200 were estimated to be 37.1 $\pm$ 0.1 and 61.8 $\pm$ 0.1 °C. In order to assign the two melting transitions for Watson−Crick and Hoogsteen base-pair formations, the thermal denaturation was also traced at 295 nm (Figure 3B). A single sigmoidal UV melting curve was observed, which is typical of a DNA structure containing Hoogsteen base pairs.[24] Moreover, T_m's estimated from the UV melting curves at 295 nm with and without 20 wt % PEG 200 were 37.0 $\pm$ 0.4 °C and 34.0 $\pm$ 0.3 °C, respectively, identical to those estimated from the melting curves at 260 nm. Therefore, it is clear that the transition observed at the lower temperature in the UV melting curves at 260 nm (Figure 3A) corresponds to a dissociation of Hoogsteen base pairs in the intramolecular triplex structure, while the higher-temperature transition corresponds to dissociation of Watson−Crick base pairs. Furthermore, these results indicate that the Hoogsteen and Watson−Crick base-pair formations in the triplex are stabilized and destabilized by molecular crowding, respectively, consistent with the previous results.[17,18]

Next, we investigated the stability of these base pairs in the intramolecular duplexes of H- and W-strands, which had identical sequences. Figure 3C shows normalized UV melting curves of H-strand at 260 nm in buffers containing 0−20 wt % PEG 200. T_m was observed to increase from 13.0 to 16.7 °C as the PEG 200 concentration increased from 0 to 20 wt %. Since melting transitions with the same T_m's were observed at 295 nm (Figure S3A, Supporting Information), this transition corresponds to dissociation of Hoogsteen base pairs. However, normalized UV melting curves of W-strand at 260 nm showed that T_m decreased from 67.4 to 62.2 °C (Figure 3D). No melting transition was observed at 295 nm for W-strand (Figure S3B, Supporting Information), indicating that the transition observed at 260 nm corresponds to dissociation of Watson−Crick base pairs. These results demonstrate that Hoogsteen and Watson−Crick base-pair formations in the intramolecular duplex structures are

(23) Liu, K.; Todd Miles, H.; Frazier, J.; Sasisekharan, V. *Biochemistry* **1993**, *32*, 11802–11809.
(24) Mergny, J. L.; Li, J.; Lacroix, L.; Amrane, S.; Chaires, J. B. *Nucleic Acids Res.* **2006**, *33*, e138.

Table 1. Thermodynamic Parameters for Hoogsteen and Watson−Crick Base-Pair Formations of T-Strand[a]

PEG 200 (wt %)	$\Delta H°$ (kcal mol^{-1})	$T\Delta S°$ (kcal mol^{-1})	$\Delta G°_{25}$ (kcal mol^{-1})	T_m (°C)
		Hoogsteen Base-Pair Formation		
0	−65.4 ± 4.3	−63.5 ± 4.2	−1.89 ± 0.13	33.9 ± 0.2
5	−66.2 ± 4.2	−64.1 ± 4.0	−2.11 ± 0.21	34.8 ± 0.5
10	−67.0 ± 4.0	−64.7 ± 4.0	−2.35 ± 0.10	35.8 ± 0.3
15	−68.5 ± 3.7	−65.9 ± 3.5	−2.55 ± 0.13	36.5 ± 0.1
20	−69.6 ± 3.3	−66.9 ± 3.2	−2.71 ± 0.11	37.1 ± 0.1
		Watson−Crick Base-Pair Formation		
0	−86.0 ± 1.5	−75.2 ± 1.3	−10.8 ± 0.2	67.7 ± 0.4
5	−85.7 ± 1.4	−75.3 ± 1.2	−10.4 ± 0.2	65.8 ± 0.3
10	−85.0 ± 1.7	−75.0 ± 1.5	−10.0 ± 0.2	64.7 ± 0.2
15	−84.6 ± 1.7	−75.0 ± 1.6	−9.6 ± 0.2	63.2 ± 0.2
20	−84.1 ± 2.0	−74.8 ± 1.8	−9.2 ± 0.2	61.8 ± 0.1

[a] All experiments were carried out in buffers containing NaCl (100 mM), Na_2HPO_4 (10 mM; pH 7.0), Na_2EDTA (1 mM), and various concentrations of PEG 200. Values are mean ± standard deviation from at least three measurements.

