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Effects of deficient of the Hoogsteen base-pairs on the G-quadruplex stabilization and binding mode of a cationic porphyrin

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ABSTRACT

Background: In stabilization of the G-quadruplex, formation of a Hoogsteen base-pair between the guanine (G) bases is essential. However, the contribution of each Hoogsteen base-pair at different positions to whole stability of the G-quadruplex has not been known. In this study, the effect of a deficiency of the Hoogsteen type hydrogen bond in the G-quadruplex stability was investigated. Spectral properties of *meso*-tetrakis(1-methylpyridinium-4-yl)porphyrin (TMPyP) associated with various G-quadruplexes were also examined.

Methods: The thermal stability of the thrombin-binding DNA aptamer $5'G_1G_2TTG_5G_6TG_8TG_{10}G_{11}TTG_{14}G_{15}$ G-quadruplex, in which the guanine (G) base at 1, 2, 5, 6 and 8th positions was replaced with an inosine (I) base, one at a time, was investigated by circular dichroism (CD). The absorption, CD and fluorescence decay curve for the G-quadruplex associated TMPyP were also measured.

Results: The transition from the G-quadruplex to a single stranded form was endothermic and induced by an increase in entropy. The order in stability was $0 > 8 > 6 > 2 > 5 > 1$, where the numbers denote the position of the replacement and 0 represents no replacements of the G base, suggesting the significant contribution of the G_1 base in the stability of the G-quadruplex. Alteration in the spectral property of TMPyP briefly followed the order in thermal stability.

Conclusions: Replacement of a G base with an I base resulted in destabilization of the G-quadruplex. The missing hydrogen bond at position 1 destabilized the G-quadruplex most efficiently. TMPyP binds near the I base-replaced location namely, the side of the G-quadruplex.

General significance: The Hoogsteen base-pairing is confirmed to be essential in stabilization of G-quadruplex. When G is replaced with I, the latter base is mobile to interact with cationic porphyrin.

1. Introduction

Guanine-rich tracts of nucleic acids can fold into a four-stranded secondary structure called the G-quadruplex, in which four G-bases are connected *via* Hoogsteen type base pairing in the same plane to form a G-quartet in a monovalent cation-containing aqueous solution. The stacking interactions between the G bases and other electrostatic interactions also help stabilize the G-quartet in the G-quadruplex in addition to hydrogen bonding. The structure and dynamics of the G-quadruplexes have attracted considerable attention because of their biological importance [1–6]. The existence of these structures *in vivo*, such as at the telomeric ends of chromosomes and oncogene regulatory regions, is evident. They influence a variety of biological processes, such as the prevention of telomerase binding, promoter activation, and gene rearrangement, and are related to cell aging and cancer development. The G-quadruplex can adopt a variety of structural variations. The length and number of individual G-quartets as well as the length and sequence of the linker moiety affect the structure of the G-quadruplex [7–11]. The unimolecular G-quadruplex structure of the thrombin-binding DNA aptamer, $5'G_2T_2G_2TGTG_2T_2G_2$ (Fig. 1), was reported in the early 1990s based on nuclear magnetic resonance (NMR) spectroscopy and X-ray studies, which revealed the structure of the aptamer $5'G_2T_2G_2TGTG_2T_2G_2$ to be "antiparallel" [12–14]. This G-quadruplex adopts a highly compact and symmetrical structure consisting of two G-quartets and three loops. The residues of the G-quartet adopted an anti-syn–anti-syn conformation.

A number of molecules that binds selectively to the G-quadruplex have been reported, including metal salphen complexes [15,16], metal terpyridine complexes [17,18] and Ru(II) complexes with planar aromatic polycyclic rings [19–22]. Cationic porphyrin derivatives are one of the important classes that bind to the

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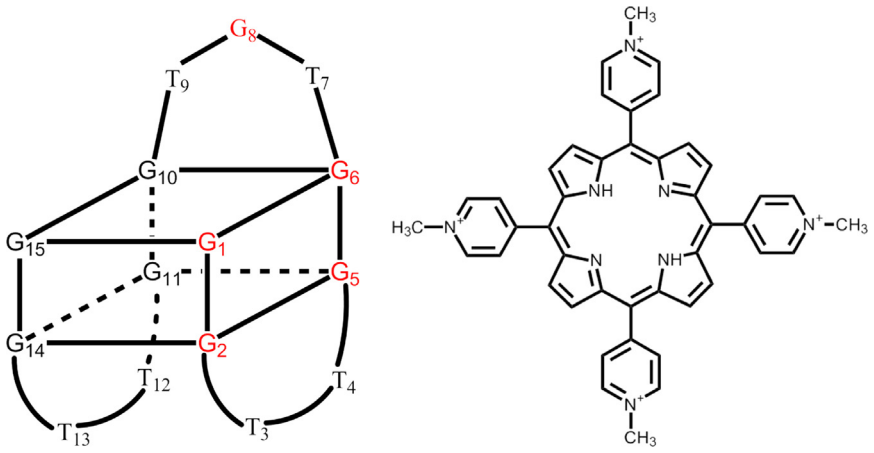

