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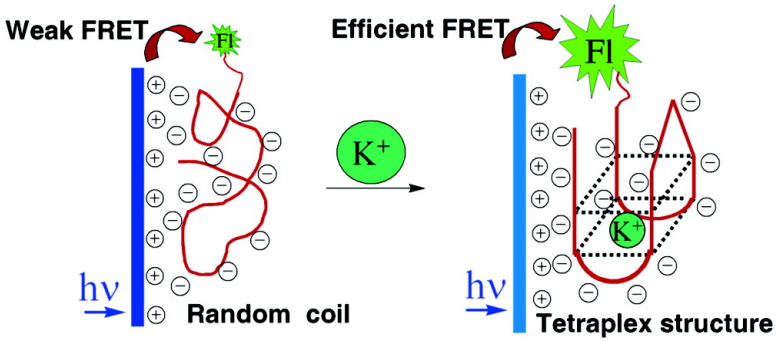
J. Am. Chem. Soc., **2005**, 127 (35), 12343-12346• DOI: 10.1021/ja051507i • Publication Date (Web): 12 August 2005



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Fluorescent Amplifying Recognition for DNA G-Quadruplex Folding with a Cationic Conjugated Polymer: A Platform for Homogeneous Potassium Detection

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Abstract: Single-stranded DNA with G-rich sequences can fold into secondary structures, G-quadruplexes, via intramolecular hydrogen-bonding interactions. This conformational change can be detected by a homogeneous assay method based on fluorescence resonance energy transfer (FRET) from a water-soluble cationic conjugated polymer (CCP) to a fluorescein chromophore labeled at the terminus of the G-quadruplex DNA. The space charge density around the DNA controls the efficiency of FRET from the CCP to the fluorescein. The higher FRET efficiency for the CCP/G-quadruplex pair is correlated to the stronger electrostatic interactions between the more condensed G-quadruplex and the CCP in comparison to the CCP/ssDNA pair. Since the potassium ion can specifically bind to the G-quadruplex DNA, the G-quartet-DNA/CCPs assembly can also be used as a platform to sense the potassium ion in water with high selectivity and sensitivity.

Introduction

Single-stranded DNA with G-rich sequences is able to fold into secondary structures via intramolecular hydrogen-bonding interactions, and such structures are named G-quadruplex.[1] Among these G-quadruplex sequences, the G-rich human telomere strand is of special interest.[2−4] The G-quadruplex is an unusual tetraplex conformation of the telomere DNA, which has been shown to directly inhibit telomerase activity in immortalized and most cancer cells.[5] Efficient recognition of the G-quadruplex structure is a key requirement for the rational design and development of telomerase inhibitors for cancer, HIV, and other diseases.[6,7] UV melting curves analysis, circular dichroism, gel electrophoresis, and NMR methods are widely used and serve as direct G-quadruplex folding probes;[7−9] however, they require large DNA quantities and lack sensitivity. Fluorescent methods based on fluorescence resonance energy transfer (FRET) require the difficult process of dual tagging the same DNA strand.[10] These considerations provide motivation for developing new assay methods that probe the DNA G-quadruplex structures.

G-quadruplex formation is known to be promoted by the presence of monovalent cations, especially by potassium ions.[11] It also provides one a chance to design and develop a selective and sensitive fluorescent sensor for the potassium ion using the same assay system that could probe the G-quadruplex structure.[10] Potassium ions play a key role in biological systems, such as the maintenance of extracellular osmolarity and the regulation of the concentration of other ions in the living cell, and an unbalance of K^+ ions is associated with arrythmia disease.[12,13] Although many fluorescent K^+ assays based on artificial receptors, such as fluoroionophores PBFI, [222] cryptand, B15C5 crown ether, and γ-cyclodextrin (γ-CD) have been reported,[14−16] the lack of high selectivity against sodium ions or the nonaqueous assay requirements for most of these systems prevents their clinical application.

