J|A|C|S COMMUNICATIONS

Published on Web 05/06/2006

Quadruplex-to-Duplex Transition of G-Rich Oligonucleotides Probed by Cationic Water-Soluble Conjugated Polyelectrolytes

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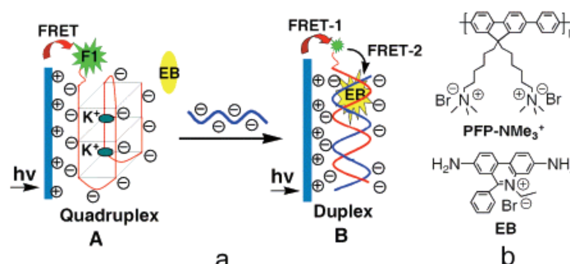
G-quadruplexes are tetraplex conformations of telomere DNA that directly inhibit telomerase activity in immortalized and most cancer cells[1] and have the potential to control gene expression.[2] The structural stability of the G-quadruplexes realizes their function. It has been demonstrated that G-quartet DNA can predominantly convert to duplex form in the presence of its complementary strand under physiological conditions.[3] Efficient probing of the quadruplex-to-duplex transition can thus provide useful insight for designing and developing telomerase inhibitors for cancer, HIV, and other diseases.[1b,4] Conformational changes involved in quadruple-to-duplex transitions have also been used to demonstrate actions akin to nanomolecular machines.[5]

Assays for probing quadruplex-to-duplex transitions take advantage of UV melting curves, circular dichroism (CD), polyacrylamide gel electrophoresis, Raman spectroscopy, surface plasmon resonance, electrospray mass spectrometry, and NMR techniques.[6] These methods are time-consuming and laborious or require strand concentrations that may favor formation of higher-order aggregates. Techniques based on fluorescence resonance energy transfer (FRET) have also appeared;[7] however, they require doubly labeled DNA or PNA probes, which are expensive. There is thus a need for more sensitive and convenient methods to examine quadruplex/duplex transitions.

Conjugated polymers (CPs) have light-harvesting properties that, in conjunction with reporter fluorophores attached to high-specificity probes, can be used in the function of very sensitive optical biosensors.[8] The CP coordinates the action of a large number of absorbing units with efficient intrachain and interchain energy-transfer mechanisms.[9] Recently, we and others have utilized these optical properties to detect DNA, RNA, proteins, and metal ions.[10−17] As demonstrated here, it is also possible to use CPs to design a sensitive, simple, homogeneous, and real-time protocol that responds to quadruplex/duplex transitions in G-rich DNA.

The overall strategy is illustrated in Scheme 1a. G-quartet DNA is labeled with fluorescein (Fl) at the 5′-terminus (G-quadruplex-Fl). One begins with a solution that contains the cationic CP (CCP) $PFP-NMe_3^+$ (poly{(1,4-phenylene)-2,7-[9,9-bis(6′-*N,N,N*-trimethylammonium)hexyl fluorene]diiodide}), G-quadruplex-Fl, and ethidium bromide (EB, an intercalator of double-stranded DNA, dsDNA). As shown in Scheme 1a-A, electrostatic interactions between G-quadruplex-Fl and $PFP-NMe_3^+$ keep them in close proximity, allowing for FRET from $PFP-NMe_3^+$ to Fl. EB does not intercalate into the G-quadruplex (Supporting Information). Situation B in Scheme 1a illustrates how addition of a complementary strand results in the transition from G-quadruplex to dsDNA-Fl. Under these conditions the EB intercalates into the grooves of dsDNA-

Scheme 1. (a) Schematic Representation of the Assay for G-Quadruplex/dsDNA Transitions; (b) Chemical Structures: $ssDNA_C$ Is Complementary to G-rich DNA, the $ssDNA_{6NC}$, $ssDNA_{3NC}$, and $ssDNA_{1NC}$ are Strands that Are Complementary to G-rich DNA with Six, Three and One-Base Mismatches (highlighted in red)



Fl. Excitation of $PFP-NMe_3^+$ leads to a two-step energy transfer: from $PFP-NMe_3^+$ to dsDNA-Fl (FRET-1), followed by FRET from dsDNA-Fl to EB (FRET-2). Direct FRET from $PFP-NMe_3^+$ to EB is not favored due to nonoptimized transition dipole orientations of $PFP-NMe_3^+$ and EB within the CCP/dsDNA(EB) complex.[18]

Figure 1a compares the emission spectra of G-quadruplex-Fl ([G-quadruplex-Fl] = 5.0×10^{-8} M) with $PFP-NMe_3^+$ and EB ([$PFP-NMe_3^+$] = 1.25×10^{-6} M, in repeat units, [EB] = 1.5×10^{-6} M) before and after addition of complementary $ssDNA_C$ ([$ssDNA_C$] = 5.0×10^{-8} M). Measurements were performed in phosphate buffer (50 mM, pH = 7.4). The excitation wavelength was chosen at the absorbance maximum of $PFP-NMe_3^+$ (380 nm), where no significant absorption by Fl and EB occurs. In the absence of $ssDNA_C$, only FRET from $PFP-NMe_3^+$ to Fl is observed. Upon adding $ssDNA_C$, formation of the dsDNA takes place, followed by EB intercalation, and emission from EB is detectable. CD spectra were measured to confirm quadruplex-to-duplex transition (Figure 1b). For the G-quadruplex, CD spectra exhibit a positive peak at 290 nm with a shoulder at ~270 nm and a negative peak at 235 nm, which are characteristic of antiparallel G-quadruplex structures.[19] With $ssDNA_C$, the CD spectra show the disappearance of the peak at 290 nm and the appearance of positive peaks at 265 and 220 nm, with a negative peak at 240 nm, which are characteristic of dsDNA.[19a]