Table 2. Thermodynamic Parameters for the Structural Formations of H- and W-Strands[a]

PEG 200 (wt %)	$\Delta H°$ (kcal mol^{-1})	$T\Delta S°$ (kcal mol^{-1})	$\Delta G°_{25}$ (kcal mol^{-1})	T_m (°C)
		H-strand		
0	−34.4 ± 2.1	−35.8 ± 2.2	1.45 ± 0.15	13.0 ± 0.7
5	−35.2 ± 1.9	−36.6 ± 2.0	1.35 ± 0.16	14.0 ± 1.1
10	−36.1 ± 2.0	−37.3 ± 2.0	1.26 ± 0.14	15.0 ± 1.0
15	−37.0 ± 2.0	−38.1 ± 2.1	1.16 ± 0.14	15.9 ± 0.9
20	−37.9 ± 2.2	−39.0 ± 2.3	1.09 ± 0.13	16.7 ± 0.8
		W-strand		
0	−82.3 ± 3.4	−70.2 ± 3.2	−10.2 ± 0.2	67.4 ± 1.2
5	−81.4 ± 3.1	−69.9 ± 2.9	−9.8 ± 0.2	65.9 ± 1.0
10	−80.5 ± 2.3	−69.8 ± 2.2	−9.5 ± 0.1	64.8 ± 0.7
15	−79.7 ± 2.2	−69.4 ± 2.1	−9.1 ± 0.2	63.6 ± 0.4
20	−78.4 ± 1.1	−69.1 ± 1.0	−8.7 ± 0.1	62.2 ± 0.2

[a] All experiments were carried out in buffers containing NaCl (100 mM), Na_2HPO_4 (10 mM; pH 7.0), Na_2EDTA (1 mM), and various concentrations of PEG 200. Values are mean ± standard deviation from at least three measurements.

stabilized and destabilized, respectively, by the molecular crowding of PEG 200. Combining the results of the duplexes with those of the triplex, it is reasonable to conclude that the effects of molecular crowding on base pairs are qualitatively similar even when the DNA strands have different structures.

Molecular Crowding Effects on the Thermodynamic Parameters of Hoogsteen and Watson−Crick Base-Pair Formations. We further attempted to study quantitatively the effects of molecular crowding on the thermodynamics of Hoogsteen and Watson−Crick base-pair formations. Table 1 shows the values of $\Delta H°$, $T\Delta S°$, $\Delta G°$ at 25 °C ($\Delta G°_{25}$), and T_m for the formation of the Hoogsteen triplex and Watson−Crick base pairs of T-strand with various concentrations of PEG 200. When the PEG 200 concentration increased from 0 to 20 wt %, the values of $\Delta H°$, $T\Delta S°$, and $\Delta G°_{25}$ for formation of the Hoogsteen base pairs decreased, while the values for formation of the Watson−Crick base pairs increased.

The thermodynamic parameters of the Hoogsteen base-pair formations of H-strand and the Watson−Crick base-pair formations of W-strand were also estimated under dilute and molecular crowding conditions and are listed in Table 2. As observed for T-strand, molecular crowding with PEG 200 caused stabilization of Hoogsteen base pairs and destabilization of Watson−Crick base pairs: Thus, molecular crowding stabilizes and destabilizes Hoogsteen and Watson−Crick base pairs, respectively, in both intramolecular triplex and duplex structures. Moreover, the thermodynamic parameters reveal that the stabilization of the Hoogsteen base-pair formation is more significant in the triplex than the duplex, although the destabilization effect of molecular crowding on the Watson−Crick base-pair formation was almost the same in the triplex as in the duplex. The difference between Hoogsteen base pairs in the triplex and duplex will be discussed later.