It is well-known that the backbone of the conjugated polymers (CPs) consists of a large number of chromic repeat units. The excitation energy transferring to the reporter chromophore along the whole CPs backbone results in the amplification of fluorescent signals.[17,18] Recently, we[19−23] and others[9,24−27] have

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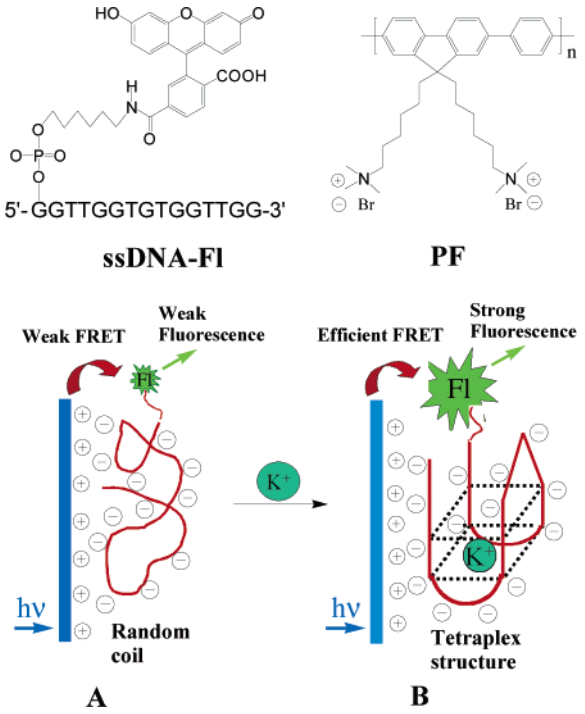
Scheme 1. Schematic Representation of the G-Quadruplex Structure and K^+ Assays and Chemical Structures of ssDNA-Fl and Cationic Polyfluorene



utilized this optical property of CPs for developing novel fluorescent biosensors based on water-soluble cationic conjugated polymers (CCPs) to detect DNA and RNA in aqueous media. In this contribution we demonstrate a new FRET-based, homogeneous technique to recognize the guanine quadruplex structure of the DNA that couples the conformation of a guanine-rich oligonucleotide strand with the optical amplification of CCPs. The G-quartet-DNA/CCPs assembly can also be used as a platform for sensing the potassium ion in water with high selectivity and sensitivity.

Results and Discussion

Our new DNA G-quadruplex and K^+ assays are illustrated in Scheme 1. The G-rich single-stranded DNA was labeled with a fluorescein at the 5′-terminus (ssDNA-Fl, see Scheme 1 for the molecular structure). The ssDNA-Fl exhibits random coil conformation in the absence of K^+.[10] The relatively weak electrostatic interactions of ssDNA-Fl with CCP keep the fluorescein far away from CCP, and FRET from CCP to fluorescein is inefficient (Scheme 1A).[28,29] The formation of a more condensed G-quadruplex upon adding KCl increases the space charge density around the DNA macromolecule[30−32] and

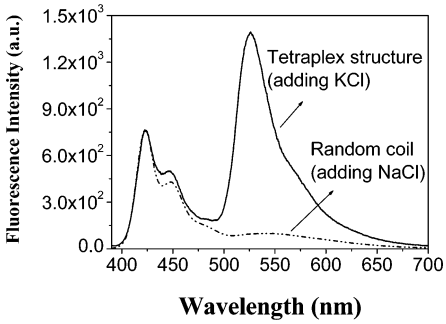


Figure 1. Emission spectra from solutions containing PF and ssDNA-Fl/KCl (solid line) and ssDNA-Fl/NaCl (dashed line), $\lambda_{ex} = 380$ nm, [KCl or NaCl] = 50 mM, [ssDNA-Fl] = 1.0×10^{-7} M, [PF] = 2.5×10^{-6} M in RUs. Measurements are performed in pure water. The spectra are normalized with respect to the emission of PF.

results in stronger G-quartet-DNA/PF electrostatic interactions, relative to ssDNA-Fl/PF. In situation B, the CCP resides in closer proximity to fluorescein, and therefore efficient FRET from CCP to fluorescein is observed.

