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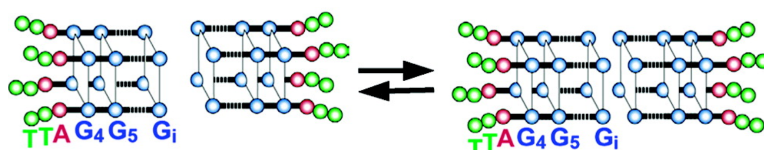

d(TTAG$_n$)	n=3	n=4	n=5
K_{eq} (mol^{-1}l)	4.9	3.4	0.9
ΔH (kJmol^{-1})	-37.2	-33.1	-28.7
ΔS (JK^{-1}mol^{-1})	-111	-103	-96.8

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Dynamics and Thermodynamics of Dimerization of Parallel G-Quadruplexed DNA Formed from d(TTAG$_n$) (n = 3−5)

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G-quadruplexed DNA, composed of stacked tetrads known as G-tetrads, can adopt several morphologies that include parallel or antiparallel configurations through either intramolecular or intermolecular organization.[1] Parallel G-quadruplex-forming sequences that have a stretch of consecutive guanines at their 3′- or 5′-termini have been shown to form extended quadruplexes by associating into higher-order structures.[2,3] Knowledge of this association is valuable for not only understanding the putative role of G-quadruplexed DNA in biological processes[4] but also for designing the molecular architecture of such a higher-order assembly to create a novel function.

The solution structure of a single repeat sequence of the human telomere, d(TTAGGG), has been shown to form a parallel G-quadruplexed DNA in the presence of low K^+ concentrations ([K^+]), which aggregates to form a higher-order structure in the presence of high [K^+].[1f,5] On the other hand, a parallel G-quadruplexed DNA formed from the d(TTAGGGT) sequence does not aggregate to form a higher-order structure even if [K^+] is increased, indicating that the extra 3′-terminal thymine prevents the aggregation of the G-quadruplexed DNA.[5b] In the present study, we have characterized the structure of an intermolecular assembly of a series of oligonucleotide sequences, d(TTAG$_n$) and d(TTAG$_n$T), where n = 3−5, in solution by means of ^{1}H NMR and a size-exclusion chromatography/multi-angle laser light-scattering system (SEC/MALLS) to gain insight into the molecular mechanism responsible for the assembly. We report herein the formation of a "dimer"[6] through end-to-end stacking of the 3′-terminal G-tetrads of parallel G-quadruplexes formed from d(TTAG$_n$) sequences, and the dynamics and thermodynamics of the dimerization.

We first examined the formation of G-quadruplexes from the d(TTAG$_n$) and d(TTAG$_n$T) sequences using ^{1}H NMR (Figure 1A).[7] Three, four, and five signals expected from the numbers of guanine bases in the d(TTAG$_n$) or d(TTAG$_n$T) sequences, where n = 3, 4, and 5, respectively, were observed in the chemical shift region characteristic of G-tetrads,[5a] confirming the formation of parallel G-quadruplexes from these sequences. Furthermore, in the spectra of the d(TTAG$_n$) sequences, minor signals were observed in addition to major ones, and the number of minor signals was exactly the same as that of major ones, suggesting that the G-tetrads of d(TTAG$_n$) sequences exist in two distinctly different chemical environments.

Next, we assigned the imino proton signals on the basis of nuclear Overhauser effect (NOE) connectivities (Figure 1B). Two independent sets of NOE connectivities, one connecting all the major signals and the other connecting all the minor ones, were observed. In addition, connectivities due to saturation transfer were also observed between the corresponding major and minor signals (Figure 1B). This finding indicated that the two different chemical environments of the G-tetrads of G-quadruplexes are in dynamic equilibrium with each other. Similar exchange behavior was also observed for the other d(TTAG$_n$) sequences. The saturation transfer