Behavior of Water Molecules on Formation of Hoogsteen and Watson−Crick Base Pairs. The thermodynamic parameters listed in Tables 1 and 2 show that the stabilization of the Hoogsteen base pairs by molecular crowding is due to the thermodynamically favorable enthalpic change which exceeds the unfavorable entropic change. The destabilization of the Watson−Crick base pairs also derives from the enthalpic change. These thermodynamic behaviors are consistent with previous reports of the effects of molecular crowding on the thermodynamics of the G-quadruplex structure[15] and suggest that hydration of the DNA structures is an important factor for the stability of the Hoogsteen and Watson−Crick base pairs in the triplex and duplex structures; we further investigated how water molecules affected the thermodynamics of these base-pair formations using the osmotic stressing method, as reported previously.[10,15,16] Figure 4A shows the plots of ln K_{obs} (an observed equilibrium constant) vs ln a_w (water activity), determined by osmotic pressure measurements, at 25 °C for formation of the Hoogsteen and Watson−Crick base pairs of T-strand, the Hoogsteen base pairs of H-strand, and the Watson−Crick base pairs of W-strand. The plots revealed that ln K_{obs} for the Hoogsteen base-pair formations in both the triplex and the duplex decreased linearly with increasing ln a_w. In contrast, ln K_{obs} for the Watson−Crick base-pair formations in both the triplex and the duplex increased linearly with increasing ln a_w. The slope of these plots is approximately equal to the constant term $-\Delta n_w$, which is the number of water molecules taken up upon formation of a structure.[16] The slopes of the plots for the formation of the Hoogsteen base pairs were estimated to be −52.7 ± 3.9 and −22.7 ± 1.5 in the triplex and duplex, respectively, which correspond to release of 1.9 ± 0.1 and 0.8 ± 0.1 water molecules per nucleotide, respectively (Figure 4B). Corresponding slopes for the Watson−Crick base pairs in the triplex and duplex structures were estimated to be 96.4 ± 4.2 and 93.4 ± 3.2, respectively, corresponding to 3.4 ± 0.2 and 3.3 ± 0.1 water molecules taken up per nucleotide, respectively. These results demonstrated dehydration (release of water molecules) and hydration (taking up water molecules) on formation of Hoogsteen and Watson−Crick base pairs, respectively, in the triplex and duplex structures.

Molecular Dynamics for Hydration of DNA Strands. In order to validate the numbers of water molecules revealed by the plots of ln K_{obs} vs ln a_w, we carried out molecular dynamics calculations of the triplex of T-strand, parallel-stranded duplex of H-strand, and antiparallel-stranded duplex of W-strand with water molecules (Figure 5). In Figure 5, we showed the water molecules only which directly bind to the DNA strands and omitted the water molecules which indirectly bind to the DNA strands for clarity. Thus, the water molecules shown in Figure 5 reflect the numbers of water molecules directly binding to the DNA strands. The number of water molecules directly binding to T-, H-, and W-strands were estimated to be 191, 123, and 186, respectively. These values correspond to 5.2, 4.9, and 7.4 water molecules per nucleotide binding to the strands. Thus, the numbers of water molecules per nucleotide binding to the DNA strands are in the following order: W-strand >

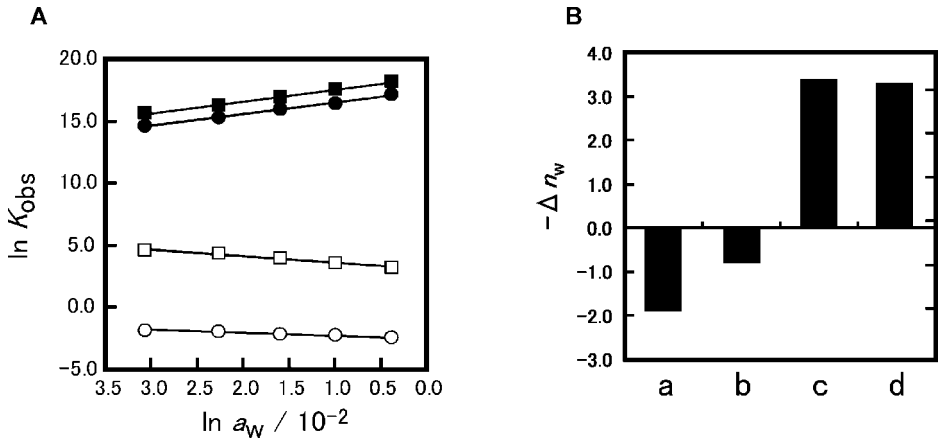

Figure 4. (A) Plots of ln K_{obs} versus ln a_w for H-strand (○), W-strand (●) and T-strand (□: Hoogsteen base pairs; ■: Watson−Crick base pairs) in buffers containing NaCl (100 mM), Na_2EDTA (1 mM) and Na_2HPO_4 (10 mM; pH 7.0) with 0, 5, 10, 15 and 20 wt % PEG 200 at 25 °C. (B) Δn_w for the Hoogsteen base pair in the triplex (a) and duplex (b), and Watson−Crick base pairs in the triplex (c) and duplex (d).