Fig. 1. Schematic diagram of (A) the G-quadruplex formed from 5′GGTTGGTGTGGTTGG and (B) chemical structure of TMPyP. The bases are numbered from 1 to 15 from the 5′-end. The guanine base that was replaced systematically by inosine is denoted by the red color. The oligonucleotide that is numbered in the text denotes the position where G is replaced. For example, oligonucleotide 8 represents the 8th G replaced by I. Oligonucleotide 0 means no G was replaced with the I base in the oligonucleotide.

G-quadruplex. As an example, certain Mn(III)porphyrin derivatives exhibited 10,000-fold selectivity for the G-quadruplex over duplex DNA [23]. Certain porphyrin derivatives were reported to inhibit the telomerase activity in HeLa cells showing the possibility of these molecules in biological applications [24–26]. In addition, cationic porphyrins have also been used as a structural probe for the G-quadruplexes. The binding mode of free base and metalloporphyrin to G-quadruplex include the intercalation between the two adjacent G-quartets [27,28], stacking on the external G-tetrads [29–31] and weak external binding [32–34] depending on nature of the G-quadruplex, nature of the central metal ion, structure of the periphery groups, and solution condition. As an example, a representative of the cationic porphyrin family, *meso*-tetrakis (1-methylpyridinium-4-yl)porphyrin (referred to as TMPyP, Fig. 1) was suggested to intercalate at each close GpG site of $AG_3(T_2AG_3)_3$, $[d(T_4G_4)]_4$ and $d(G_2T_2G_2TGTG_2T_2G_2)$ quadruplexes while binding to the parallel and antiparallel hybrid of the $AG_3(T_2AG_3)_3$ G-quadruplex by end-stacking and external groove binding mode [30,35]. The external binding mode of TMPyP to the G-quadruplex formed from the $5'G_2T_2G_2TGTG_2T_2G_2$ with 1:1 stoichiometry was also reported [36].

In stabilization of the G-quadruplex, formation of a Hoogsteen base-pair between the guanine (G) bases is essential in addition to the presence of monovalent cations, such as K^+ or Na^+. In this study, the effect of a deficiency of the Hoogsteen type hydrogen bond in the G-quadruplex stability was investigated by the systematic replacement of one of the G bases by the inosine (I) base at various positions (Figs. 1 and 2). The guanine bases only at the positions 1, 2, 5, 6 and 8 were selected to be replaced by inosine base. The similar result is expected for replacement of the guanine bases at 10, 11, 14 and 15 due to the symmetrical structure of the G-quadruplex. A comparable result is expected for the G-quadruplex in which the G_8 is replaced because it does not participate in the Hoogsteen base-pairing. The effects of the replacement on the binding mode of TMPyP were also investigated.

2. Experimental

TMPyP was purchased from Frontier Scientific Inc. (Logan, Utah). $5'G_2T_2G_2TGTG_2T_2G_2$ oligonucleotide, in which one of the G bases at the 1st, 2nd, 5th, 6th and 8th position was replaced with an I base was obtained from SBS Genetech Co., Ltd. (China). These materials were dissolved in a 5 mM cacodylate buffer at pH 7.0 and used as prepared. The concentrations of the porphyrins and

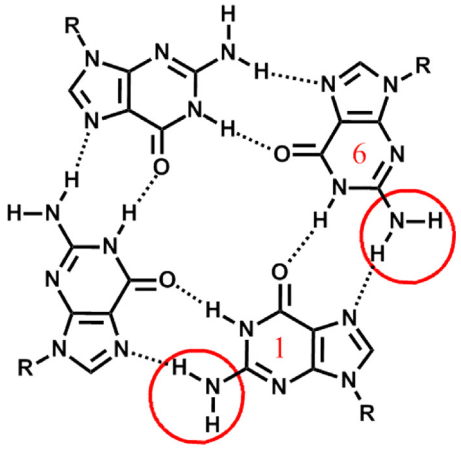

Fig. 2. Hoogsteen base pairing in the upper G-quartet viewed from top. The I bases lacking the amine group are marked by red circles.

