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JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
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Cationic Conjugated Polyelectrolytes-Triggered Conformational Change of Molecular Beacon Aptamer for Highly Sensitive and Selective Potassium Ion Detection

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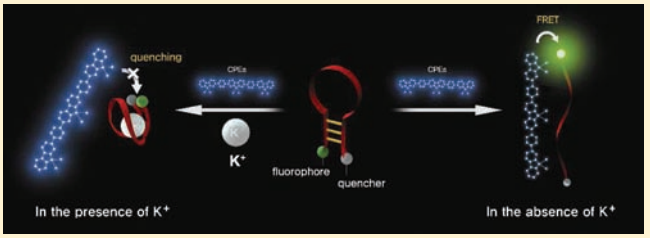
Supporting Information

ABSTRACT: We demonstrate highly sensitive and selective potassium ion detection against excess sodium ions in water, by modulating the interaction between the G-quadruplex-forming molecular beacon aptamer (MBA) and cationic conjugated polyelectrolyte (CPE). The K^+-specific aptamer sequence in MBA is used as the molecular recognition element, and the high binding specificity of MBA for potassium ions offers selectivity against a range of metal ions. The hairpin-type MBA labeled with a fluorophore and quencher at both termini undergoes a conformational change (by complexation with CPEs) to either an open-chain form or a G-quadruplex in the absence or presence of K^+ ions. Conformational changes of MBA as well as fluorescence (of the fluorophore in MBA) quenching or amplification via fluorescence resonance energy transfer from CPEs provide clear signal turn-off and -on in the presence or absence of K^+. The detection limit of the K^+ assays is determined to be ~1.5 nM in the presence of 100 mM Na^+ ions, which is ~3 orders of magnitude lower than those reported previously. The successful detection of 5′-adenosine triphosphate (ATP) with the MBA containing an ATP-specific aptamer sequence is also demonstrated using the same sensor scheme. The scheme reported herein is applicable to the detection of other kinds of G-rich aptamer-binding chemicals and biomolecules.



INTRODUCTION

Conjugated polyelectrolytes (CPEs) have established themselves as a platform in chemical and biological sensors with high sensitivity by virtue of their light harvesting properties.[1−6] Extensive studies have been performed to develop CPE-based fluorescent assays for the detection of biomolecules including DNA, RNA, and peptides with signal amplification utilizing a fluorescence resonance energy transfer (FRET) mechanism.[7−11]

Aptamers are nucleic acid ligands that bind to specific target molecules with high affinity and specificity.[12−14] In particular, a single-stranded DNA with guanine (G)-rich sequences can fold into a secondary structure, G-quadruplex, via intramolecular hydrogen-bonding interactions.[15−17] Its formation is known to be promoted by the presence of monovalent cations such as potassium ions.[18] Molecular beacons (MBs) are oligonucleotide hybridization probes, which are hairpin-shaped molecules labeled with a 5′-fluorophore and 3′-quencher.[19,20] MBs can undergo a structural change from a stem-loop (quenched state) to an open-chain form through hybridization with complementary target DNA, resulting in the complete recovery of fluorescence emission (fluorescent state). The potential of aptamers and MBs as novel sensing and recognition elements is currently under intense investigation.[21−29] Recently, Plaxco et al. reported that cationic CPEs can open the stem-loop structure of MBs through both electrostatic and hydrophobic interactions between the CPEs and MBs.[30] These exciting results prompted us to investigate a new homogeneous sensing scheme utilizing the interactions of a molecular beacon aptamer (MBA) and CPEs. MBAs are MB-based oligonucleotide probes with a target-specific aptamer sequence in the loop part. Combining the binding specificity of a G-quadruplex forming MBA and signal transduction via the complexation of MBA and CPEs may provide an opportunity to design and develop selective and sensitive fluorescent assays for a target species.

Potassium ions play key roles in biological systems, such as nerve transmission, maintenance of muscular strength and extracellular osmolarity, enzyme activation, apoptosis of a cell, regulation of blood pressure, pH, and the concentration of other ions in living cells.[31−36] Many previous studies have focused on developing bioassays to detect K^+ ions. Despite such notable progress, their clinical application has been challenging due to the lack of high selectivity against sodium ions (physiological condition, 135 mM), low detection sensitivity (in ranges of μM~mM), and the requirement of nonaqueous

Received: November 3, 2011
Published: January 14, 2012

   dx.doi.org/10.1021/ja210360v | J. Am. Chem. Soc. 2012, 134, 3133−3138

conditions for most previous systems.[37−48] Therefore, the development of a selective and sensitive method for the detection of potassium ions in an aqueous medium is of great importance.