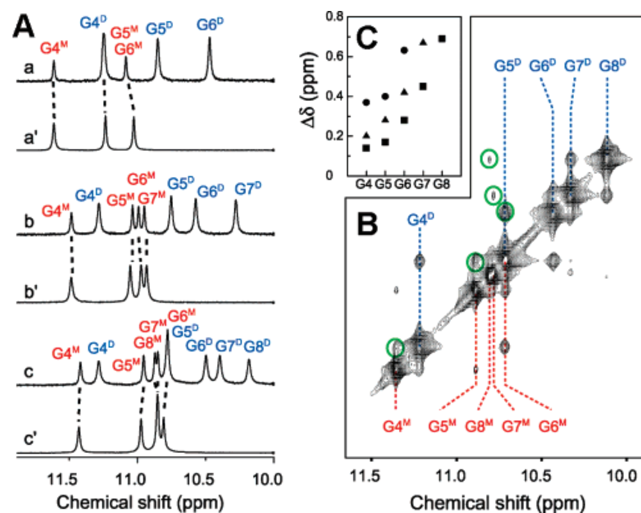

Figure 1. (A) Downfield-shifted portions of the 500 MHz ^{1}H NMR spectra of d(TTAGGGT) (a′), d(TTAGGG) (a), d(TTAGGGGT) (b′), d(TTAGGGG) (b), d(TTAGGGGGT) (c′), and d(TTAGGGGG) (c) in 90%H_2O/10%D_2O, 50 mM potassium phosphate buffer, pH 7.00, at 25 °C. The concentrations of the d(TTAG$_n$T) and d(TTAG$_n$) sequences were 2.0 and 0.25 mM, respectively. The assignments of imino proton signals are indicated with the spectra. (B) A portion of the NOESY spectrum of the d(TTAGGGGG) sequence. A mixing time of 150 ms was used to record the spectrum. The connectivities, highlighted in circles, are due to saturation transfer through the interexchange between monomers and dimers. (C) Plots of the shift difference between the corresponding signals of the monomers and dimers (Δδ) against the value of n in the d(TTAG$_n$) sequences; n = 3 (●), n = 4 (▲), and n = 5 (■).

NMR technique[8] yielded the value of (6 ± 2) × 10^{-1} s^{-1} for the rate of conversion of the major form of G-quadruplexed d(TTAGGG) to the minor one (see Supporting Information). On the other hand, only one set of signals indicative of the formation of parallel G-quadruplexes was observed in the spectra of the d(TTAG$_n$T) sequences, as demonstrated previously.[5a] The shift patterns of the minor signals for the d(TTAG$_n$) sequences were similar to those of the signals for the corresponding d(TTAG$_n$T) sequences, indicating that the minor signals in the spectra of the d(TTAG$_n$) sequences arise from parallel G-quadruplexed DNA, as in the case of the d(TTAG$_n$T) sequences. Consequently, the major signals in the spectra of the d(TTAG$_n$) sequences are attributed to higher-order structures.[5a]

To characterize the higher-order structure formed from the d(TTAGGG) sequence, we determined the weight average molecular weight (M_w) with SEC/MALLS.[7] The value of 15600 ± 900 was obtained for the M_w, which was almost the same as the value (14778) calculated for a dimer of G-quadruplexed d(TTAGGG) (see Supporting Information). The M_w of the G-quadruplexed d(TTAGGGT) was also determined, the obtained value of 8780 ± 260 being similar to the value (8601) calculated for a monomer of G-quadruplexed d(TTAGGGT). Thus, these SEC/MALLS results

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Table 1. Thermodynamic Parameters for Dimerization of d(TTAG$_n$) Sequences (n = 3, 4, or 5) in the Presence of 50 mM K^+

n	3	4	5
K_{eq}[a] ($mol^{-1}l$)	4.9	3.4	0.9
ΔH[b] ($kJ\ mol^{-1}$)	−37.2	−33.1	−28.7
ΔS[b] ($J\ K^{-1}mol^{-1}$)	−111	−103	−96.8

[a] The equilibrium constant (K_{eq}) at 25 °C. The experimental error was ±0.1. [b] The thermodynamic parameters were determined from van't Hoff plots. Six data points were collected over the temperature range of 15−65 °C, and the plots yielded a straight line with the correlation coefficient of >0.98 (see Supporting Information). The experimental errors for enthalpy (ΔH) and entropy (ΔS) were ±5 kJ mol^{-1} and ±10 J K^{-1} mol^{-1}, respectively.

not only proved that the G-quadruplexed d(TTAGGG) forms a dimer but also confirmed that the extra 3′-terminal thymine prevents the dimerization.