oligonucleotide were determined spectrophotometrically using the molar extinction coefficients: $\varepsilon_{421\ nm}=2.26\times10^5\ M^{-1}\ cm^{-1}$ and $\varepsilon_{260\ nm}=1.43\times10^5\ M^{-1}\ cm^{-1}$ for TMPyP and the oligonucleotide, respectively. The quadruplex was formed by the addition of 100 mM KCl followed by heating at 80 °C for 10 min and annealing overnight at room temperature. The formation of the quadruplex was confirmed by its characteristic CD spectrum. In this article, the number after the G-quadruplex represents the position where the G base was replaced with an I base. For example, G-quadruplex 8 denotes the replacement of the G base at the 8th position, which is in the central loop, with an I base. The G-quadruplex 0 denotes no replacement of the G base. Aliquots of a concentrated TMPyP solution were added to a 5 μM polynucleotide solution (typically few μL to 3 mL polynucleotide solution) for the absorption and CD measurements, and appropriate corrections were made for the volume change. The samples were diluted 10 fold for the fluorescence measurement to avoid the inner filter effect.

The CD spectra were obtained using either a Jasco J-715 or a J-810 spectropolarimeter (Tokyo, Japan) and the absorption spectra were recorded using a Cary 100 spectrophotometer (Palo Alto, CA). The temperature was increased by 0.2 °C for every 2 min using a built-in peltier when measuring the temperature-dependent CD intensity, which reflects the unfolding of the quadruplex. The fluorescence decay profiles were measured on a iHR320 TSCPC system constructed in the Center for Research Facilities, Kongju

National University. The sample was excited at 405 nm and the emission was detected at 650 nm.

3. Results and discussion

3.1. Destabilization of the G-quadruplex by a deficiency of a hydrogen bond in the Hoogsteen base-pair

The difference between the G and I base is the presence of the amine group at the C7 position of the G base, which is involved in the Hoogsteen type base pair with the neighboring G base by forming two hydrogen bonds. This type of hydrogen bond is essential in stabilizing the G-quadruplex in addition to the π–π stacking interaction between the G bases at different layers of the G-quartet. When the G base is replaced with an I base, one of the hydrogen bonds is impossible to form (Fig. 2). The aptamer, $5'G_2T_2G_2TGTG_2T_2G_2$, in which no G base was replaced with an I base, produced a characteristic CD spectrum in the DNA absorption region with a positive maxima at 293 nm and 248 nm, and a negative minimum at 266 nm in the presence of 100 mM KCl at 20 °C (Fig. 3A, insertion), suggesting the formation of an antiparallel type G-quadruplex. All G-quadruplexes, in which one of the G base was replaced with an I base, produced a similar CD spectrum (SI 1), indicating that all oligonucleotides formed a G-quadruplex despite the lack of a hydrogen bond. On the other hand, the CD spectrum disappeared at the high temperature region (80 °C), indicating a transition from the G-quadruplex to single-strand.