In this contribution, we report a new potassium ion detection assay using the combination of a MBA and a CPE. The K^+-specific aptamer sequence in MBA was used as the molecular recognition element. Highly sensitive and selective detection was demonstrated by combining the G-quadruplex formation of MBA with K^+ ions, electrostatic and hydrophobic interactions between the MBA and CPE components, and signal quenching or optical amplification via FRET in the presence or absence of K^+ ions. Fluorescence quenching is highly specific to K^+ ions against a range of metal ions, and the lowest detection limit of ∼1.5 nM was achieved for the first time without interference in the presence of excess Na^+ ions. This detection limit is ∼3 orders of magnitude lower than those previously reported.[44−47] A combination of a high binding specificity of biosystems and synthetic CPEs with tunable optical amplification properties provides the ultimate detection sensitivity and selectivity.

EXPERIMENTAL SECTION

General. All chemicals were purchased from Aldrich Chemical Co. and used as received unless otherwise mentioned. High pressure liquid chromatography (HPLC)-purified molecular beacon aptamers labeled with 6-carboxyfluorescein (6-FAM) and 4-(4′-dimethylaminophenylazo)benzoic acid (DABCYL) with 27 bases (5′-6-FAM-ACGC GCGG TTGG TGTG GTTG GGCG CGG-DABCYL-3′) for sensing K^+ and 30 bases (5′-6-FAM-TACA CTGG GGAG TATT GCGG AGGA AGTG TA-DABCYL-3′) for detecting ATP were obtained from Sigma-Genosys.

The ^1H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 spectrometer, with tetramethylsilane as the internal reference. The number- and weight-average molecular weights of the polymer were determined by gel permeation chromatography (GPC) using tetrahydrofuran (THF) as an eluent on a Waters GPC-150C instrument, relative to a polystyrene standard. Elemental analysis was performed by the KAIST Research Supporting Team (EA 1110 Fisons analyzer). The UV/vis absorption spectra were measured using a Jasco (V-630) spectrophotometer. The photoluminescence (PL) spectra were obtained on a Jasco (FP-6500) spectrofluorometer with a xenon lamp excitation source, using 90° angle detection for the solution samples. The fluorescence quantum yield was measured relative to a freshly prepared fluorescein solution in water at pH = 11 (Supporting Information). The circular dichroism spectra were obtained using a Jasco (J-815) 202 circular dichroism spectrometer.

Synthesis of Poly[(9,9′-bis(4-(6-*N,N,N*-trimethylammonium-hexyloxy)phenyl)fluorene-2,7-diyl)-*alt*-1,4-phenylene dibromide] (PPFP−Br). To a mixture of 2,7-dibromo-9,9′-bis(4-(6-bromohexyloxy)phenyl)fluorene (0.30 g, 0.36 mmol) and 1,4-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzene (0.12 g, 0.36 mmol) was added 15 mg of $(PPh_3)_4Pd(0)$ in a drybox. Subsequently, degassed 2 M aqueous potassium carbonate (4 mL) and a phase transfer catalyst, Aliquat 336 in toluene (8 mL), were transferred to the above flask via a cannula. The reaction mixture was heated at 80 °C for 24 h. Bromobenzene (6.0 mg, 0.038 mmol) in 1 mL of toluene was used as an end-capper and reacted further for 12 h. The reaction mixture was cooled down to room temperature and precipitated into 200 mL of methanol with vigorous stirring. The polymer fibers were collected by filtration and purified by Soxhlet extraction in acetone for 2 days. A neutral precursor (**3**) was obtained as a gray solid. Yield: 66% (180 mg). ^1H NMR (400 MHz, $CDCl_3$, ppm): 7.81 (b, 2H), 7.60 (m, 8H), 7.18 (d, 4H), 6.68 (d, 4H), 3.88 (b, 4H), 3.38 (m, 4H), 1.83 (b, 8H), 1.45 (b, 8H).