Then we analyzed the shift changes ($\Delta\delta$) between the dimer and monomer signals for the corresponding imino proton to characterize the molecular assembly in the dimer. The dimer signals were upfield-shifted by 0.2−0.7 ppm relative to the corresponding monomer ones (Figure 1C). In addition, for a given d(TTAG$_n$) sequence, the $\Delta\delta$ value was larger for the imino proton of the G-tetrad closest to the 3′-terminus and was dependent solely upon the distance of the G-tetrad from the 3′-terminus. These results were consistent with assembly through interaction between the 3′-terminal G-tetrads. Furthermore, the equivalence of the corresponding G-tetrads of the constituent G-quadruplexes within the dimers, as manifested in the number of dimer signals, supported dimerization through end-to-end stacking of the 3′-terminal G-tetrads, as has been demonstrated for the assembly of two hairpins formed from the d(GGGTTAGGG) sequence.[9] Simple end-to-end stacking of the 3′-terminal G-tetrads does not account for the upfield shifts of all the imino proton signals of the dimer relative to the corresponding signals of the monomer, as previously pointed out.[5a] Structural features of G-quadruplexes, such as the conformation of the individual G-tetrads, the relative orientation of the adjacent G-tetrads, and the coordination of K^+ to G-tetrads, might be affected by the dimerization. A tetranucleotide sequence, d(GGGT), has been shown to self-assemble into an interlocking G-quadruplex dimer.[3] The difference in assembly between G-quadruplexes of the d(TTAG$_n$) and d(GGGT) sequences could arise from the presence of adenine residues adjacent to G-tetrads in the former, which has been shown to form an A-tetrad stacking with an adjacent G-tetrad.[5e] Bulky adenine bases are likely to prevent the formation of quadruplexes with a slipped strand essential for assembly of the interlocking G-quadruplex dimer.

The slow interexchange between monomers and dimers of the G-quadruplexes of the d(TTAG$_n$) sequences allowed the quantitative characterization of the energetics of the process. The equilibrium constant (K_{eq}) for the dimerization can be obtained from the NMR signal intensities for the two forms, and then the temperature dependence of the K_{eq} value allows the determination of thermodynamic parameters associated with the process (Table 1). The enthalpy change (ΔH) clearly indicated that the dimerization of the G-quadruplexes is largely enthalpic in origin, as expected from the hydrophobic interaction through the end-to-end stacking of the 3′-terminal G-tetrads. Furthermore, the entropy change (ΔS) was negative, as also expected for the dimerization reaction. Interestingly, both the ΔH and ΔS values contribute to a decrease in the K_{eq} value, and hence the monomer becomes more favored, with an increasing number of consecutive G-tetrads at the 3′-terminus. ΔH and ΔS values of −256 kJ mol^{-1} and −763 J K^{-1} mol^{-1}, respectively, were determined for the formation of the G-quadruplexes of the d(TTAGGGT) sequence in the presence of similar $[K^+]$[5e] and have been attributed predominantly to the formation of core G-tetrads.[10] Assuming that three G-tetrads equally contribute to the ΔH value, −85 kJ mol^{-1} was estimated for the ΔH value for the formation of a single G-tetrad. The ΔH value (−37.2 kJ mol^{-1}) obtained for dimerization of the G-quadruplexes was close to a half of that estimated for the formation of a single G-tetrad, reflecting the significant contribution of the stacking of G-tetrads to the stability of the G-quadruplexes.

In the present study, we characterized the dynamics and thermodynamics of the dimerization of the G-quadruplexes formed from d(TTAG$_n$) sequences (n = 3, 4, or 5), which occurs through end-to-end stacking of 3′-terminal G-tetrads. Determination of the thermodynamic properties for the self-assembly of G-quadruplexes is of significance from the viewpoints of understanding both the diversity in higher-order G-quadruplex structures and the molecular recognition of G-tetrads.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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(6) Each "monomer G-quadruplex" is a tetramer of a d(TTAG$_n$) sequence; hence, a "dimer G-quadruplex" is in fact an octamer of the sequence.

(7) Oligonucleotides were obtained from Espec Oligo Service, Co. The formation of G-quadruplexes from the sequences used in the study was also confirmed by circular dichroism spectra (see Supporting Information). NMR spectroscopy and phase-sensitive two-dimensional nuclear Overhauser effect-correlated spectroscopy (NOESY) were performed with Bruker Avance 500 and 400 FT NMR spectrometers operating at the ^1H frequencies of 500 and 400 MHz, respectively. Chemical shifts are given in ppm downfield from sodium 2,2-dimethyl-2-silapentane-5-sulfonate, with the residual H^2HO as an internal reference. Shodex PROTEIN KW-802.5 and 804 columns (Showa Denko K. K.) were used for SEC. A DAWN EOS (Wyatt Technologies), equipped with a K5 flow cell and a gallium-arsenide 690-nm laser light source, was used as the MALLS detector.

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