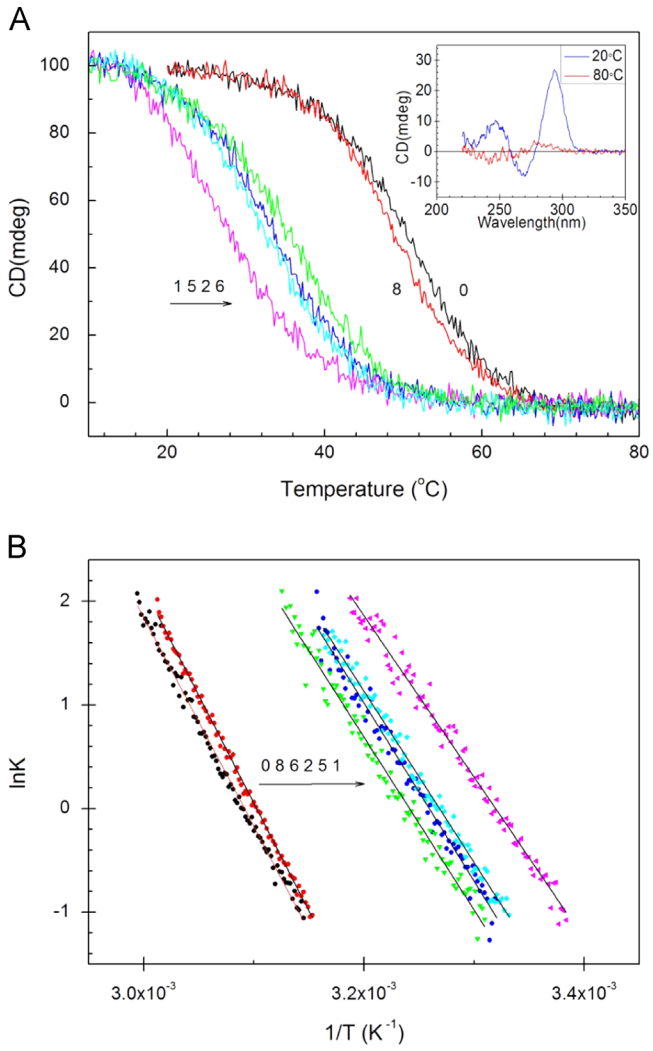

Fig. 3. (A) Temperature-dependent change in CD intensity at 294 nm and (B) the van't Hoff plot for denaturation of the various G-quadruplex. The numbers denote the position, where the G base is replaced with an I base. The concentration of the G-quadruplex was 5 μM. The CD spectrum of the oligonucleotide 0 at 20 °C and at 80 °C is inserted in panel (A). The average of five measurements are shown for all G-quadruplexes.

$$G-\text{quadruplex} \rightleftharpoons \text{Single strand} \quad (1)$$

The destabilization of the G-quadruplex by a deficiency of a hydrogen bond in the G-quartet was investigated by thermal denaturation. Fig. 3A shows the change in the CD intensity at 294 nm with respect to a temperature change. The temperature at which a 50% transition occurred (T_m) was 51.3 °C for G-quadruplex 0. The T_m for the G-quadruplex 8, whose G-base at the central loop was replaced by an I base, was slightly lower being at 48.8 °C. This suggests that the G8 base at the central loop in the quadruplex stabilization makes a minimal contribution. On the other hand, the T_m for the G-quadruplex 1 was the lowest at 28.2 °C, and those for quadruplex 6, 2 and 5 were 35.4 °C, 33.8 °C and 32.2 °C, respectively. The replacement of any G base with an I base resulted in destabilization as expected from the lack of a hydrogen bond. The order of the contribution in the quadruplex stabilization was quadruplex $1 > 5 > 2 > 6$.

The temperature-dependent change in CD reflects the shift in the equilibrium from the G-quadruplex to a single stranded $5'G_2T_2G_2TGTG_2T_2G_2$ oligonucleotide denoted in Eq. (1). The equilibrium constant, K, for this transition can be calculated easily. Fig. 3B presents the van't Hoff plot, in which the logarithm of the temperature-dependent equilibrium constant was plotted as a function of the reciprocal absolute temperature between the G-quadruplex and single stranded oligonucleotide.

$$\ln K = -\frac{\Delta H^0}{R}\left(\frac{1}{T}\right) + \frac{\Delta S^0}{R} \quad (2)$$

The R in this equation denotes the gas constant. The enthalpy and entropy for the G-quadruplex to single strand transition can be calculated from the slope and y-intercept of the plot. Table 1 lists the resulting thermodynamic parameters. At a glance, the unfolding of the G-quadruplex is endothermic with a negative slope in the van't Hoff plot and the entropy change was positive in all cases. The enthalpy and entropy changes for the transition of the quadruplex 0 to single strand were 2.42 ± 0.02 kJ mol^{-1} and 7.49 ± 0.07 J mol^{-1} K^{-1}, respectively. Therefore, the favorable change in entropy causes unfolding of the G-quadruplex, despite its unfavorable change in enthalpy. In other words, as the thermal energy was provided, the quadruplex became a less ordered single strand. Similar values were observed for quadruplex 8: ΔH and ΔS were 2.50 ± 0.01 kJ mol^{-1} and 7.77 ± 0.04 J mol^{-1} K^{-1}, respectively. In addition to the thermal melting curve, the similar thermodynamic parameter indicates that the replacement of a G base with an I base at position 8 had little effect on the stability of the G-quadruplex.

Table 1
Enthalpy and entropy change for the G-quadruplex to single strand transition for various G-quadruplexes, in which the G-base is replaced with an I base at denoted position.

Oligonucleotide	ΔH, kJ mol^{-1}	ΔS, J mol^{-1} K^{-1}
0	2.42 ± 0.02	7.49 ± 0.07
8	2.50 ± 0.01	7.77 ± 0.04
1	2.00 ± 0.02	6.50 ± 0.09
2	2.06 ± 0.03	6.73 ± 0.09
5	1.98 ± 0.02	6.50 ± 0.07
6	1.87 ± 0.02	6.23 ± 0.08

This suggests that the carbonyl group at the C5 of the G8 base does not contribute the G-quadruplex stability. On the other hand, the entropy changes of the other G-quadruplexes were ~6.5 J mol^{-1} K^{-1}. Assuming that the degree of disorder of the single stranded oligonucleotide with similar bases are in the same range, the similar entropy change observed for the quadruplex to a single strand transition suggests that the degree of disorder for the quadruplexes at which the I base is replaced were similar. As expected, the degree of disorder of quadruplexes 0 and 8 was lower than those of the quadruplex 1, 2, 5, and 6. This disorder originated from the missing hydrogen bond involved in the Hoogsteen base pairs. Therefore, the I base possesses a considerable degree of freedom in the G-quartet when it replaces the G base, as expected from number of hydrogen bonds.