The final water-soluble PPFP−Br was prepared through a simple quaternization reaction with trimethylamine. Condensed trimethylamine (3 mL) was added dropwise to a solution of the neutral precursor polymer (100 mg) in THF (10 mL) at −78 °C. The mixture was allowed to warm slowly to room temperature for 24 h. The precipitate was redissolved by the addition of excess methanol, and an additional 2 mL of trimethylamine was added at −78 °C. The resulting mixture was stirred further for 24 h at room temperature. After removing the solvents under reduced pressure, the polymer was dissolved in methanol and reprecipitated into diethyl ether several times. Yield: 87% (101 mg). ^1H NMR for PPFP−Br (400 MHz, DMSO, ppm): 8.07 (b, 2H), 7.70 (m, 8H), 7.14 (b, 4H), 6.85 (b, 4H), 3.89 (b, 4H), 3.23 (m, 4H), 3.01 (s, 18H), 1.78 (b, 8H), 1.34 (m, 8H).

2,7-Dibromo-9,9′-bis(4-(6-bromohexyloxy)phenyl)fluorene (1). Compound **1** was synthesized by modifying a previous procedure.[49] ^1H NMR (400 MHz, $CDCl_3$, ppm): 7.57 (d, 2H), 7.46 (s, 2H), 7.44 (d, 2H), 7.05 (d, 4H), 6.76 (d, 4H), 3.90 (t, 4H), 3.40 (t, 4H), 1.87 (m, 4H), 1.76 (m, 4H), 1.48 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): 158.09, 153.67, 137.81, 136.31, 130.71, 129.21, 128.97, 121.75, 121.51, 114.31, 67.61, 64.32, 33.77, 32.63, 29.03, 27.86, 25.26. Anal. calcd for $C_{37}H_{38}Br_4O_2$: C, 53.26; H, 4.59; Br, 38.31. Found: C, 53.45; H, 4.61.

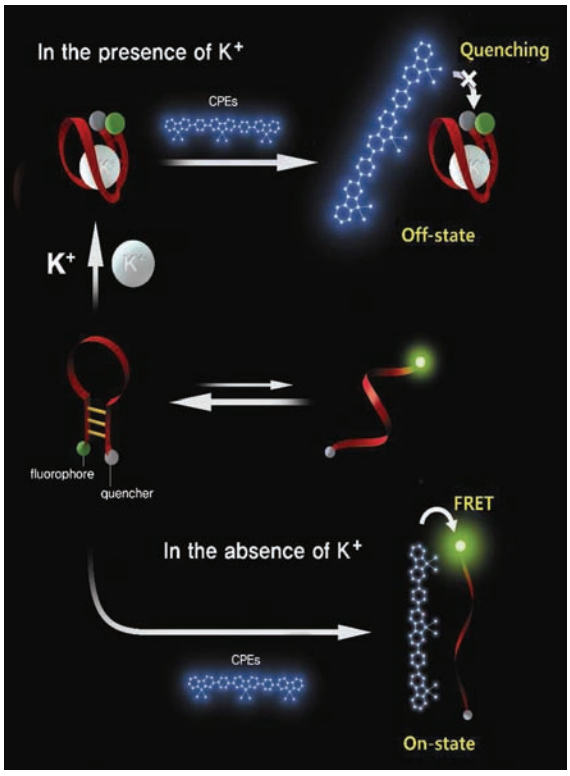
Potassium Ion Assay Protocols. All PL experiments were performed in 20 mM Tris−HCl buffer (pH = 7.4) containing 100 mM NaCl as a salt. A stock solution (10^{-5} M) of MBA was prepared in deionized water. The MBA stock solution (4 μL) was added to 2 mL of buffer, and the resulting solution was incubated at 60 °C for 20 min in the presence or absence of potassium ions. The annealed solution was cooled slowly to room temperature for 1 h. PPFP−Br (20 μL, 10^{-4} M) was then added to the above solution, and the PL spectra were measured by exciting either the polymer or 6-FAM. The final concentrations of MBA and PPFP−Br in the assay system were 2.0×10^{-8} M and 9.9×10^{-7} M, respectively. The same procedures were repeated in the presence of NaCl, $CaCl_2$, LiCl, $MgCl_2$, NH_4Cl, $CuCl_2$, $FeCl_3$, $ZnCl_2$, and $AlCl_3$ instead of KCl to assess the selectivity against other metal ions.