Poly(9,9-bis(6′-*N*,*N*,*N*-trimethylammonium)hexyl)fluorenylene phenylene (PF)[33] is used as CCP in energy-transfer experiments (see Scheme 1 for the molecular structure). Fluorescein, with an absorption maximum at 488 nm and an emission maximum at 518 nm, was chosen since its absorption overlaps with the emission of PF. Irradiation at 380 nm selectively excites PF, and FRET from PF (donor) to fluorescein (acceptor) is favored.[20]

Figure 1 compares the emission spectra observed upon addition of PF ([PF] = 2.5×10^{-6} M in repeat units (RUs)) to solutions of ssDNA-Fl ([ssDNA-Fl] = 1.0×10^{-7} M) in the presence of 50 mM KCl or NaCl. The DNA resumes a G-quadruplex and random coil structures in the presence of KCl and low concentration of NaCl, respectively.[10] In these experiments the ssDNA-Fl was premixed with 50 mM KCl or NaCl at 4 °C, respectively. Addition of PF and subsequent comparison of the resulting fluorescence from fluorescein obtained by excitation at 380 nm reveals a significantly higher turn-on signal for the ssDNA-Fl/KCl, relative to the ssDNA-Fl/NaCl pair. These FRET differences demonstrate the specificity for G-quadruplex structure of the detection method. Furthermore, the fluorescein emission using 380 nm excitation is more than 10 times larger than that obtained by direct excitation at the ssDNA-Fl absorption maximum (480 nm). The increased ssDNA-Fl emission by the FRET (Scheme 1, situation B) shows the optical amplification by the CCP macromolecule.

In additional experiments energy-transfer optimization by varying the concentration of KCl was examined. Figure 2 shows that at a concentration of [ssDNA-Fl] = 1.0×10^{-7} M and [PF] = 2.5×10^{-6} M, addition of KCl causes a concomitant increase in the energy transfer from PF to ssDNA-Fl with an equilibrium shift from the random coil structure of ssDNA-Fl toward the G-quadruplex conformation. The FRET ratio of the intensity at 527 nm to that at 422 nm (I_{527}/I_{422}) is about 16, 3.4, and 2 times higher for the PF/ssDNA-Fl in the presence of 50 mM KCl, relative to those in the presence of 0, 8.5, and 30 mM KCl, respectively. Because the dominant interaction

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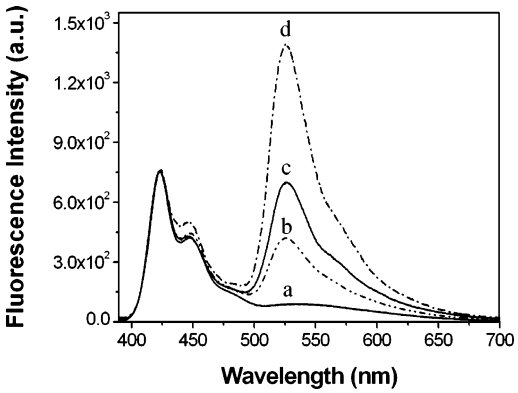
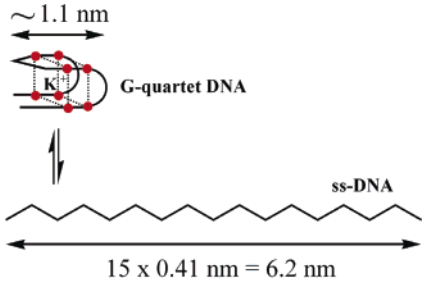


Figure 2. Emission spectra of solutions of PF/ssDNA-Fl with addition of KCl. The concentration of KCl ranges from 0 to 50 mM with $a = 0$ mM, $b = 8.5$ mM, $c = 30$ mM, $d = 50$ mM. [ssDNA-Fl] $= 1.0 \times 10^{-7}$ M, [PF] $= 2.5 \times 10^{-6}$ M in RUs, $\lambda_{ex} = 380$ nm. Measurements are performed in pure water. The spectra are normalized with respect to the emission of PF.