3.2. *Effect of I bases on the binding mode of TMPyP*

The interaction of cationic porphyrins with the G-quadruplex has been studied extensively for their biological potential and as a marker for DNA conformation. TMPyP, a representative of this class of molecules, is one of the most widely studied cationic porphyrins. A variety of binding modes of porphyrins to the G-quadruplex has been reported, including the intercalation of planar porphyrin between two adjacent G-quartets [27,28], stacking on the external G-tetrads [29–31] and weak external binding [32–34] *via* electrostatic interactions. The binding mode of TMPyP to the 5′$G_2T_2G_2TGTG_2T_2G_2$ quadruplex was also reported [36]. Based on the relatively small change in the absorption spectrum in the Soret absorption region, a positive CD spectrum and a larger accessibility of the I^- fluorescence quencher compared to that of intercalated TMPyP to double stranded DNA suggest that TMPyP is not intercalated to the G-quadruplex formed from the 5′$G_2T_2G_2TGTG_2T_2G_2$ aptamer. TMPyP was concluded to bind to the exterior of the G-quadruplex.

The UV/vis absorption spectra reported in this study were invariant for the [TMPyP]/[G-quadruplex]=0.1, 0.2, 0.4, 0.6, 0.8, and 1.0, suggesting that the binding mode of TMPyP to the G-quadruplex is homogeneous at those mixing ratios. Therefore, only those for [TMPyP]/[G-quadruplex]=1.0 is presented for clarity. Fig. 4 shows the absorption spectrum of TMPyP associated with the G-quadruplex, in which the I base was replaced at various positions. As reported, TMPyP produced a 5 nm red shift (from 422 nm in the absence of a G-quadruplex to 427 nm) and ~17% hypochromism in the Soret absorption region upon binding to quadruplex 0. The change in the absorption spectrum upon the association with quadruplex 0 is in contrast to that intercalated to the double stranded DNA, which was reported to produce a ~20 nm red shift and ~47% hypochromism [37,38]. A larger area of overlap, *i.e.*, larger π–π interactions for the G-quadruplex-intercalated TMPyP compared to that to double stranded DNA is expected. If TMPyP is intercalated to the G-quadruplex a larger or, at least, similar extent of change in the absorption spectrum is expected. Therefore, the possibility of a TMPyP intercalation to the G-quadruplex may be very small. A slight further hypochromism of ~21% and the absorption maximum at 427.5 nm was observed for TMPyP bound to quadruplex 8. In both quadruplexes 2 and 6, the absorbance decreased by ~25%. The maxima were at 428 nm and 429 nm for quadruplexes 6 and 2, respectively. TMPyP associated with quadruplexes 1 and 5 exhibited similar hypochromism of ~29% and the absorption maximum at 429 nm and 430 nm for the quadruplex 1 and 5, respectively. These changes in the absorption spectrum suggest that TMPyP is unlikely to intercalate between the G-quartets. The TMPyP absorption spectrum changes with increasing concentration of quadruplex 0 (Fig. 5A). The absorption maximum shifted to a longer wavelength

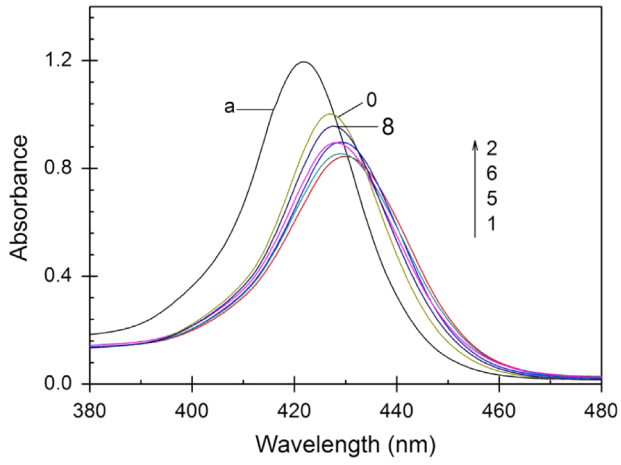

Fig. 4. Absorption spectrum of TMPyP in the presence of various G-quadruplexes in the Soret region at 20 °C. Curve *a* represent the absorption spectrum of TMPyP in the absence of the G-quadruplex. The concentration of [TMPyP]/[G-quadruplex]=1. [G-quadruplex]=5 μM.