RESULTS AND DISCUSSION

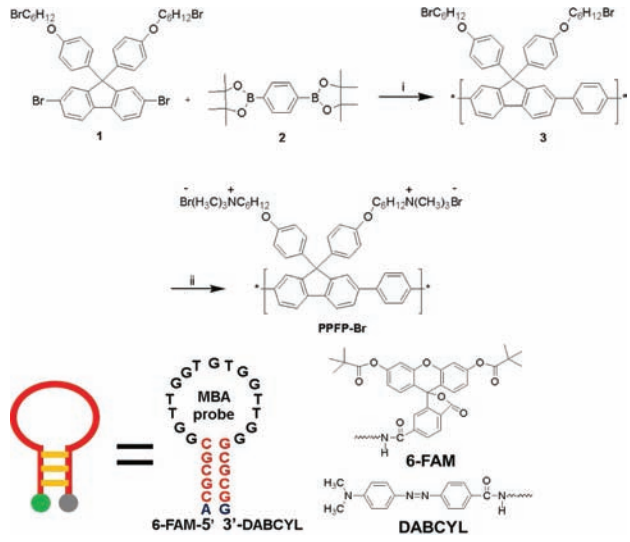
Scheme 1 illustrates our strategy for K^+ ion detection. A molecular beacon containing a K^+-specific aptamer base sequence (MBA, 5′-6-FAM-ACGC GCGG TTGG TGTG GTTG GGCG CGG-DABCYL-3′) was designed as a probe. The base sequence of GGTTGGTGTGGTTGG is known to form a quadruplex structure with a K^+ ion.[43,44] In the stem part, five GC pairs were introduced to induce a stable hairpin formation at equilibrium at room temperature. The MBA was labeled with 6-FAM and DABCYL at the 5′- and 3′-termini, respectively (Scheme 2).

The MBA exists mainly as a hairpin-type structure at equilibrium, which shows highly quenched emission via efficient energy transfer from a fluorophore (6-FAM) to quencher (DABCYL) due to direct contact of 6-FAM and DABCYL.[50] When the CPE is added to the MBA solution, 6-FAM emission is recovered due to a conformational change of the MBA into an open-chain form induced by complexation with the CPE. In this CPE/MBA complex, the increased intermolecular separation between 6-FAM and DABCYL leads to reduced PL quenching.[30] In addition, the opposite charges on the polymer backbone and MBA allow the formation of an electrostatic complex, CPE/MBA, enabling facile FRET from the polymer to 6-FAM in MBA.[51] In the open-chain conformation of MBA without K^+, efficient FRET from CPE to 6-FAM can occur, and the FRET induced 6-FAM emission can be amplified by exciting the polymer (on state). On the other hand, in the presence of K^+ ions, the MBA forms a tightly bound G-quadruplex with K^+, in which a G-rich specific aptamer sequence offers a unique K^+ binding site in the intramolecular tetraplex.[44] The addition of the CPEs induces no conformational change due to the stronger interaction

Scheme 1. Schematic for K^+ Ion Detection



Scheme 2. Synthetic Routes to PPFP−Br and Molecular Structure of MBA[a]



[a]Reagents and conditions: (i) $(PPh_3)_4Pd(0)$, 2 M K_2CO_3, toluene, 80 °C, 36 h; (ii) trimethylamine in THF/methanol, room temperature, 48 h.

between the MBA and K^+, in which the resulting 6-FAM emission signal is strongly quenched by exciting either the polymer or 6-FAM directly (off state). Sensitive and selective detection of K^+ ions is possible because the distance of both termini of the MBA is dramatically changed in the G-quadruplex and open-chain structures, and their formation is strongly dependent on $[K^+]$.