Scheme 2. Schematic Representation of the Persistence Lengths of G-Quadruplex DNA and Random Coil ssDNA



between PF and DNA is electrostatic in nature,[20,28] to explain this behavior one may consider that G-quadruplex DNA has a much higher space charge density than single-strand DNA since the persistence length of random coil ssDNA with 15 bases is about 6.2 nm (the base spacing value is 0.41 nm at the neutral pH)[30,31] and that of more condensed G-quadruplex DNA is only about 1.1 nm,[32] while they possess same charge numbers (Scheme 2). According to the Förster theory,[29] Förster energy transfer has a $1/r^6$ distance dependence; thus, as the electrostatic interactions between the oppositely charged DNA and PF become stronger (decreasing the average distance), the efficiency of energy transfer will increase. The results in Figure 2 confirm the importance of electrostatic interactions in determining the success of Scheme 1.

Figure 3 shows the emission spectra and FRET ratio (I_{527}/I_{422}) of the ssDNA-F1/PF in the presence of various metal ions. For Na^+, Li^+, and NH_4^+ FRET is very weak due to their weak inducing abilities to form G-quadruplex,[10] and therefore, less fluorescence of fluorescein is observed. For the divalent cations Ca^{2+} and Mg^{2+}, only a small increase in fluorescence of fluorescein is observed (Figure 3a) since Ca^{2+} and Mg^{2+} can also stabilize the G-quadruplex structure. However, their binding to G-quartet-DNA is also much weaker than that of K^+.[34] The value of the FRET ratio (I_{527}/I_{422}) for K^+ is approximately 16 times higher than those for Na^+, Li^+, and NH_4^+ and 6 times higher than those for Ca^{2+} and Mg^{2+} (Figure 3b). High selectivity for K^+ over Na^+, Li^+, NH_4^+, Ca^{2+}, and Mg^{2+} is observed.

For the K^+ detection, one of the essential requirements is minor or no interference from Na^+.[12] To study the Na^+ interference, the dependence of the FRET ratio (I_{527}/I_{422}) of the sensor was examined at different concentrations of Na^+ and K^+ in pure water (Figure 4). With an increase in the concentration of KCl from 0 to 50 mM, the FRET ratio (I_{527}/I_{422}) increases dramatically; for NaCl almost no increase in the value of I_{527}/I_{422} is observed in this concentration range (Figure 4 insert). These results show that the sensor responds only to K^+ in the concentration range from 0 to 50 mM, although it also responds to the Na^+ at a concentration of beyond 200 mM. It is noteworthy that our sensor can achieve high selectivity for K^+

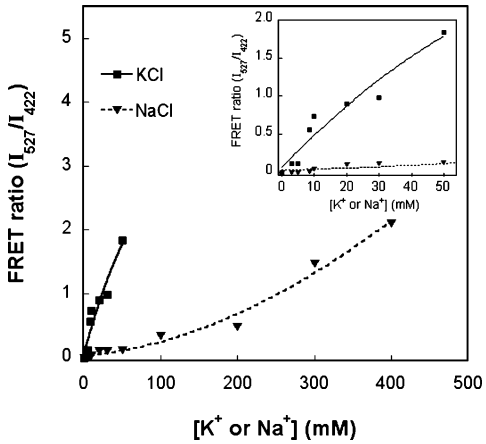
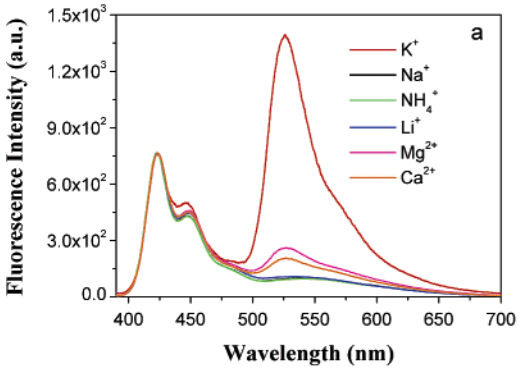


Figure 4. The dependence of the FRET ratio (I_{527}/I_{422}) on the concentration of K^+ and Na^+ in pure water. $\lambda_{ex} = 380$ nm, [ssDNA-Fl] $= 1.0 \times 10^{-7}$ M, [PF] $= 2.5 \times 10^{-6}$ M in RUs. (Insert) Low K^+ and Na^+ concentration (0−50 mM) profiles.



