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Fluorescence properties of 2-aminopurine in human telomeric DNA†

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The substitution of 2-aminopurine (Ap) for A7 in the human telomeric sequence d[AGGG(TTAGGG)$_3$] resulted in a significant increase in the fluorescence intensity of Ap for the conformational change from duplex to quadruplex.

Telomeres, which are the ends of the linear chromosomes of eukaryotes, contain tandem repeats of short G-rich DNA sequences that form structures based on the G-quartet.[1–3] An intramolecular basket-type quadruplex was observed by the folding of the human telomeric sequence d[AGGG(TTAGGG)$_3$].[4] Recent discoveries suggest that telomeres exist in at least two different states or architectures; *i.e.*, 'open' and 'closed' complexes.[5] The 'closed' quadruplex form seems to represent the state that caps and protects the chromosome end. For direct observation of the quadruplex folding, fluorescence resonance energy transfer (FRET) between the fluorophores attached at each end of the telomeric DNA was measured.[6–8] FRET efficiently occurs in the 'closed' state, in which two fluorophores are in close proximity. In the 'open' structure, the two fluorophores are separated and no FRET is observed. However, a bulky substituent such as a fluorophore may inhibit the DNA structural transitions, making it difficult to study the dynamics of the telomeric DNA.[9]

Here, we selected Ap as the structure-sensitive fluorescence probe of the quadruplex. Ap is a fluorescence adenine isomer able to form a Watson–Crick base-pair with thymine.[10,11] The fluorescence properties of Ap in the singlet excited state (^{1}Ap*) in DNA are strongly influenced by the electron transfer quenching process from G.[12–15] Hence, Ap has been widely employed as a fluorescence probe of the protein-induced local conformational changes in DNA.[16–18] To test the possibility of using Ap as a probe for the human telomeric quadruplex, we have prepared a set of human telomeric DNA sequences containing site-specific Ap substitution (Fig. 1A, Table 1).

First, the effects of the Ap substitution on the stability of the quadruplex were evaluated by measuring the melting temperature of each oligodeoxynucleotide (ODN) by UV thermal denaturation and the CD spectra (see electronic supplementary information†). The melting temperatures (T_m) of the Ap modified quadruplexes were not significantly lower than that of the unmodified one. The CD spectra of Ap modified quadruplexes showed two peaks around 240 and 290 nm, and a negative band near 260 nm, which are typical for an antiparallel quadruplex structure.[19] Therefore, the quadruplex can also form in the presence of Ap. We then measured fluorescence intensities for the steady-state fluorescence emission of Ap in duplexes (F_{dup}) and quadruplexes (F_{quad}). An important observation was that F_{quad} was consistently higher than F_{dup} for all the studied sequences. Because π-stacking within the loop by the TTA segments in a quadruplex is highly distorted, the electron transfer quenching of ^{1}Ap* by G in the quadruplex seemed to decrease. In P7 ODN, a significant increase of over 30-fold was observed in the fluorescence intensity of Ap for the conformational change from the duplex to quadruplex (Table 1). Interestingly, in the quadruplex form, the fluorescence intensity of Ap at the A7 position (P7) was 2–3 times higher than that at the other positions. It is known that the human telomeric quadruplex is stabilized by three stacked G-quartets which are connected by two lateral loops and a central diagonal loop.[4] Among the four grooves that are formed, one is wide, two are of medium width and one is narrow. Fig. 1B shows that Ap at the A7 position of the quadruplex faces one wide and one medium groove (P7). On the other hand, Ap at the A13 (P13) and A19 (P19) positions of the quadruplexes face one narrow and one medium groove. Therefore, Ap at the A7 position is likely to be more hydrated compared with Ap at the other positions. Thus, the solvent accessible surface area of Ap may also be responsible for the fluorescence properties of Ap, since the

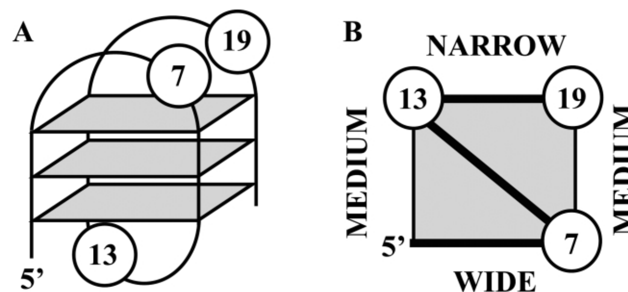

Fig. 1 (A) Schematic diagram of the human telomeric quadruplex in which the numbers correspond to the positions of the adenines that were individually replaced by Ap in this study. (B) Schematic top view indicating the position of Ap and G-quartet groove widths.

