Detection of the G-quadruplex-TMPyP4 complex by 2-aminopurine modified human telomeric DNA†

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2-Aminopurine (Ap) modified human telomere sequences were used to monitor the specific complex formation of the G-quadruplex and 5,10,15,20-tetrakis(*N*-methyl-4-pyridyl)-porphyrin (TMPyP4).

The G-rich sequences in the linear chromosome of the eukaryotes can form a G-quadruplex structure.[1,2] The antiparallel G-quadruplex with the lateral and diagonal loops has one wide groove, two medium grooves, and one narrow groove.[3] The groove widths vary among the different antiparallel G-quadruplexes, which may provide the structural diversities needed for the specific G-quadruplex recognition. Since a ligand generally binds by externally stacking to the G-quartet, the structures of the loops and adjacent regions might also influence the binding specificity.

Recently, we reported that Ap can be used to monitor the duplex to quadruplex conformational change of the human telomeric DNA sequence.[4] Ap is a fluorescent adenine isomer able to form a Watson–Crick base-pair with thymine.[5] In this study, we investigated the possibility of using Ap as a probe for G-quadruplex–ligand interactions. We used cationic porphyrin TMPyP4 (Fig. 1B) as a G-quadruplex-interactive agent to form the specific G-quadruplex–porphyrin complexes. TMPyP4 was reported to be a telomerase inhibitor.[6] It is known that TMPyP4 selectively interacts with the G-quadruplex.[7,8] We have prepared a set of Ap modified G-quadruplex-TMPyP4 complexes (Table 1, P7, P13, and P19, Fig. 1). The titration of TMPyP4 into a solution of the Ap modified G-quadruplexes showed a relative increase in the Ap fluorescence. The estimated saturation concentration of TMPyP4 by fluorescence titration studies was approximately 4 μM (Fig. 2A). This result indicates that TMPyP4 forms a 1 : 1 complex with the Ap modified G-quadruplex (single strand conc. 3.6 μM). Notably, a significant increase in the fluorescence intensity of Ap at **P13** of the G-quadruplex was observed in the presence of TMPyP4. The fluorescence enhancement of Ap at **P13** was over 3-fold higher compared with that at **P7** and **P19** in the G-quadruplex–TMPyP4 complex. Hurley *et al.* reported the binding mode of the G-quadruplex–TMPyP4 complex.[9] The complex was constructed by inserting TMPyP4 between a central diagonal loop and the G-quartet for d(AG$_3$[T$_2$AG$_3$]$_3$), and removing the counter ion closest to the pyrimidinium groups of

Table 1 Sequences and melting temperatures[a] (T_m) of the Ap modified G-quadruplex and the Ap and ^{dz}G modified G-quadruplex

Oligo.	Sequences	T_m/°C
P0	AGGGTTAGGGTTAGGGTTAGGG	61.8
P7	AGGGTT*Ap*GGGTTAGGGTTAGGG	55.8
P13	AGGGTTAGGGTT*Ap*GGGTTAGGG	58.3
P19	AGGGTTAGGGTTAGGGTT*Ap*GGG	57.4
PZ7	AGGGTT*Ap*^{dz}**G**GGTTAGGGTTAGGG	54.8
PZ13	AGGGTTAGGGTT*Ap*^{dz}**G**GGTTAGGG	53.7
PZ19	AGGGTTAGGGTTAGGGTT*Ap*^{dz}**G**GG	54.1

[a] Thermal denaturation profiles were recorded on a Jasco V-530 UV/VIS spectrophotometer. Absorbance of the samples was monitored at 260 nm from 10 to 80 °C. DNA samples are at a concentration of 3.6 μM (single strand conc.), sodium chloride of 100 mM, and pH 7, buffered by 5 mM sodium phosphate solution, at 7 °C in the following conditions.

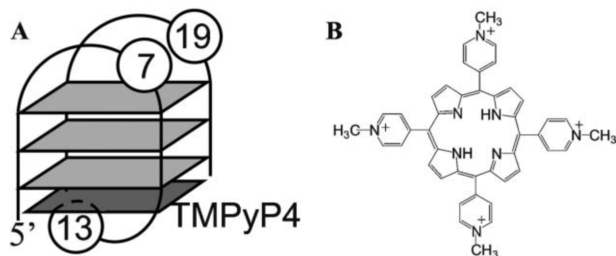

Fig. 1 (A) Schematic diagram of the G-quadruplex–TMPyP4 complex in which the numbers correspond to the positions of the adenines that were individually replaced by Ap in this study. (B) The structure of TMPyP4.

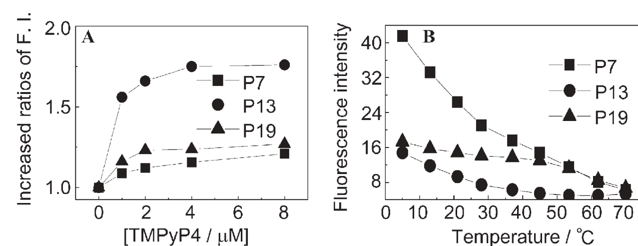

Fig. 2 (A) The fluorescence titration (λ_{ex} = 305 nm) of the Ap modified G-quadruplex; **P7**, **P13**, and **P19** with TMPyP4 (0, 1, 2, 4, and 8 μM). DNA samples are at a concentration of 3.6 μM (single strand conc.), sodium chloride of 100 mM, and pH 7, buffered by 50 mM sodium phosphate solution, at 5 °C in the following conditions. (B) The thermal melting profiles of the Ap modified G-quadruplexes. The samples were monitored the fluorescence emission (λ_{em} = 370 nm) of Ap from 5 to 70 °C.