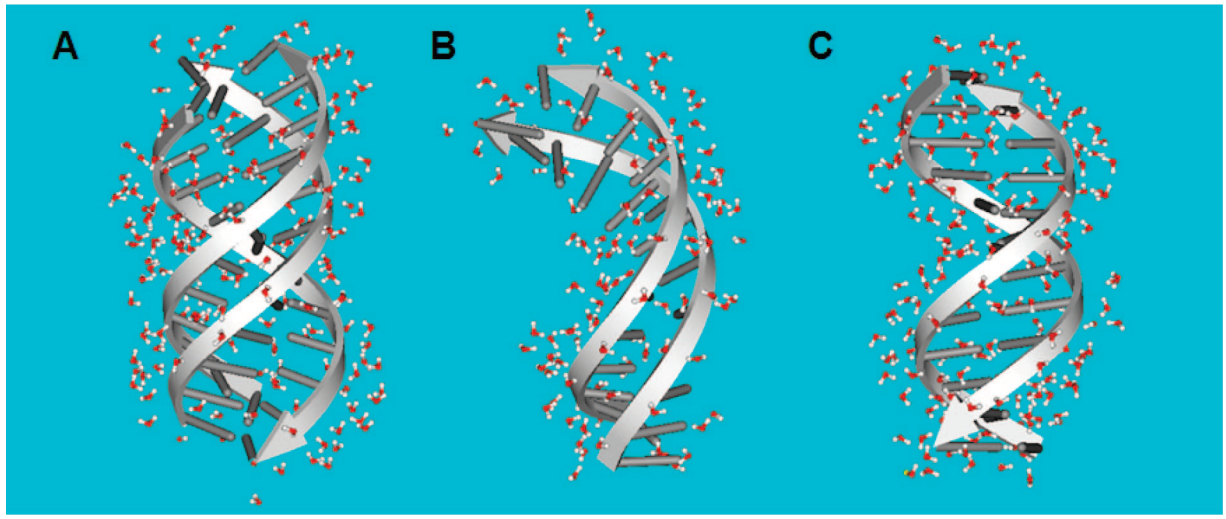

Figure 5. Molecular dynamics calculations for the parallel-stranded triplex of T-strand (A), parallel-stranded duplex of H-strand (B), and antiparallel-stranded duplex of W-strand (C) with the surrounding water molecules. Water molecules that did not form a hydrogen bond directly with DNA strands were omitted for clarity. The Insight II package was used for building and molecular dynamics of the DNA structures..

T-strand > H-strand. As shown in Figure 4B, the plots of ln K_{obs} vs ln a_w showed that 3.4 water molecules per nucleotide were taken up on formation of the Watson−Crick base pairs in the triplex and 1.9 water molecules per nucleotide were released on formation of the Hoogsteen base pairs in the triplex. Thus, 1.5 (= 3.4 − 1.9) water molecules per nucleotide were taken up through the formation of the whole triplex structure of T-strand. The plots showed that the number of water molecules per nucleotide taken up through the formation of the Hoogsteen base pairs of H-strand was −0.8, and that of the Watson−Crick base pairs of W-strand was 3.3 per nucleotide (Figure 4B). It should be mentioned that the molecular dynamics calculations evaluate the number of water molecules binding to the final (structured) DNA strands, whereas Δn_w, quantified by osmotic pressure measurements, is the difference in the number of water molecules binding to the initial (random coil) state and the final (structured) state of the DNA strands. Although the number of water molecules per nucleotide binding to these DNA strands in the random coil state is unknown, the order of the numbers of water molecules taken up through the structural formations of these strands is as follows: W-strand > T-strand > H-strand, which is the same order as that estimated from molecular dynamics calculations, although the absolute values are different. Therefore, the molecular dynamics studies of the DNA structures with water molecules support qualitatively the results of the numbers of water molecules taken up and released through the base-pair formations. In addition, it is notable that the numbers of water molecules quantified by osmotic pressure measurements can be different from the numbers of water molecules observed in the crystal structure since the water molecules which affect thermodynamics of the DNA structures are quantified by osmotic pressure measurements.

Effects of Co-solute Properties on the Number of Water Molecules. The osmotic stressing method used in this study to estimate the number of water molecules has also revealed the behavior of water molecules in various biomolecular processes such as protein folding and catalytic reactions.[25−29] Although this method can be most effective when the co-solutes used to

(25) Colombo, M. F.; Rau, D. C.; Parsegian, V. A. *Science* **1992**, *256*, 655–659.
(26) Parsegian, V. A.; Rand, R. P.; Rau, D. C. *Methods Enzymol.* **1995**, *259*, 43–94.
(27) Parsegian, V. A.; Rand, R. P.; Rau, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 3987–3992.