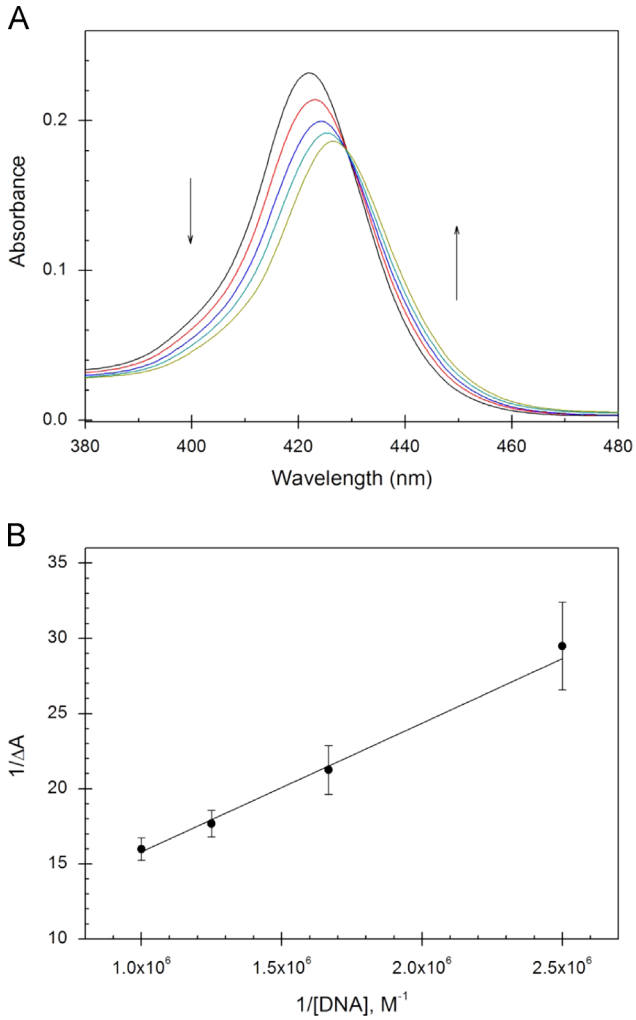

Fig. 5. (A) Change in the absorption spectrum of TMPyP in the Soret region with increasing concentration of the G-qaudruplex formed from 5′$G_2T_2G_2TGTG_2T_2G_2$ (quadruplex 0). (B) Benesi–Hildebrand plot constructed from the absorbance change at 440 nm for the association of TMPyP with the quadruplex 0. The other oligonucleotide produced similar plots. The concentration of TMPyP was fixed at 1 μM while that of the G-quadruplexes were varied from 0 to 1 μM with an increment of 0.2 μM. The absorption spectrum for the [G-quadruplex]=0.8 μM was similar to [G-quadruplex]=1.0 μM and is not shown for clarity. The error bars denote the standard deviation from the five measurements.

and the absorbance decreased. This change in the absorption spectrum was accompanied by an isosbestic wavelength at 429 nm. An increase in double stranded-DNA concentration also caused an increase in absorbance at the longer wavelengths. The presence of an isosbestic wavelength indicated that the system consisted of the two species: free and the quadruplex-bound TMPyP, unless the bound species having two distinctive binding modes produces coincidently the same absorption spectrum. If this change occurs between the two states, the equilibrium constant can be calculated using a simple Benesi–Hildebrand equation.

$$\frac{1}{\Delta A_{440\ \mathrm{nm}}} = -\frac{1}{(\varepsilon_b-\varepsilon_f)[L_t]} + \frac{1}{(\varepsilon_b-\varepsilon_f)[L_t]K_{BH}[\mathrm{Quadruplex}]} \quad (3)$$

In this equation, ε is the molar extinction coefficient and the subscripts b, f and t denote the bound, free and total metal complexes, respectively. $[L_t]$ and $\Delta A_{322\ \mathrm{nm}}$ are the total TMPyP concentration and the change in absorbance at 440 nm, respectively. The association constant for the formation of quadruplex–TMPyP adducts, K_{BH}, was calculated from the slope to intercept ratio of the Benesi–Hildebrand plot of the reciprocal absorbance with respect to the reciprocal DNA concentration (Fig. 5B). The equilibrium constant was $7.4 \pm 2.3 \times 10^5$ for complex formation between TMPyP and quadruplex 0. The equilibrium constant obtained from other quadruplexes were in the same range without a recognizable pattern: $K_{BH} = 8.0 \pm 0.9 \times 10^5$ for quadruplex 3 and was $4.5 \pm 3.0 \times 10^5$ for quadruplex 8. All TMPyP can be considered to be bound to the quadruplexes at these high equilibrium constants.

