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Detection of structure-switching in G-quadruplexes using end-stacking ability

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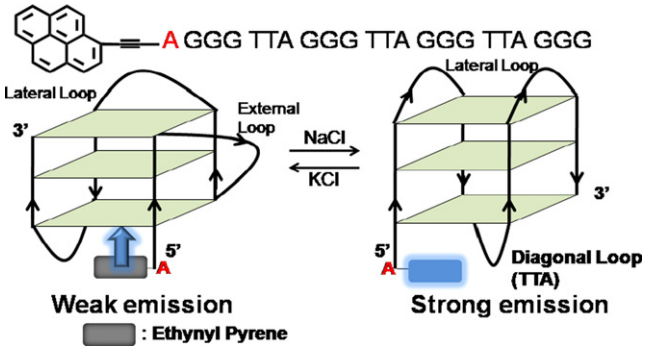
ABSTRACT

G-quadruplex-forming ODNs containing nonpolar aromatic fluorophore moiety, $\mathbf{A^{PY}}$ at the dangling ends undergo π-stacking on surface of G-quadruplex, and the fluorescence change can be used to distinguish the structure-switching between the mixed parallel/antiparallel structure and antiparallel structure.

Structural transition of G-quadruplex DNA induced by changes in K^+/Na^+ ratios may play an important role in controlling gene expression, such as DNA replication, transcription of particular genes, and telomere function.[1] A number of reports have also documented altered K^+/Na^+ ratios in tumor cells.[2] Human telomeres fold in a manner that is fundamentally distinct from that of lower organisms.[3] The antiparallel fold of human telomeric quadruplex sequences is a consequence of Na^+ ions,[4] whereas the more strongly bound K^+ ions induce a transition to a mixed parallel/antiparallel arrangement.[5] The mixed formed G-quadruplex structure was defined recently.[6] Inspection of the structures of mixed-structure and antiparallel-structure G-quadruplexes suggests that there is a significant difference in the end stack position in the two structures.[6] In the mixed parallel/antiparallel structure, the G-quadruplex surface is exposed for stacking with a planar aromatic fluorophore whereas, in the antiparallel structure, the diagonal loop hinders the interaction of fluorophore with G-quadruplex surface.

We expected that this difference could be monitored via a pyrene-attached deoxyadenosine ($\mathbf{A^{PY}}$), which was developed by our research group[7]: $\mathbf{A^{PY}}$ showed efficient planar aromatic stacking ability in the end stacking position of duplex DNA. The fluorescence of $\mathbf{A^{PY}}$ has been used to probe charge transfer within DNA duplex, in which the G bases function as a quencher in the end stacking position (Scheme 1).[8]

Several researchers have adopted a structure-based design approach to develop a G-quadruplex probing system.[9] Here we first report a fluorescent probing system[10] to detect the G-quadruplex structural change from an antiparallel to a mixed parallel/antiparallel structure.



Scheme 1. Design of a structure-switching signaling G-quadruplex probing system.

We synthesized the 22-mer oligonucleotide, **S1** (5′-$\mathbf{A^{PY}}$GGG TTA GGG TTA GGG TTA GGG-3′) and **N1** (5′-AGGG TTA GGG TTA GGG TTA GGG-3′) using phosphoramidite chemistry and a DNA synthesizer (Fig. 1).[11]

S1 5′-$\mathbf{A^{PY}}$ GGG TTA GGG TTA GGG TTA GGG
N1 5′-A GGG TTA GGG TTA GGG TTA GGG

We added NaCl to the solution of **S1** to induce the conformational change from single strand to G-quadruplex (antiparallel structure). We investigated the conformational change of the $\mathbf{A^{PY}}$-containing **S1** using circular dichroism (CD) spectroscopy (Fig. 2A). The antiparallel structure showed characteristic bands at around 260 nm (negative band) and 295 nm (positive band)

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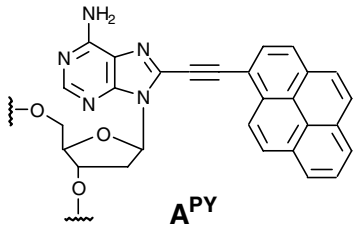

Figure 1. Structures of $\mathbf{A}^{PY}$ and the oligonucleotide sequences used in this study.

after the addition of NaCl. In contrast, after adding KCl to a solution of **S1** to induce the mixed parallel/antiparallel type G-quadruplex, **S1** showed a strong positive band at around 290 nm with weak negative bands near 255 and 235 nm, indicating the presence of mixed G-quadruplex.[6] We also measured the CD spectra about the natural sequence **N1**, and we could compare with the case of the modified sequence **S1** (Fig. 2B). From this CD experiment, we confirmed that $\mathbf{A}^{PY}$ incorporation does not interfere with the conformational stability of the G-quadruplexes.