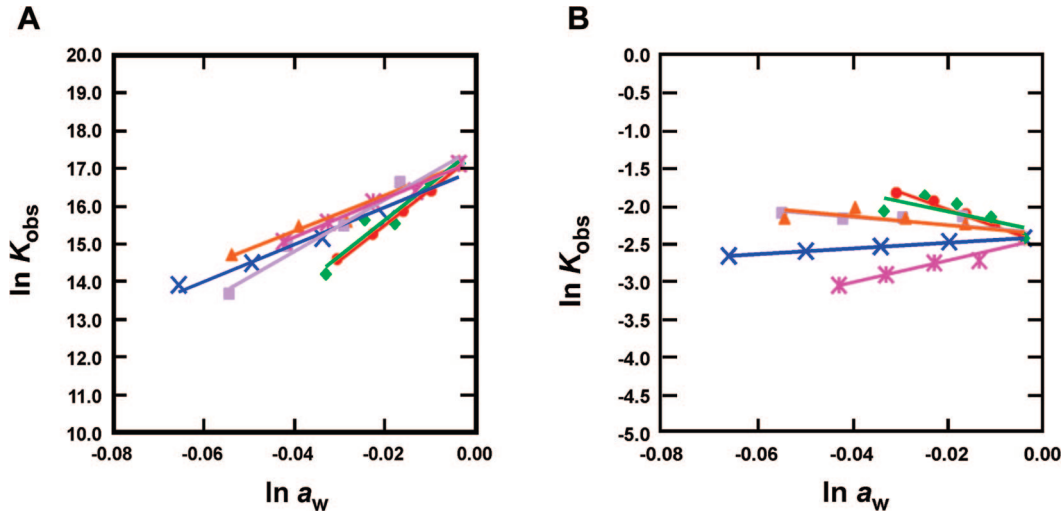

Figure 6. Plots of ln K_{obs} for the W-strand (A) and H-strand (B) versus ln a_w in buffers containing NaCl (100 mM), Na_2EDTA (1 mM) and Na_2HPO_4 (10 mM; pH 7.0) with 0, 5, 10, 15, and 20 wt % glycerol (pink *), ethylene glycol (blue ×), 1,3-propanediol (orange ▲), 2-methoxyethanol (purple ■), 1,2-dimethoxyethane (green ◆), or PEG 200 (red ●) at 25 °C.

induce molecular crowding conditions are inert,[25−27,30] it has further demonstrated that the number of water molecules depends on the properties of co-solutes (structure, size, charge, functional group, etc.).[15,16,31,32] Thus, the osmolytes used in the previous studies were not inert; they interacted with or otherwise affected the target molecules.[30] In order to assess the microscopic behaviors of co-solutes and water molecules around Watson−Crick or Hoogsteen base pairs, we further studied the effects of co-solute properties on the thermodynamic stability of the DNA structures.

We carried out UV melting analysis of the DNA strands in the presence of various co-solutes. Figure 6A shows the plots of ln K_{obs} vs ln a_w at 25 °C for the formation of the Watson−Crick base pairs of W-strand. The plots demonstrated that all co-solutes tested here [ethylene glycol (MW 62), 1,3-propanediol (MW 76), 2-methoxyethanol (MW 76), 1,2-dimethoxyethane (MW 90), glycerol (MW 92), as well as PEG 200 (average MW 200)] destabilized Watson−Crick base pairs linearly with increasing ln a_w. The slopes in the presence of these co-solutes were estimated to be 49.3 ± 6.2, 48.0 ± 4.8, 68.4 ± 5.9, 95.7 ± 15.1, 50.9 ± 3.1, and 93.4 ± 3.2, respectively. The order of the number of water molecules (destabilization of the Watson−Crick base pairs of W-strand) is as follows: 1,2-dimethoxyethane ≈ PEG 200 > 2-methoxyethanol > glycerol ≈ ethylene glycol ≈ 1,3-propanediol. The number of hydroxyl groups in the vicinal position in 1,2-dimethoxyethane, PEG 200, 2-methoxyethanol, glycerol, ethylene glycol, and 1,3-propanediol is 0, 0, 1, 3, 2, and 1, respectively. Therefore, these results reveal a tendency for a co-solute with fewer hydroxyl groups in the vicinal position to cause more water molecules to be taken up during Watson−Crick base-pair formation. These results are consistent for intermolecular antiparallel duplexes of various lengths.[16]