Previously, Plaxco et al. studied the ability to open the beacon using three kinds of cationic polymers, including a CPE, poly[9,9′-bis(6-*N*,*N*,*N*-trimethylammoniumhexyl)fluorene-*alt*-1,4-phenylene dibromide] (PFP−Br) and two nonconjugated polymers, poly(diallyldimethylammonium chloride) and poly(allylamine hydrochloride). In this study, they reported that only PFP−Br can induce the conformational change of beacons from stem-loop to single-stranded structure, emphasizing the hydrophobic interactions between cationic polymers and MBs play an important role in the formation of linear extended complex.[30] According to this report, we introduced additional phenylene groups into the PFP structure as a side chain to increase the hydrophobic properties of the polymeric backbone. As a FRET donor, a cationic polyfluorene-based CPE, poly[(9,9′-bis(4-(6-*N*,*N*,*N*-trimethylammoniumhexyloxy)-phenyl) fluorene-2,7-diyl)-*alt*-1,4-phenylene dibromide] (PPFP−Br) was synthesized via the Suzuki coupling of 2,7-dibromo-9,9′-bis(4-(6-bromohexyloxy)phenyl)fluorene (**1**) and 1,4-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzene (**2**) using $(PPh_3)_4Pd(0)$ as a catalyst in toluene/H_2O (2:1, vol) at 80 °C for 36 h (yield 66%), followed by a quaternization reaction (yield 87%) by treating the neutral precursor polymer (**3**) with condensed trimethylamine (Scheme 2). The number average molecular weight was determined to be M_n = 16 100 g/mol (polydispersity index = 2.71) with the neutral precursor (**3**) of PPFP−Br using gel permeation chromatography (solvent: THF) relative to a polystyrene standard.

Figure 1(a) shows the UV−vis absorption and photoluminescence (PL) spectra of PPFP−Br and 6-FAM in a 20

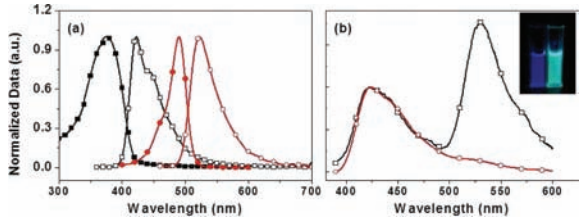


Figure 1. (a) Normalized absorption and PL spectra of PPFP−Br (black) and 6-FAM (red) in buffer. (b) Normalized FRET-induced PL spectra of PPFP−Br/MBA in the absence (black) and presence (red) of K^+ ions by exciting at 380 nm. $[KCl]$ = 50 mM; $[PPFP-Br] = 9.9 \times 10^{-7}$ M; $[MBA] = 2.0 \times 10^{-8}$ M. Inset shows the photograph of the solutions with (blue) and without K^+ ions (sky blue) under UV illumination at 365 nm.

mM Tris−HCl buffer solution. The maxima in the absorption and PL of the polymer were measured at λ_{abs} = 375 nm and λ_{PL} = 423 nm, respectively. The emission of the CPE as a FRET donor shows good spectral overlap with the absorption of 6-FAM (λ_{abs} = 495 nm; λ_{PL} = 518 nm) as a FRET acceptor. The PL quantum efficiency of PPFP−Br was determined to be ~46% relative to the freshly prepared fluorescein in water at pH = 11. Figure 1(b) compares the FRET-induced PL spectra of the MBA solution upon addition of CPE in the absence and presence of KCl. The PL spectra were measured in 20 mM Tris−HCl buffer (containing 100 mM NaCl as a salt, pH = 7.4). The MBA ($[MBA] = 2.0 \times 10^{-8}$ M) was added to the buffer in the presence or absence of K^+ ions (50 mM) and incubated at 60 °C for 20 min. The solution was slowly cooled down to room temperature for 1 h to induce G-quadruplex folding with K^+. Finally, the CPE ($[PPFP-Br] = 9.9 \times 10^{-7}$ M based on a repeat unit, charge ratio ([+] in CPE: [−] in MBA) = 3.7:1, in which the maximum FRET ratio (I_{533}/I_{423}; I_{533} is PL intensity at 533 nm, and I_{423} is intensity at 423 nm) was

dx.doi.org/10.1021/ja210360v | J. Am. Chem. Soc. 2012, 134, 3133−3138

obtained, Supporting Information, Figure S1) was added to the above solution, and the FRET PL spectra were measured by exciting the polymer at 380 nm. In the absence of K^+ ions, the strong FRET signal was measured by forming an open-chain electrostatic complex, PPFP−Br/MBA (on state). In the presence of K^+ ions, the MBA formed a tight G-quadruplex, and no 6-FAM emission was measured by exciting PPFP−Br (off state). This indicates that the polymer cannot open the G-quadruplex structure of MBA and K^+, in which 6-FAM and DABCYL are in sufficiently close contact to induce strong PL quenching.