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† Electronic supplementary information (ESI) available: Fluorescence titration experiments of Ap modified G-quadruplex and Ap and ^{dz}G modified G-quadruplex with TMPyP4 at various concentrations. CD spectra of Ap and ^{dz}G modified G-quadruplexes. See DOI: 10.1039/b514526k

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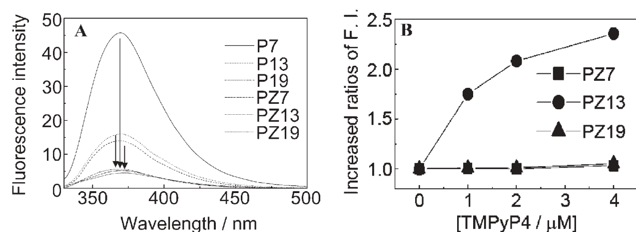

Fig. 3 (A) Fluorescence spectra of the Ap modified G-quadruplexes and the Ap and ^{dz}G modified G-quadruplexes. (B) The fluorescence titration of the Ap and ^{dz}G modified G-quadruplex; **PZ7**, **PZ13**, and **PZ19** with TMPyP4 (0, 1, 2, and 4 μM).

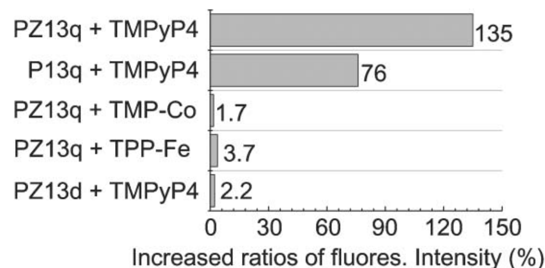

Fig. 4 Specific recognition of TMPyP4 by **PZ13** G-quadruplex (PZ13q). The concentrations of free and metal porphyrins (TMPyP4, TMP-Co, and TPP-Fe) are 4 μM.

TMPyP4. Therefore, Ap at **P13** in the diagonal loop adjacent to the porphyrin may be significantly affected by the TMPyP4 stacking, leading to the increase in the Ap fluorescence intensities at **P13** because of the decrease in the efficiency of the electron transfer quenching of Ap at **P13** by neighboring bases (Fig. 1A).

In order to investigate the denaturation profiles of the G-quadruplex, we performed a fluorescence melting study using the Ap modified G-quadruplex. The melting-induced decrease in the fluorescence intensities of Ap in the G-quadruplexes was clearly observed with the increase in the solution temperature (Fig. 2B). Electron transfer quenching of Ap in the singlet excited state (^{1}Ap*) by neighboring guanines may be enhanced in the linear single strand compared with that in the G-quadruplexes, since distortion of the strand in the G-quadruplexes was relaxed by thermal denaturation to produce an efficient interaction between ^{1}Ap* and the neighboring nucleobases.[10–11]

To design a more sensitive probe of the G-quadruplex–TMPyP4 complex, ^{dz}G was incorporated into the G-quartet adjacent to Ap (Table 1, **PZ7**, **PZ13**, and **PZ19**). ^{dz}G has a much lower oxidation potential than G, and is expected to amplify the effects of electron transfer during the Ap fluorescence quenching.[12] First, the effects of the ^{dz}G substitution on the stability of the G-quadruplex were examined by measuring the melting temperature and the CD spectra (see ESI†). The results showed that the ^{dz}G substitution does not significantly destabilize the G-quadruplex structure. The incorporation of ^{dz}G resulted in a decreased fluorescence intensity of Ap, showing that ^{1}Ap* was efficiently quenched by ^{dz}G (Fig. 3A). Therefore, we confirmed that the fluorescence intensity of Ap in the loop of the G-quadruplex is affected by the G-quartet. The titration of TMPyP4 into a solution of **PZ13** resulted in about a 2.5-fold increase in the fluorescence intensity of Ap, showing that **PZ13** serves as a better probe than **P13**. In contrast, as for the other ^{dz}G substituted ODNs (**PZ7** and **PZ19**), the fluorescence intensity of Ap was not affected by the presence of TMPyP4 (Fig. 3B). Thus, the results clearly demonstrate that the increase in the fluorescence intensity of Ap is due to the binding of TMPyP4 between the G-quartet and diagonal loop.

In order to examine the ability of **PZ13** to detect the G-quadruplex-TMPyP4 complex formation, fluorescence titration experiments were performed with a number of transition-metal complexes of porphyrins (5,10,15,20-tetrakis(4-methoxyphenyl)-21*H*,23*H*-porphyrin cobalt(II); TMP-Co and 5,10,15,20-tetrakis-(pentafluorophenyl)porphyrin iron(III); TPP-Fe). In results, no change in the Ap fluorescence was observed in the presence of the transition-metal complexes of the porphyrin (Fig. 4). The fluorescence intensity of Ap was also unaffected by the addition of TMPyP4 when PZ13 forms a duplex (PZ13d) with the complementary strand. These results clearly showed that PZ13 can be used for the detection of the G-quadruplex–TMPyP4 complex.

In conclusion, Ap modified human telomere sequences were used to monitor the specific complex formation of the G-quadruplex and TMPyP4. These probes are not only useful to detect G-quadruplex–TMPyP4 complex formation, but may also provide information about the denaturation process of the G-quadruplex. It will be useful for studying the biological role of the G-quadruplexes.

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