In the same manner, the relationship was studied between ln K_{obs} and ln a_w at 25 °C for the formation of Hoogsteen base pairs of H-strand (Figure 6B). Surprisingly, the plots demonstrated that 1,2-dimethoxyethane and PEG 200 stabilized Hoogsteen base pairs, whereas the other co-solutes had a destabilizing effect. The stabilization of Hoogsteen base pairs increased linearly with increasing ln a_w. The slopes for glycerol, ethylene glycol, 1,3-propanediol, 2-methoxyethanol, 1,2-dimethoxyethane, and PEG 200 were estimated to 14.6 ± 2.2, 3.9 ± 0.1, −6.0 ± 2.8, −5.1 ± 2.4, −13.3 ± 7.4, and −22.7 ± 1.5, respectively. The order is roughly opposite to that estimated for Watson−Crick base pairs. These results are consistent with previous results for the DNA triplex,[10,17,18] in which it was suggested that hydration was a critical determinant of the themodynamics of the DNA triplex structure. Moreover, the order of the values for Watson−Crick and Hoogsteen base pairs indicates that co-solutes containing fewer hydroxyl groups are excluded from the DNA surface because the DNA surface prefers interaction with water molecules over the co-solutes. In the case of co-solutes with a larger number of hydroxyl groups, the DNA surface may interact preferentially with the co-solutes rather than water molecules.

Discussion

Hydration of DNA Strands Determined by Base Pair Type. The number of water molecules taken up or released on structure formation, estimated with inert PEG 200, as shown in Figure 4B, reveals the following two important points: (1) Dehydration and hydration proceed through the formation of Hoogsteen and Watson−Crick base pairs, respectively, which are independent of the DNA structures. (2) The number of water molecules taken up on the formation of the Watson−Crick base pairs in the triplex structure is almost the same as that in the duplex structure, while the number of water molecules released on the formation of the Hoogsteen base pairs in the triplex structure (1.9 per nucleotide) is significantly larger than that in the duplex structure (0.8 per nucleotide).

For the first point, we reported previously that the number of water molecules released on formation of an intramolecular antiparallel-stranded G-quadruplex with Hoogsteen base pairs

(28) Robinson, C. R.; Sligar, S. G. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 2186–2191.
(29) Webb, J. N.; Webb, S. D.; Cleland, J. L.; Carpenter, J. F.; Randolph, T. W. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 7259–7264.
(30) Shimizu, S. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 1195–1199.
(31) Timasheff, S. N. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 9721–9726.
(32) Courtenay, E. S.; Capp, M. W.; Anderson, C. F.; Record, M. T., Jr. *Biochemistry* **2000**, *39*, 4455–4471.

between guanine bases was estimated to be 4.0 ± 0.1 per nucleotide.[15] Spink and Chaires reported that the number of water molecules taken up per nucleotide through the formation of Hoogsteen base pairs in an intermolecular triplex formation of poly(dT)•(dA)•(dT) was approximately 0.5, leading to destabilization of the triplex under molecular crowding conditions with small co-solutes, whereas the triplex was stabilized by molecular crowding conditions with larger co-solutes such as PEG 400, implying dehydration on triplex formation.[10] We also reported that solvation of nucleotides by co-solutes with more hydroxyl groups eliminates the uptake or release of water molecules through formation of the quadruplex.[15,16] Therefore, there is no contradiction between the previous results for the triplex and the results obtained here for Hoogsteen base pairs. In the same way, the number of water molecules per nucleotide taken up through the formation of Watson−Crick base pairs in the duplex and triplex are consistent with those in the intermolecular *Escherichia coli* (long) duplex, poly(dT)•(dA) duplex, and the short duplex reported previously.[10,16] The numbers of water molecules taken up through the formation of the antiparallel duplex evaluated here and evaluated previously with totally different sequence are 3.3 and 7.7, respectively.[16] These numbers indicate that the hydration of the antiparallel duplex is observed independently on its sequence, although the number of water molecules taken up may depend on its sequence. These considerations lead to the conclusion that the type of base pair (Hoogsteen or Watson−Crick) regulates the behavior of water molecules, which directly affects the thermodynamics of DNA structures.