We observed a dramatic difference between the fluorescence emission properties of the antiparallel and mixed structures of the pyrene-modified G-quadruplex. The antiparallel structure G-quadruplex exhibited a increase in fluorescence relative to the single strand (Fig. 3A). In case of adding KCl to a solution of **S1**, emission is decreased in log scale (Fig. 3B and C). We think that the changes in fluorescence emission that occur during the transition from antiparallel structure to mixed parallel/antiparallel structure must arise from changes in the electrostatic interactions between the $\mathbf{A}^{PY}$ and the neighboring nucleobases. Deoxyguanine is a well-known fluorescence quencher.[12] In the mixed parallel/antiparallel structure of G-quadruplex the deoxyguanosine interacts effectively with the terminal π-stacking pyrene, and the fluorescence is decreased. On the other hand, in the antiparallel structure, there seems to be increasing in fluorescence intensity. This is because the diagonal loop prevents the interaction between fluorophore and G-quadruplex. We believe that this property allows our system to discriminate between the antiparallel and mixed structures of G-quadruplex.

We measured the melting temperatures to determine the strengths of the stacking interactions. The G-quadruplexes containing nonpolar aromatic termini $\mathbf{A}^{PY}$ were more stable than their natural counterparts. The melting temperature for the natural N1 with a KCl salt (100 mM, mixed parallel/antiparallel structure G-quadruplex) was 56 °C, but for the modified S1 with a KCl salt

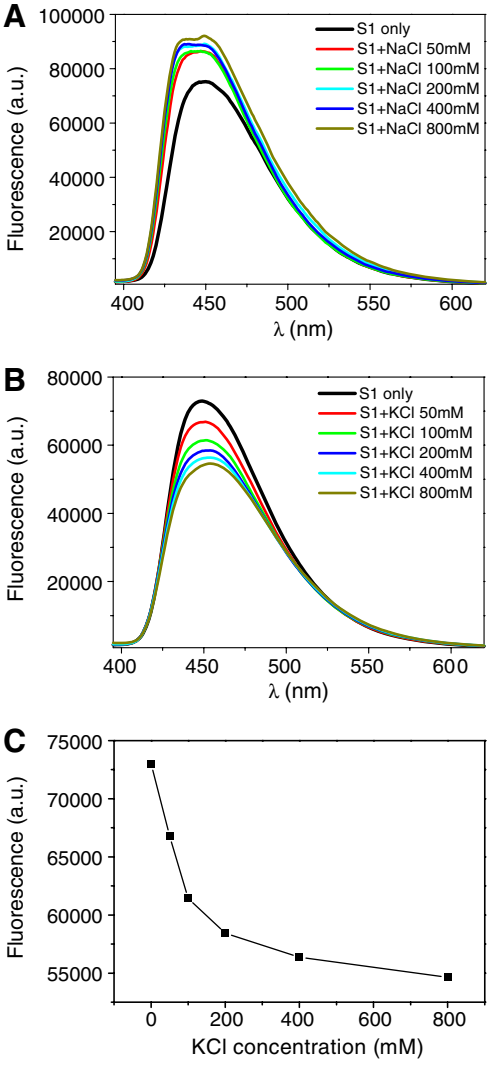

Figure 3. (A) Fluorescence spectra of the single-stranded **S1** (1.5 μM, black line) and **S1** with NaCl (50–800 mM,[9] red to dark yellow line). (B) Fluorescence spectra of the single-stranded **S1** (1.5 μM, black line) and **S1** with KCl (50–800 mM, red to dark yellow line). (C) Maximum emission intensity depending from the concentration of KCl. All samples were prepared at a concentration of 1.5 μM in 25 mM Tris–HCl buffer (pH 7.2) at 20 °C and excited at 386 nm.