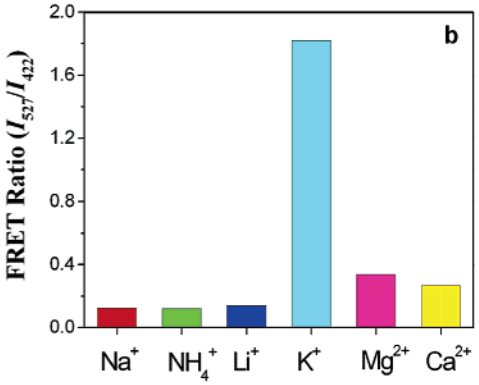


Figure 3. (a) Emission spectra from solutions containing PF, ssDNA-Fl, and 50 mM metal ions. The spectra are normalized with respect to the emission of PF. (b) The dependence of FRET ratio (I_{527}/I_{422}) on the various metal ions. $\lambda_{ex} = 380$ nm, [metal ion] $= 50$ mM, [ssDNA-Fl] $= 1.0 \times 10^{-7}$ M, [PF] $= 2.5 \times 10^{-6}$ M in RUs. Measurements are performed in pure water.

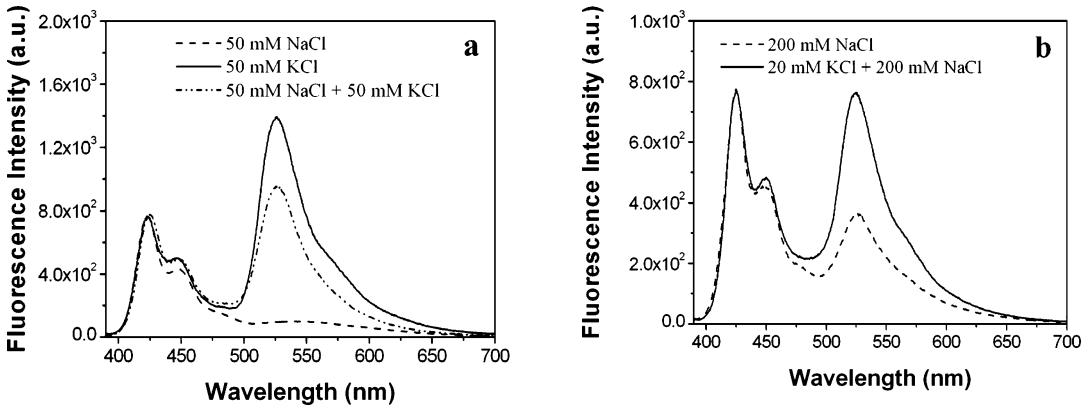


Figure 5. Emission spectra of solutions of PF/ssDNA-Fl in: (a) 50 mM NaCl, and 50 mM KCl with the presence or absence of 50 mM NaCl, respectively; (b) 200 mM NaCl, and 20 mM KCl with the presence of 200 mM NaCl, respectively. [ssDNA-Fl] = 1.0×10^{-7} M, [PF] = 2.5×10^{-6} M in RUs, λ_{ex} = 380 nm. Measurements are performed in pure water. The spectra are normalized with respect to the emission of PF.

in the presence of Na^+. Figure 5a shows that the FRET ratio (I_{527}/I_{422}) gives similar value to 50 mM K^+ in the presence and absence of 50 mM Na^+. More importantly, our sensor can still detect 20 mM K^+ in the presence of 200 mM Na^+, where the FRET ratio (I_{527}/I_{422}) is 2.5 times higher than that for 200 mM Na^+ only (Figure 5b). The minor interference from Na^+ clearly shows the PF/ssDNA-Fl assembly can be used as a K^+ probe in water.