We further considered the second important point. It has been demonstrated by experimental and theoretical studies that the spine of hydration is formed in the minor groove of Watson−Crick base pairs in the duplex structure.[2] The water molecules included in the spine of hydration in the minor groove result in more water molecules binding to the duplex of W-strand. Watson−Crick base-pair formation in the triplex of T-strand proceeds in the same manner as that in the duplex of W-strand, because the Hoogsteen base pairs of T-strand cannot exist at the temperature at which Watson−Crick base-pair formation occurs. Moreover, thermodynamic parameters ($\Delta H°$, $T\Delta S°$, and $\Delta G°_{25}$) of Watson−Crick base-pair formation of T-strand are almost identical to those of Watson−Crick base-pair formation of W-strand. The parameters suggest that not only the base-pair formation but also the hydration pattens are very similar in the duplex and triplex. Thus, it is appropriate that the number of water molecules taken up per nucleotide on the Watson−Crick base pairs in the triplex and duplex are the same.

It is possible to consider the difference in the numbers of water molecules released through formation of the Hoogsteen base pairs in the triplex and duplex structures from the structural information for the parallel-stranded triplex, which has three grooves (major, minor, and narrow ones between the PyW, PyC, and PyH regions) (Figure 1B). Radhakrishnan and Patel showed with NMR that the narrow groove between PuC and PyH in a triplex structure contained an ordered network of water molecules.[33] They also showed that the major groove between PyW and PyH in the triplex structure harbored several water molecules.[33] Ouali et al. found a spine of water molecules bound between thymine O4's of the PyW strand and the PyH strand at the major groove in a triplex structure.[34] These water molecules binding to the narrow and major grooves of a triplex can lead to an increased number of water molecules binding to the DNA strand (hydration) through Hoogsteen base-pair formation in the triplex structure. On the other hand, the water molecules binding to the major groove of the of the PyW-PuC duplex are expected to be pushed away when the third strand (PyH) binds to the major groove, leading to fewer water molecules binding to the DNA strand (dehydration). A combination of the hydration of the minor groove in the triplex structure and dehydration from the major groove by the third strand binding results in 1.9 water molecules per nucleotide are released upon Hoogsteen base-pair formation in the triplex structure. As a result, it can be considered that only 0.8 water molecule per nucleotide is released upon Hoogsteen base-pair formation in the duplex structure, whereas 1.9 water molecules per nucleotide are released in the triplex structure.

Properties of Co-solutes Affecting Preferential Hydration. It has previously been demonstrated with analysis based on the Kirkwood−Buff theory that the osmotic stressing method often overestimates the number of water molecules taken up by biomolecules.[30,35] This overestimation is due to interactions between the co-solutes inducing molecular crowding as well as their direct interactions with target molecules.[30,35] Although analysis based on the Kirkwood−Buff theory can provide quantitative information about the behavior of water molecules, it requires details of the three-dimensional structures of the target molecule before and after the process.[30] Thus, the structures of the unfolded (random coil) and folded (duplex or triplex) DNA states are required. However, structures of the duplex and triplex have not yet been reported; in particular, the details of the parallel-stranded duplex remain unclear.

How can we evaluate in detail the behavior of water molecules when we do not have structural information? Rau and co-workers demonstrated with the osmotic stressing method that water-structuring forces dominated the interaction between co-solutes and target macromolecules, leading to preferential hydration where the number of included waters varied substantially with the properties, such as size, shape, and functional groups, of the co-solute and macromolecular surface.[36] Furthermore, the osmotic stressing method does not require structural information for its basic analysis. Therefore, the osmotic stressing method with various co-solutes compares favorably with the Kirkwood−Buff treatment for evaluation of the hydration of biomolecules, although the osmotic stressing method may be less quantitative than the Kirkwood−Buff treatment for some systems.

It was reported that the size of alcohols utilized as co-solutes had little effect on exclusion from spermidine-condensed DNA arrays.[37] It was further proposed that co-solutes included in the arrays minimized the unfavorable energies associated with the distorted water network confined around the arrays. Our results obtained here showed that the number of water molecules depended on the properties of the co-solutes. This is totally consistent with the previous postulate because of the following two reasons: (1) The number of water molecules is strongly

(33) Radhakrishnan, I.; Patel, D. J. *Biochemistry* **1994**, *33*, 11405–11416.
(34) Ouali, M.; Gousset, H.; Geinguenaud, F.; Liquier, J.; Gabarro-Arpa, J.; Le Bret, M.; Taillandier, E. *Nucleic Acids Res.* **1997**, *25*, 4816–4824.
(35) Smith, P. E. *Biophys. J.* **2006**, *91*, 849–856.
(36) Sidorova, N.; Muradymov, S.; Rau, D. C. *J. Biol. Chem.* **2006**, *281*, 35656–35666.
(37) Stanley, C.; Rau, D. C. *Biophys. J.* **2006**, *91*, 912–920.

related to the number of co-solute hydroxyl groups. (2) The hydroxyl groups not only interact with the DNA but also mediate the hydrogen-bonded network of water molecules. Thus, co-solutes containing fewer hydroxyl groups are excluded from the DNA surface because it prefers interaction with water molecules over the co-solutes; in the case of co-solutes with more hydroxyl groups, the DNA surface interacts preferentially with the co-solutes.