(100 mM, mixed parallel/antiparallel structure G-quadruplex), it was much higher (77 °C). The modified S1 with a NaCl salt (100 mM, antiparallel structure G-quadruplex) melted at 72 °C,

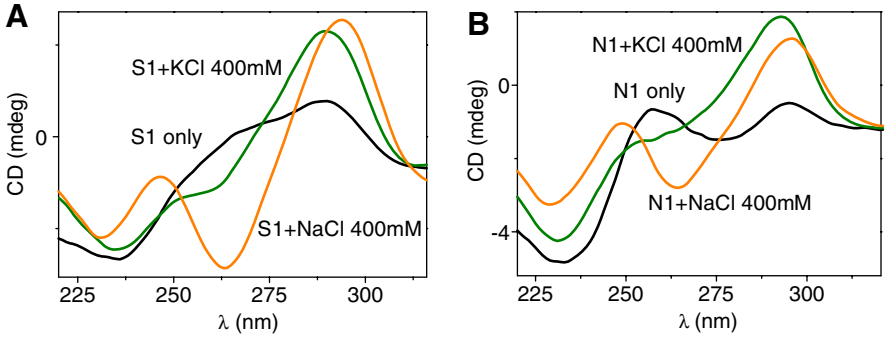

Figure 2. (A) CD spectra of **S1** (single strand) of **S1** with 400 mM NaCl (antiparallel structure G-quadruplex), and of **S1** with 400 mM KCl (mixed parallel/antiparallel structure G-quadruplex). (B) CD spectra of **N1** (single strand), of **N1** with 400 mM NaCl (antiparallel structure G-quadruplex), and of **N1** with 400 mM KCl (mixed parallel/antiparallel structure G-quadruplex). All samples were prepared at a concentration of 1.5 μM in 25 mM Tris–HCl buffer (pH 7.2) at 20 °C.

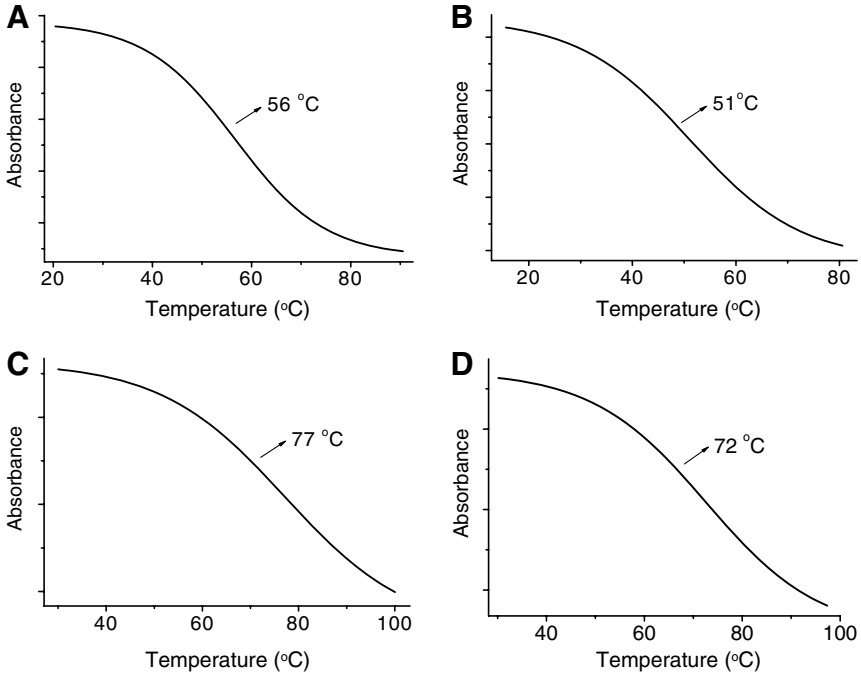

Figure 4. Melting temperature of (A) **N1** and (C) **S1** with 100 mM KCl, and (B) **N1** and (D) **S1** with 100 mM NaCl. All samples were prepared in buffer 25 mM Tris–HCl (pH 7.2), 1.5 μM, under irradiation at 295 nm.

and the natural one of N1 with a NaCl salt (100 mM, antiparallel structure G-quadruplex) melted at 51 °C (Fig. 4).

We observed that the mixed parallel/antiparallel structure of the G-quadruplex was more stable than the antiparallel structure of the G-quadruplex, in both the unmodified G-quadruplex **N1** and the modified G-quadruplex **S1** (about 5 °C difference). We also observed remarkable differences between the stabilities of the unmodified G-quadruplex **N1** and the fluorophore-modified G-quadruplex **S1**. The melting temperature of the fluorophore-modified G-quadruplex **S1** was higher (about 21 °C) than that of the unmodified G-quadruplex **N1**. These observations suggest that aromatic stacking of the $\mathbf{A^{PY}}$ units at the dangling end, both in the antiparallel and the mixed parallel/antiparallel structures of the G-quadruplex, does increase stability. It seems that the hydrophobicity of the pyrene-modified bases $\mathbf{A^{PY}}$ contributes favorably to enhance the stacking ability in both the antiparallel and mixed parallel/antiparallel structures.