Conclusion

In summary, we report a homogeneous fluorescence amplification assay for G-quadruplex structures of DNA that interfaces DNA association with the light-harvesting properties of conjugated polymers. The higher FRET efficiency for the CCP/G-quadruplex pair is correlated to the stronger electrostatic interactions of G-quadruplexes with CCP in comparison to the CCP/ssDNA pair. Alternatively, our technique is able to detect potassium ion in aqueous solution with high sensitivity and selectivity, combining the binding specificity of G-quadruplex DNA and signal transduction of CCPs. In principle, the technique shows the potential to detect G-rich aptamer-binding proteins.

Experimental Section

The oligonucleotides (ssDNA-F1) were purchased from Shanghai Sangon Biological Engineering Technology & Service Co., Ltd. The ssDNA-F1 concentration was determined by measuring the absorbance at 260 nm in a 250-μL quartz cuvette. UV−vis absorption spectra were taken on a Hitachi U-3010 spectrophotometer. The fluorescence measurements were recorded on a Hitachi F-4500 spectrophotometer equipped with a Xenon lamp excitation source at about 4 °C. All fluorescence spectra were measured at an excitation wavelength of 380 nm. The water was purified using a Millipore filtration system.

Assay for G-Quadruplex Folding of the DNA. To two 1.5 mL Eppendorf cups were respectively added 10 μL of ssDNA-F1 (1.0×10^{-5} M) and then 0.5 μL of 1.0 M KCl into one Eppendorf cup (sample 1) and 0.5 μL of 1.0 M NaCl into the other one (sample 2). The two cups were incubated for 48 h at 4 °C. After incubation, 5 μL of PF (5.0×10^{-4} M) was added, respectively. The samples were treated at 0 °C for 1 h to form complexes; 940 μL of water and 50 μL of 1.0 M KCl were added to sample 1, and 940 μL of water and 50 μL of 1.0 M NaCl were added to sample 2. The fluorescence spectra were measured in a 3-mL quartz cuvette at 4 °C.

K^+ Sensor. Into a 1.5 mL Eppendorf cup were added 10 μL of ssDNA-F1 (1.0×10^{-5} M) and 0.5 μL of 1.0 M KCl. The cup was incubated for 48 h at 4 °C. After incubation, 5 μL of PF (5.0×10^{-4} M) was added. The samples were treated at 0 °C for 1 h to form complexes. Then the fluorescence spectra were measured in a 3-mL quartz cuvette at 4 °C after adding 940 μL of water and 50 μL of 1.0 M KCl. The assay procedures for sodium, ammonium, lithium, calcium, and magnesium ions are same as that for the potassium assay, except for using NaCl, NH_4Cl, LiCl, $CaCl_2$, and $MgCl_2$ instead of KCl.

Sensitivity for K^+ Sensor. Into seven 1.5 mL Eppendorf cups was added 10 μL of ssDNA-F1 (1.0×10^{-5} M), respectively. And then 0.3, 0.5, 0.8, and 1.0 μL of 0.1 M KCl and 0.2, 0.3, and 0.5 μL of 1.0 M KCl were added into the cups, respectively (samples 1−7). The samples were incubated for 48 h at 4 °C. After incubation, 5 μL of PF (5.0×10^{-4} M) was added. The samples were treated at 0 °C for 1 h to form complexes. Then the fluorescence spectra were measured in a 3-mL quartz cuvette at 4 °C after the samples 1−7 were diluted to 1000 μL with 3, 5, 8, 10, 20, 30, and 50 mM KCl aqueous solution, respectively.

Acknowledgment. We are grateful for the financial supports from the "100 talents" program of Chinese Academy of Sciences and Institute of Chemistry, Chinese Academy of Sciences.

JA051507I

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