The selective G-quadruplex formation of the MBA with K^+ was confirmed by circular dichroism (CD) analysis, as shown in Figure 2. The characteristic absorption bands at 270 and 290

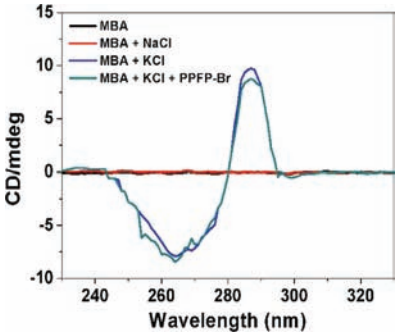


Figure 2. Circular dichroism measurements of MBA solutions containing Na^+ and K^+ ions before and after addition of PPFP−Br. [MBA] = 1.0×10^{-6} M; [NaCl] = [KCl] = 50 mM; [PPFP−Br] = 5.0×10^{-5} M.

nm of the quadruplex were observed only in the presence of K^+ ions.[37] The CD spectra also show that the addition of PPFP−Br does not interfere with the G-quaruplex formation of MBA/K^+.

The relative binding strength between the CPE and the MBA and between the MBA and K^+ ions was also tested. First, PPFP−Br was added to the MBA solution to form an open-chain electrostatic PPFP−Br/MBA complex (on state) followed by the addition of K^+ ions. Interestingly, the 6-FAM signal was quenched completely after annealing at 60 °C, which indicates the formation of the MBA/K^+ quadruplex. This clearly shows that the binding interaction between the MBA and K^+ is stronger than the interaction between the CPE and MBA (Figure 3). Therefore, FRET emission clearly differentiates the presence and absence of K^+ ions in the sample.

The PL spectra were also measured by exciting 6-FAM directly at 490 nm from the solutions containing the MBA and PPFP−Br with or without K^+ ions. As shown in Figure 4, the 6-FAM signal was enhanced with increasing [PPFP−Br], through a conformational change of the MBA in the absence of K^+ ions. The red-shifted emission upon addition of PPFP−Br is due to increased effective conjugation by forming the CPE/MBA complex. In the presence of K^+, a negligible PL spectral change (quenched state) was observed upon the addition of the polymer. However, a relatively weak PL signal with small signal turn on/off ratio was measured in the absence and presence of K^+, compared to the data via FRET.

Figure 5 shows the normalized FRET emission with changing [KCl] = 0−50 mM. With increasing [K^+], the PL intensity around 420 nm increased, and the emission around 520 nm decreased with a concomitant decrease in the resulting

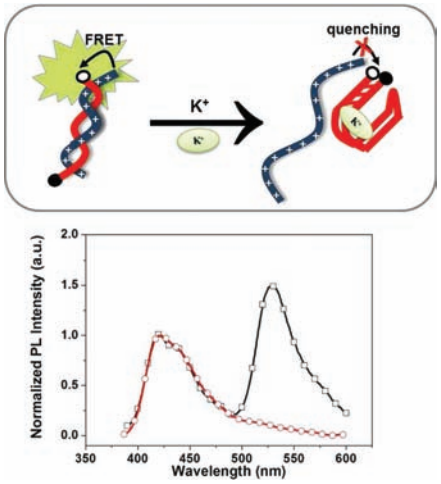


Figure 3. Normalized FRET-induced PL spectra of PPFP−Br/MBA without K^+ ions (black) and after annealing at 60 °C with K^+ ions (red) by exciting at 380 nm. [KCl] = 50 mM; [PPFP−Br] = 9.9×10^{-7} M; [MBA] = 2.0×10^{-8} M.

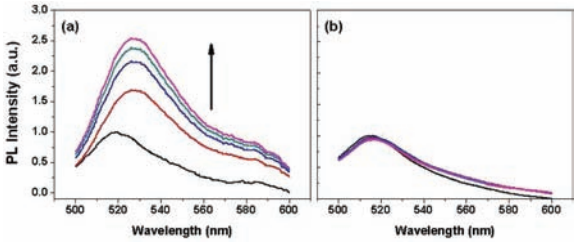


Figure 4. PL spectra of the MBA with increasing [PPFP−Br] = 0−2.0×10^{-6} M in the absence (a) and presence (b) of K^+ ions ([K^+] = 50 mM) by exciting 6-FAM at 490 nm. [MBA] = 2.0×10^{-8} M.