To deduce the origin of this emission spectral feature, that is, to determine whether it arose from a structural change (from mixed parallel/antiparallel to antiparallel or vice versa), we recorded the fluorescence spectra (Fig. 5A) and CD spectra (Fig. 5B and C) of **S1** at various concentration ratios of NaCl and KCl. Increasing the concentration of NaCl resulted in an decrease in the intensity of the negative band at 260 nm, indicating the presence of the antiparallel structure. In contrast, increasing the concentration of KCl resulted in an increase in the intensity of the band at around 260 nm, indicating the presence of the mixed parallel/antiparallel structure. When the concentration ratios KCl to NaCl were 1:2 and 1:3, the structure would change to the mixed parallel/antiparallel type. We observed same changes in the fluorescence spectra at the same salt concentration ratios. The fluorescence intensity of **S1** increased proportionately upon increasing the ratio of the antiparallel conformation (by increasing the NaCl concentration), while the fluorescence of **S1** decreased upon increasing the ratio of the mixed conformation (increasing the KCl concentration). This fluorescence intensity change is consistent with the structural change confirmed by CD.

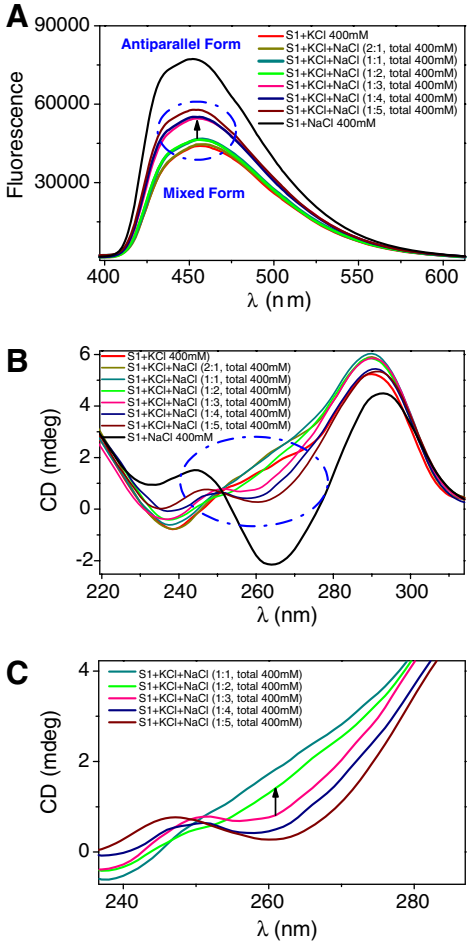

Figure 5. (A) Fluorescence spectra of **S1** at various salt concentration ratios. (B) CD spectra of **S1** at various salt concentration ratios. (C) Magnification of the circled region in Figure 5B. Total salt concentration is 400 mM. All samples were prepared at a concentration of 1.5 μM in 25 mM Tris–HCl buffer (pH 7.2) at 20 °C and excited at 386 nm.

These results indicate that the decreased fluorescence of $\mathbf{A^{PY}}$ in the mixed parallel/antiparallel structure of G-quadruplex is in accordance with a stacking interaction between the $\mathbf{A^{PY}}$ and the surface of G-quadruplex with charge transfer. The intense fluorescence signal of **S1** in the antiparallel conformation may be explained as being due to the disruption of the charge transfer by the diagonal loop (TTA), which is in close proximity to the pyrene.

In conclusion, we have successfully used $\mathbf{A^{PY}}$ units as nonpolar aromatic fluorophore moieties that undergo π-stacking at the dangling ends of G-quadruplex. Such π-stacking is shown to stabilize the mixed parallel/antiparallel and antiparallel structures of these modified G-quadruplexes, as compared to unmodified G-quadruplexes. Most importantly, the terminal π-stacking induces a fluorescence decrease in the mixed parallel/antiparallel structure of G-quadruplex, but not in the antiparallel structure; this phenomenon allows us to differentiate the two states visually. Using the fluorescence signal change we can also monitor the reversible transformation from mixed parallel/antiparallel to antiparallel structure, and vice versa. Thus, the pyrene-labeled oligodeoxyadenylates have the potential to be used as new types of sensors for detecting the structure-switching of G-quadruplexes.

Acknowledgment

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Supplementary data

MALDI-TOF mass spectral data, HPLC data, and denaturing gel electropherograms of ODNs. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.06.033.

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