Figure 2 shows the EB fluorescence intensity at 600 nm as a function of the G-quadruplex-Fl/$ssDNA_C$ incubation time. In these experiments, a solution of G-quadruplex-Fl ([G-quadruplex-Fl] = 5.0×10^{-8} M), $PFP-NMe_3^+$ ([$PFP-NMe_3^+$] = 1.25×10^{-6} M) and EB ([EB] = 1.5×10^{-6} M) was prepared at room temperature,

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10.1021/ja058075w CCC: $33.50 © 2006 American Chemical Society

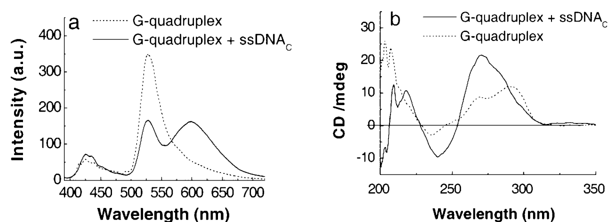

Figure 1. (a) Fluorescence spectra of G-quadruplex-Fl in the presence of $PFP-NMe_3^+$ and EB before and after addition of $ssDNA_C$, [G-quadruplex-Fl] = $[ssDNA_C] = 5.0 \times 10^{-8}$ M, $[PFP-NMe_3^+] = 1.25 \times 10^{-6}$ M, [EB] = 1.5×10^{-6} M; excitation wavelength is 380 nm; (b) CD spectra of G-quadruplex at 10 °C before and after addition of $ssDNA_C$, [G-quadruplex] = $[ssDNA_C] = 5.0 \times 10^{-6}$ M.

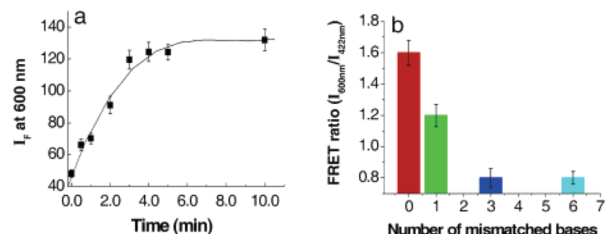

Figure 2. (a) The fluorescence intensity of EB against incubating time of G-quadruplex-Fl with its complementary $ssDNA_C$; (b) the FRET ratio as a function of the number of mismatched bases in the complementary strand. [G-quadruplex-Fl] = $[ssDNA_C] = [ssDNA_{1NC}] = [ssDNA_{3NC}] = [ssDNA_{6NC}] = 5.0 \times 10^{-8}$ M, $[PFP-NMe_3^+] = 1.25 \times 10^{-6}$ M, [EB] = 1.5×10^{-6} M; excitation wavelength is 380 nm. Error bars represent the standard deviation of four measurements.

and then the $ssDNA_C$ ($[ssDNA_C] = 5.0 \times 10^{-8}$ M) was added. Fluorescence spectra were then measured after a specific incubation period. As shown in Figure 2a, the EB emission intensity increases in the time range from 0 to 3 min, after which it reaches a plateau. This curve provides a measure of the rate for the G-quadruplex-to-duplex transition promoted by the presence of $ssDNA_C$. The CCP-based assay thus makes it possible to probe the transition between the G-quadruplex and duplex structures in real time and to provide kinetic measurements of structural transformations.

The effect of base mismatch in the complementary strand was also studied by adding $ssDNA_C$, $ssDNA_{6NC}$, $ssDNA_{3NC}$, and $ssDNA_{1NC}$ to G-quadruplex-Fl solutions under conditions similar to those in Figure 1, and the fluorescence spectra were measured after 5 min. As shown in Figure 2b, the ratio of the EB to $PFP-NMe_3^+$ fluorescence intensity follows the order: $ssDNA_{6NC} = ssDNA_{3NC} < ssDNA_{1NC} < ssDNA_C$. Increasing the number of mismatches inhibits the G-quadruplex-to-duplex transition.

In conclusion, the results described here demonstrate a real-time protocol to probe the transition between the G-quadruplex and duplex structures. Distinguishing aspects of this assay include the signal amplification of conjugated polymers, which should allow examination of more dilute concentrations of substrate, the intercalation of EB into dsDNA, and the FRET constraints brought about by the $PFP-NMe_3^+$/dsDNA supramolecular structure, which is static within the time scale of the excited states.[20] Furthermore, the assay provides a measure of how the number of mismatched bases in the complementary strand inhibits the transition from G-quadruplex to duplex conformation of DNA.

Acknowledgment. We are grateful for the financial support from the "100 Talents" program of Chinese Academy of Sciences, the National Natural Science Foundation of China (20421101 and 20574073), the National Institutes of Health (GM62958-01), and the National Science Foundation (DMR-0097611).

Supporting Information Available: Details for fluorescence experiments and the fluorescence titrations of EB as a function of [G-quadruplex] and [dsDNA]. This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) We recognize that inter-polyelectrolyte aggregates may be present in the assay solutions. However, the recognition processes remain operative, even under these circumstances, see ref 18.

JA058075W