FRET ratio. This suggests that the concentration of G-quadruplex (MBA/K^+) increases gradually with increasing [K^+]. In addition, the PL intensity of free MBA was quenched gradually with increasing [K^+], which is related to the equilibrium shift to G-quadruplex formation (Supporting Information, Figure S2). The FRET ratio was ∼18 times smaller for the solution containing 50 mM KCl relative to that in the absence of KCl. To determine the detection limit of K^+ ions, the FRET ratio was examined by varying [KCl]. The FRET ratio showed a clear dependence on [K^+] with a linear relationship (R = 0.994) in the range of 5.0−30 nM. The detection limit of the K^+ assays was determined to be ∼1.5 nM (3σ/slope, σ is the standard deviation in 5 independent measurements of slope in Figure 5b)[52,53] in the presence of 100 mM Na^+ ions, which is the lowest reported thus far. Moreover, the sensing scheme was unaffected by the presence of excess Na^+ ions.[54] The typical PL spectra at [K^+] = 500 nM are included in the Supporting Information (Figures S3 and S4).

To investigate specific binding of the probe MBA for K^+ ions, we measured the FRET-induced PL spectra with a series of MBAs with 1 base mismatch (MBA1), 3 bases mismatch (MBA2), and a nonspecific base sequence (MBA3) in the loop (aptamer) part. Table S1 of the Supporting Information shows the detailed base sequences for the MBAs tested. In the presence of K^+ ions (50 mM), the FRET ratio with MBA1 is ∼7 fold higher than the value with K^+-specific MBA. For MBA2, the FRET ratio is almost similar with compared to nonspecific MBA (MBA3), indicating that the perfectly

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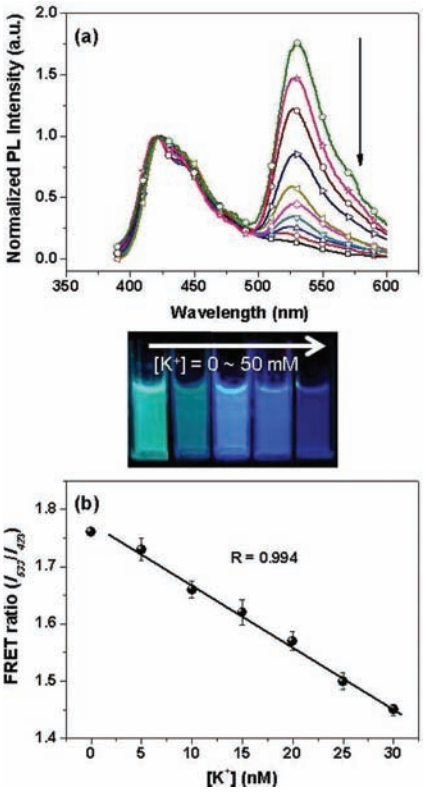

Figure 5. (a) Normalized FRET-induced PL spectra of PPFP−Br/MBA with changing $[K^+]$ by exciting at 380 nm. [KCl] = 0−50 mM. The photograph of the solutions represents fluorescence emission with varying the $[K^+]$ under UV illumination at 365 nm. (b) FRET ratio (I_{533}/I_{423}) vs $[K^+]$ shows linearity in a range of $[K^+]$ = 5−30 nM. [PPFP−Br] = 9.9×10^{-7} M; [MBA] = 2.0×10^{-8} M. The error bars represent the standard deviation of five measurements.

matched aptamer sequence is necessary for highly selective detection of K^+ ions (Supporting Information, Figure S5).

Figure 6 shows the selectivity of this CPE/MBA sensing system for various metal ions. The FRET ratio was measured in

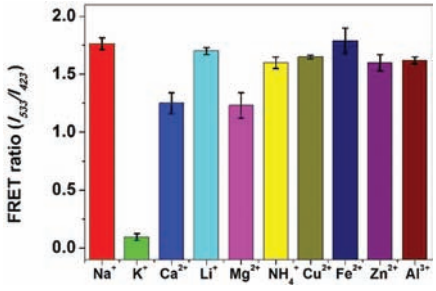

Figure 6. FRET ratio (I_{533}/I_{423}) for various metal ions. [Metal ion] = 50 mM; [PPFP−Br] = 9.9×10^{-7} M; [MBA] = 2.0×10^{-8} M. The error bars represent the standard deviation of five measurements.