† Electronic supplementary information (ESI) available: CD spectra of Ap modified and unmodified human telomeric quadruplex. See http://www.rsc.org/suppdata/cc/b4/b403913k/

Table 1 Relative fluorescence intensities[a] at 370 nm (F), lifetimes (τ) and melting temperatures (T_m) of Ap modified duplexes and quadruplexes[b]

No.	Sequences	F_{dup}[c]	F_{quad}	$K/\times10^3$ M^{-1}	$\tau_{1\,(dup)}$/ns (α_1)	$\tau_{2\,(dup)}$/ns (α_2)	$\tau_{1\,(quad)}$/ns	T_m/°C[d]
P0	5′-AGGGTTAGGGTTAGGGTTAGGG							61.8
P7	5′-AGGGTT*Ap*GGGTTAGGGTTAGGG	1	32	4.2[e] / 0.95[f]	0.043 (0.82)	0.644 (0.18)	0.532	55.8
P13	5′-AGGGTTAGGGTT*Ap*GGGTTAGGG	0.93	11	2.0[e] / 0.88[f]	0.048 (0.76)	0.531 (0.24)	0.340	58.3
P19	5′-AGGGTTAGGGTTAGGGTT*Ap*GGG	1.0	13	2.4[e] / 1.0[f]	0.052 (0.81)	0.639 (0.19)	0.354	57.4

[a] Relative fluorescence intensities were evaluated based on the fluorescence intensity of the P7 duplex. The fluorescence quantum yields of Ap in duplex and quadruplex were up to 0.005 and 0.06, respectively. [b] Measurement conditions are similar to Fig. 2. [c] Ap modified telomeric sequences were hybridized with the complementary DNA strand (5′-CCCTAACCCTAACCCTAACCCT). [d] Thermal denaturation profiles were recorded using a Jasco V-530 UV/VIS spectrophotometer with a thermoelectrically controlled sample holder. Absorbance of the samples was monitored at 260 nm from 2 to 80 °C at a heating rate of 1 °C min^{-1}. [e] Measured for quadruplex structure. [f] Measured for duplex structure.

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fluorescence quantum yield of Ap is higher in polar solvents.[20] To test the microenvironment dependency of the Ap fluorescence properties, fluorescence quenching of ^{1}Ap* by the water-soluble antioxidant ascorbic acid[21–23] was investigated. In Fig. 2, the ratio F^0/F was plotted *versus* the concentration of ascorbic acid where F^0 and F are the fluorescence intensities of Ap in the presence and absence of ascorbic acid as the quencher. The slopes (K) of these plots can be assumed to reflect the solvent accessibility of Ap. The K values were in the order $P7_{quad} >> P19_{quad} > P13_{quad} >> P7_{dup}$.[24] Therefore, Ap in the P7 quadruplex is more exposed to solvent than Ap at the other positions in the quadruplex, or Ap in the duplex, resulting in a high fluorescence intensity.

Time-resolved fluorescence measurements with a femtosecond laser were also performed to measure the fluorescence lifetime of ^{1}Ap*, showing results consistent with the steady-state fluorescence measurements.[25] The lifetime of ^{1}Ap* was significantly different in the duplex and quadruplex. In the case of P7 ODN, the lifetime of ^{1}Ap* in the quadruplex ($\tau_{1\,(quad)}$) was 14-fold longer than that in the duplex ($\tau_{1\,(dup)}$). The lifetime of ^{1}Ap* in the P7 quadruplex ($\tau_{1\,(quad)}$) was over 1.5-fold longer than that in the other Ap containing quadruplexes. The longer lived components ($\tau_{2\,(dup)}$) in duplexes are attributed to poorly stacked minor conformers.[26]

Our results showed that Ap can be used to monitor the duplex to quadruplex conformational change of the human telomeric DNA sequence. The fluorescence property of Ap in the quadruplex depended on the DNA stacking interactions and solvent accessibility. Since human telomeric DNA sequences are known to interact with several proteins,[27,28] Ap may serve as a useful fluorescence probe to study such telomere–protein interactions.

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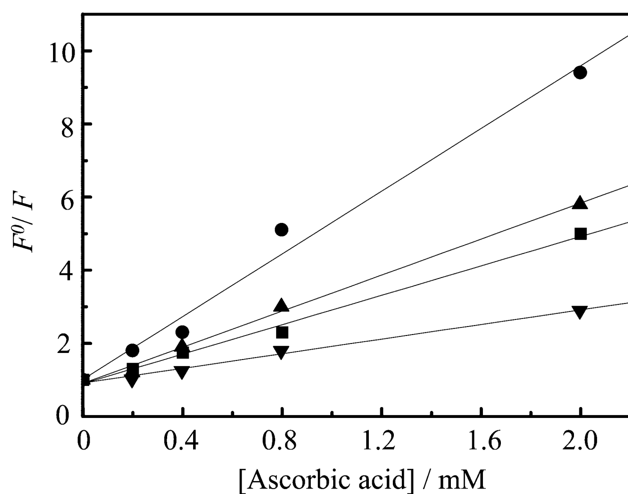

Fig. 2 Fluorescence quenching of ^{1}Ap* in the quadruplexes and duplex by ascorbic acid. F^0/F was plotted *versus* the concentration of ascorbic acid, where F^0 and F are the fluorescence intensities at 370 nm (λ_{ex} = 300 nm) of Ap in the presence and the absence of ascorbic acid; $P7_{quad}$ (●), $P19_{quad}$ (▲), $P13_{quad}$ (■), $P7_{dup}$ (▼). Sample concentration: 100 μM (base conc.) ODN, 50 mM pH 7.0 sodium phosphate buffer, and 100 mM sodium chloride at 8 °C.

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