CD spectroscopy is a very useful technique for determining the binding mode of cationic porphyrins to DNAs. Although TMPyP is an achiral molecule, it produces a strong CD signal in the Soret absorption region when associated with various DNAs. The origin of this CD spectrum is the interaction between the two transition moments of porphyrin, the B_x and B_y transitions, and the chirally arranged DNA bases. TMPyP prefers to intercalate between the GC base pairs, producing a negative CD signal [36–38]. On the other hand, TMPyP binds at the minor groove of AT-rich DNAs at a low [porphyrin]/[DNA] ratio. As the porphyrin concentration increases, they begin to stack in the major groove. A strong positive CD signal is associated with the former binding mode, whereas TMPyP with the latter binding mode produces a bisignate CD spectrum in the Soret absorption band [39,40]. A positive CD signal was apparent when TMPyP was complexed with the G-quadruplex 0, which was formed from aptamer 5′$G_2T_2G_2TGTG_2T_2G_2$, as it was previously reported (Fig. 6A) [36]. Similar to the absorption spectra, the CD spectra for the various complexes were similar regardless of the [TMPyP]/[quadruplex] ratio. Therefore, only those of the highest TMPyP concentration ([TMPyP]/[quadruplex] = 1.0) is shown for clarity. The positive CD spectrum in the Soret band was assigned to the bindings exterior of quadruplex 0 *via* electrostatic interactions based on the spectroscopic evidences in addition to the CD spectrum. The shape of the CD spectrum was similar when the G base at position 8 was replaced with an I base, suggesting that the interaction of TMPyP and the G-base at the 8th position is negligible. A similar positive CD spectrum with somewhat lower intensity was observed for quadruplex 6 (Fig. 6B). The association of TMPyP with quadruplexes 1 and 5 produced almost identical positive CD signals (Fig. 6B). Although the shapes of CD are similar, the intensities of these complexes were significantly low compared to those of quadruplexes 1 and 8. A unique bisignate CD spectrum with its negative minimum at ~420 nm and positive maximum at 432 nm was produced upon the binding of TMPyP to quadruplex 2. Although bisignate CD is normally assigned to the stacked TMPyP along the DNA stem, it may be understood by the different interactions of the two electric transition moments of TMPyP with the bases in the G-quartet

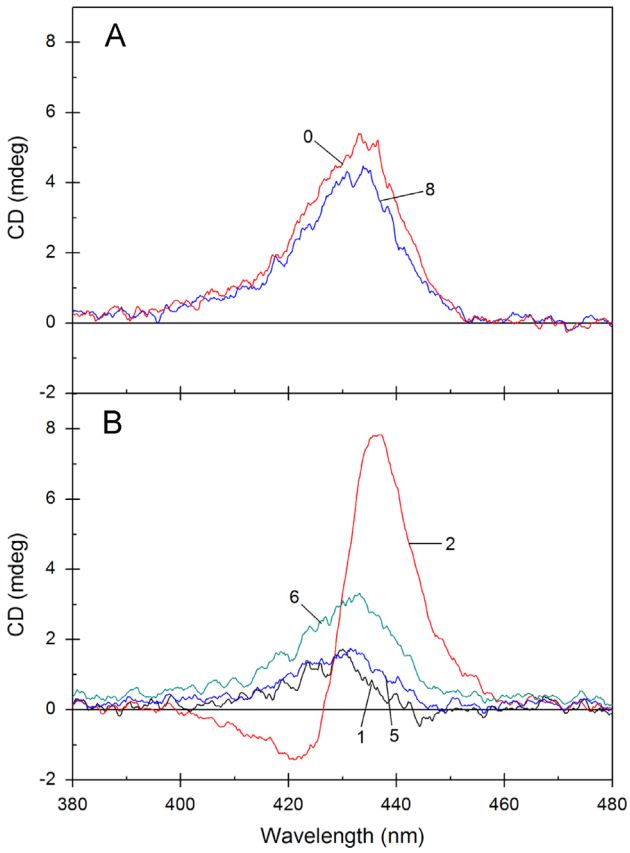

Fig. 6. (A) CD spectrum of TMPyP associated with oligonucleotides 0 and 8, and (B) those with oligonucleotide 1, 2, 5, and 6. [TMPyP]/[G-quadruplex] = 1.0. [G-quadruplex] = 5 μM.

because the G-quadruplex adopted in this study possesses only two layers of the G-quartet, which may be insufficient for TMPyP to stack. In summary, the shapes of the CD spectrum of TMPyP complexed with the G-quadruplexes are similar, suggesting that the interaction between TMPyP and G-quartet is essentially the same in nature, with the exception of quadruplex 2. The intensity was in the order of quadruplex $0 \approx 8 > 6 > 1 \approx 5$.