the presence of [metal ion] = 50 mM in the same manner used for K^+ ions. For Na^+, Li^+, NH_4^+, Cu^{2+}, Fe^{2+}, Zn^{2+}, and Al^{3+}, the strong FRET-induced 6-FAM emission was observed due to the low binding affinity of the MBA to metal ions and little tendency to form a G-quadruplex.[37] For divalent cations, Ca^{2+} and Mg^{2+}, a relatively small FRET ratio was observed because Ca^{2+} and Mg^{2+} can also stabilize the G-quadruplex structure. However, their binding affinity to the MBA is much weaker than that of K^+.[33] The resulting FRET ratio for K^+ is approximately 14 times smaller than those for Ca^{2+} and Mg^{2+}. The selectivity test for K^+ ions was also performed in the presence of coexisting other ions (Supporting Information, Figure S6). With the solution containing (K^+ + Ca^{2+}) and (K^+ + Li^+) ions, a similar FRET ratio was obtained with the sample containing K^+. Similar data were also measured with two coexisting metal ions, showing a clear PL quenching at 533 nm by forming the complex MBA/K^+ with negligible interference by other ions. The strong binding affinity of the MBA for K^+ induces almost complete PL quenching only in the presence of K^+ ions by forming a G-quadruplex. These results clearly show that this MBA/CPE-based sensory system selectively detects K^+ ions against a range of metal ions.

More importantly, this sensing scheme is able to detect other metal ions and biomolecules by combining the specificity of the G-quadruplex-forming MBA and signal transduction by CPEs via FRET. The successful detection of 5′-adenosine triphosphate (ATP) with the MBA containing an ATP-specific aptamer sequence (5′-6-FAM-TACA CTGG GGAG TATT GCGG AGGA AGTG TA-DABCYL-3′) was also demonstrated using the same sensor scheme (Figure 7).[55−57] The clear signal

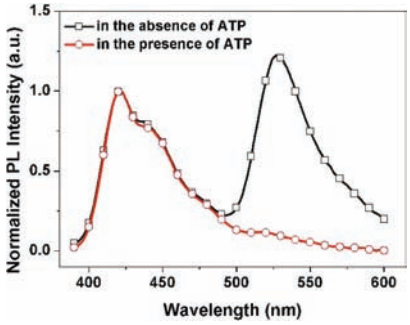

Figure 7. FRET-induced PL spectra of PPFP−Br/MBA in the absence (black) and presence (red) of ATP by exciting at 380 nm. [ATP] = 50 mM; [PPFP−Br] = 9.9×10^{-7} M; [MBA] = 2.0×10^{-8} M.

turn on and off was measured in the absence or presence of ATP. Therefore, the CPE and MBA-based detection scheme might be suitable for detecting a wide range of target species that can form a G-quadruplex with a specific aptamer sequence, with high sensitivity and selectivity.

CONCLUSION

In summary, we have developed a highly sensitive and selective homogeneous sensory system for K^+ ions based on CPE and MBA that operates in an excess presence of Na^+ ions. The hairpin-type MBA labeled with a fluorophore and quencher at both termini undergoes a conformational change (by complexation with CPEs) to either an open-chain form or a G-quadruplex in the absence or presence of K^+ ions. This ion selective event induces the amplified FRET-based PL signal from the fluorophore without K^+ (on state), whereas the almost complete quenching of the fluorophore emission was observed in the presence of K^+ (off state). The potassium ion-specific G-rich base sequence in the MBA provides the selectivity with remarkable sensitivity via optical amplification by the CPEs. A detection limit of ~1.5 nM was achieved, which is the lowest among reported thus far. The scheme reported herein can be extended further to sensing other types of G-rich aptamer-binding chemicals and biomolecules.

dx.doi.org/10.1021/ja210360v | J. Am. Chem. Soc. 2012, 134, 3133−3138

■ ASSOCIATED CONTENT

Supporting Information

Additional PL spectra, FRET ratios in presence of K^+ ions and coexisting metal ions, base sequence in MBAs, and information concerning the measurement of PL quantum yield. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENTS

This research was supported by the World Class University program and Basic Science Research Program through the National Research Foundation (NRF) of Korea funded by the Ministry of Education, Science and Technology, Korea (grant nos. R31-2008-000-20004-0 and 2009-0085182).

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