Implications for Nucleic Acid Structures under Cell-like Conditions. Water is critical to the structure and function of biomolecules because the thermodynamic stability of the biomolecules comprises the sum of contributions not only from the free energy changes of their intramolecular and intermolecular interactions but also from the free energy changes of interactions between the biomolecules and the surrounding water molecules.[38] In fact, water molecules have been identified at the interfaces of protein−protein interactions,[38,39] and are involved in the catalytic functions of enzymes.[40,41] Because of the importance of the hydration shell of the biomolecules, quantitative analysis of the numbers of water molecules bound to nucleic acids has been carried out by experimental approaches including X-ray diffraction, neutron scattering, NMR, FTIR, and molar volume measurements.[42−46] Compared with such methods, one of the advantages of the osmotic stressing method used here is that it can estimate the number of water molecules that directly affect the thermodynamics of structural formation. The results reported here, as well as previous results,[15,16] for the duplex and G-quadruplex demonstrated dehydration and hydration through the formation of Hoogsteen and Watson−Crick base pairs, respectively, especially with the larger co-solutes such as PEG 200. Thus, it appears that molecular crowding conditions, where water activity decreases and hydration is unfavorable, stabilize DNA structures containing Hoogsteen base pairs and destabilize those with Watson−Crick base pairs, leading to structural polymorphism of various DNA sequences under molecular crowding conditions that mimic those found in cells. It is noteworthy that the destabilization of DNA structures with Watson−Crick base pairs seems to be a general phenomenon, since it has been independently observed with various small co-solutes of different properties. On the other hand, we have found that the stability of Hoogsteen base pairs under molecular crowding conditions depends on the properties of the small co-solutes. Thus, Hoogsteen base pairs seem to be sensitive to coexsisting molecules in a living cell.

Acknowledgment. This work was supported in part by Grants-in-Aid for Scientific Research and the "Academic Frontier" Project (2004−2009) of the Ministry of Education, Culture, Sport, Science and Technology (MEXT) of Japan, by Long-range Research Initiative, and by the Hirao Taro Foundation of the Konan University Association for Academic Research.

Supporting Information Available: CD spectra of W-strand in dilute and molecular crowding conditions; gel electrophoresis of H-strand and W-strand with varying concentrations of PEG 200; normalized UV melting curves at 295 nm for H-strand and W-strand with varying concentrations of PEG 200. This material is available free of charge via the Internet at http://pubs.acs.org.

JA805972A

(38) Timasheff, S. N. *Annu. Rev. Biophys. Biomol. Struct.* **1993**, *22*, 67–97.
(39) Pal, S. K.; Peon, J.; Zewail, A. H. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 1763–1768.
(40) Suck, D.; Oefner, C. *Nature* **1986**, *321*, 620–625.
(41) Breyer, W. A.; Matthews, B. W. *Nat. Struct. Biol.* **2000**, *7*, 1125–1128.
(42) Hu, Y.; Yang, X.; Yin, H. D.; Mahadevan, J.; Kuczera, K.; Schowen, L. R.; Borchardt, T. R. *Biochemistry* **2001**, *40*, 15143–15152.
(43) Woods, K. K.; Lan, T.; McLaughlin, W. L.; Williams, D. L. *Nucleic Acids Res.* **2003**, *31*, 1536–1540.
(44) Parvathy, V. R.; Bhaumik, S. R.; Chary, K. V. R.; Govil, G.; Liu, K.; Howard, F. B.; Miles, H. T. *Nucleic Acids Res.* **2002**, *30*, 1500–1511.
(45) Liepinsh, E.; Leupin, W.; Otting, G. *Nucleic Acids Res.* **1994**, *22*, 2249–2254.
(46) Ai, X.; Zhou, Z.; Bai, Y.; Choy, W.-Y. *J. Am. Chem. Soc.* **2006**, *128*, 3916–3917.