Fig. 7 presents the time-dependent fluorescence decay profiles of the TMPyP–quadruplex complexes. The observed decay curves for TMPyP in the presence of quadruplexes 0, 8 and 6 were indistinguishable, consisting of two decay times of ~2.5 ns and ~10.4 ns, which is in the same range as the reported values [35,36]. The relative amplitudes were ~0.25 for short decay times and ~0.75 for long decay times. In the quadruplex 2 case, the decay times were ~2.9 ns and ~10.1 ns with amplitudes of 0.32 and 0.68, respectively. The decay profile of TMPyP when associated with quadruplexes 1 and 5 were similar; ~2.4 ns and ~9.5 ns with amplitudes of 0.38 and 0.62, respectively. The deviation in the decay times measured in this study for various quadruplexes is similar within experimental error. The observed differences in the fluorescence decay curves shown in Fig. 7 may originate from the fractional contribution, f_i, which is determined by the following equation [41]:

$$f_i = a_i\tau_i / \sum_i a_i f_i \quad (4)$$

where a_i and τ_i denote the relative amplitude and the decay time of the ith component. The contribution of the short component was 0.073 for quadruplexes 0, and 6, whereas it was 0.119 for complex 2. In the quadruplex 5 and 1 case, the largest contribution of 0.134 was found. The contribution of the short decay time

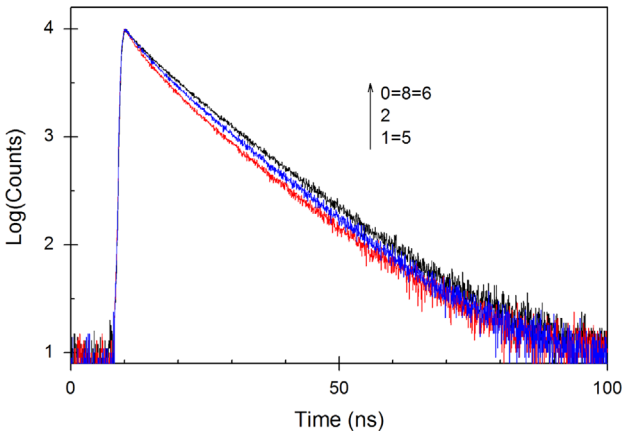

Fig. 7. Fluorescence decay profile of TMPyP bound to the G-quadruplexes. The decay curves appeared to be identical for oligonucleotides 1, 8 and 6. Oligonucleotides 1 and 5 also produced an identical decay profile. [TMPyP]/[G-quadruplex]=1. [TMPyP]=0.5 μM. The excitation and emission wavelengths were 405 nm and 650 nm, respectively.

increased in the order of quadruplex $0 \approx 8 \approx 6 > 2 > 5 \approx 1$. Although precise elucidation of the fluorescence decay behavior requires further study, they appear to be related to the stability of the quadruplexes, particularly for the quadruplex 1 case. Quadruplex 1 is the least stable, as shown in the thermal dissociation experiment. This suggests that the I base at position 1 has the largest degree of freedom, from which it has a greater chance to interact with TMPyP.

In summary, the order of variation, $0 \approx 8 > 6 > 5 > 1$, in the absorption and CD spectral property as well as the changes in the fluorescence decay property of the quadruplex bound TMPyP roughly coincides. That order also coincides with the thermal stability of the quadruplex. Although unclear at this stage, the degree of freedom of the I base at the 1 position was the largest, and that the order of freedom followed the order of the spectral variations.

4. Conclusion

Replacement of a G base with an I base resulted in destabilization of the G-quadruplex, except for the G8 base, as expected from the lack of a hydrogen bond. The missing hydrogen bond at position 1 in the upper G-quartet destabilized the G-quadruplex most efficiently. Replacement also affects the binding mode of TMPyP suggesting that TMPyP binds at the side of the G-quadruplex. A change in the binding mode was most pronounced when the G1 bases was replaced, suggesting that this base is most flexible when replaced with an I base.

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Appendix A. Transparency document

Transparency document associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2015.03.012.

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