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An aptamer-based fluorescent biosensor for potassium ion detection using a pyrene-labeled molecular beacon

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ABSTRACT

A novel and sensitive biosensor based on aptamer and pyrene-labeled fluorescent probes for the determination of K^+ was developed. The aptamer was used as a molecular recognition element and a partially complementary oligonucleotide with the aptamer was labeled by pyrene moieties at both ends to transduce the binding event of K^+ with aptamer. In the presence of K^+, the complementary oligonucleotides were displaced from aptamers, which was accompanied by excimer fluorescence of pyrenes because the self-hairpin structure of the complementary oligonucleotide brought pyrene moieties into close proximity. However, it gave only monomer emission in the absence of K^+. Under optimum conditions, the relative fluorescence intensity of pyrene was proportional to the concentration of K^+ in the range of 6.0×10^{-4} to 2.0×10^{-2} M. A detection limit of 4.0×10^{-4} M was achieved. Moreover, this method was able to detect K^+ with high selectivity in the presence of Na^+, NH_4^+, Mg^{2+}, and Ca^{2+} ions of biological fluids. In brief, the assay may have great potential applications, especially in a biological environment because of its simplicity, sensitivity, and specificity.

Potassium ions (K^+) play an important role in living organisms. They exist in extracellular spaces, tissue of animals and plants, biological fluids, and so on [1]. Since the abnormal K^+ concentration can lead to several diseases, the sensitive detection of K^+ has become a very important issue. So far, although numerous studies concerning K^+ sensors were reported, most of them prevent their clinical applications. For example, K^+ detection in biological fluids based on fluoroionophores PBFI [2] and coumarin diacid cryptand [2.2.2] [3] has been reported, but K^+ selectivity against Na^+ was shown to be very poor. They were tolerated in biological fluids for 1.5- and 3.4-fold excess of Na^+ ions, respectively. Lee et al. [4] reported on the development of practical polydiacetylene (PDA)[1] liposome-based microarrays to selectively detect potassium ions in the presence of Na^+. The detection limit from the microscope images for 30 min of incubation was 0.5 mM. Other fluorescent probes for K^+ detection were based on the quadruplex-forming oligonucleotides and FRET (fluorescence resonance energy transfer), in which aptamers specific to K^+ were modified by two fluorophores at both ends [5–7]. However, the majority of modified aptamers binding to targets are unable to yield FRET, and even slight modifications on aptamers may cause significant reduction of affinity and specificity, therefore greatly limiting their applicability [8–11].

Pyrene molecules are one of the more interesting dyes for the development of fluorescent oligonucleotide probes [12–14]. Its fluorescence wavelength is about 395 nm for the pyrene monomer. When two pyrene molecules are brought into close proximity to form an excimer, the fluorescence wavelength is switched to about 485 nm [15,16]. The emission wavelength switching reduces the probe background signal that occurs with FRET molecular probes. Importantly, the pyrene excimer has a longer fluorescent lifetime (40 ns) than most biological environment species (<5 ns) [17,18]. These two special features of pyrene excimer enable it to have great potential applications in a biological environment. Previous pyrene-labeled oligonucleotides have been used to detect cDNA [19] and biomarker protein-platelet-derived growth factors [17] by monitoring the monomer and excimer emission fluctuations and time-resolved measurements were used to eliminate the influence from a biological environment.

Herein, we reported a generic design method for aptamer-based optical biosensors, in which aptamer was unmodified as the molecular recognition element. A full or partially complementary oligonucleotide modified by pyrene moieties at both ends with aptamer served for transducting binding events by the pyrene excimer emission. In the paper, we used potassium ion as an example for the method. The thrombin-binding aptamer was chosen for K^+ detection because it formed a chair-type quadruplex structure on

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[1] *Abbreviations used:* FRET, fluorescence resonance energy transfer; PDA, polydiacetylene.

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binding to K^+ [20–22]. Therefore, a hairpin complementary oligonucleotide was able to organize its 5′ and 3′ ends so that the two attached pyrene moieties gave a new broad excimer band around 485 nm, accompanied by quenching of the monomer emission at 395 nm in the presence of K^+ [23].

Materials and methods

Materials

All oligonucleotides were synthesized by Shanghai Sangon Biological Engineering Technology & Service Co., Ltd., and purified by HPLC. The thrombin-binding aptamer was the sequence d(GGT *TGG TGT GGT TGG*) for detection of K^+ ion. The partially complementary oligonucleotide with aptamer was the sequence d(TGG TGT **CAA CCA CAC CA**), which was modified by two pyrene moieties at both ends. The sequence in boldface was complementary with the italic portion in the aptamer sequence. All chemicals were analytical grade and used without further treatment. All solutions were prepared in deionized water.

Apparatus

Fluorescence intensities were recorded on a Hitachi F-4500 spectrophotometer (Tokyo, Japan) equipped with a xenon lamp. UV–Vis measurements were performed with a Cary 50 UV–Vis-NIR spectrophotometer (Varian) for oligonucleotide quantitation.

Fluorescence measurements

Aptamers and the partially complementary oligonucleotide were diluted from 1.0×10^{-5} M stock solution to 0.4 μM in 5 mM Tris–HCl (pH 7.2) containing 20 mM NaCl and 2 mM $MgCl_2$, and incubated at room temperature for 15 min to form a heteroduplex. Following the addition of different concentrations of K^+, the solutions were incubated at room temperature of 5 min for competitive binding of K^+ ions with aptamer. All fluorescence spectra were measured at an excitation wavelength of 340 nm and emission wavelength at 485 nm. The slit width was 10 nm. The assay procedures for sodium, ammonium, calcium, and magnesium ions were the same as those for the K^+ ions, except that NaCl, NH_4Cl, $CaCl_2$, and $MgCl_2$ were used instead of KCl. All the experiments were performed in triplicate.

Results and discussion

Preparation of the complementary oligonucleotide probe

The relative stabilities of the heteroduplex and the self-structure of complementary oligonucleotide were predicted by the mfold program (available at the web site, http://mfold.bio-info.rpi.edu/cgi-bin/dna-form1.cgi) to help select the appropriate complementary sequence [24,25]. On the basis of thermodynamic prediction and K^+ ion reaction buffer, a 17-bp complementary oligonucleotide modified by pyrene moieties at both ends was designed. The number of complementary bases of aptamer with complementary oligonucleotide was 11.

Fluorescence measurements to detect target K^+ ions

The principle for the new complementary oligonucleotide modified by pyrene moieties for K^+ ions assays is illustrated in Fig. 1. In the presence of K^+ ions, the excimer fluorescence of pyrenes was produced by complementary oligonucleotides displaced from aptamers by targets, whereas only monomer emission was generated in the absence of K^+ ions. Consequently, it was expected to exhibit an excimer-emission intensity depending on the K^+ ions concentration. To assess the sensitivity of the strategy, the detection of K^+ ions based on thrombin-binding DNA aptamer was tested by fluorimetric titration with KCl (Fig. 2). As the protocols described under Materials and methods, Fig. 2 showed the fluorescence intensities observed on the addition of different concentrations of K^+ ions. The fluorescence spectrum in the absence of K^+ ion exhibited only a weak monomer band. With the increase of K^+ ion concentration, the resulting excimer-emission intensity increased owing to release of the free complementary oligonucleotide to give a self-structure in hairpin configuration. At the same time, two small peaks at 380 and 395 nm represented the fraction of unquenched monomer fluorescence in the two attached pyrene moieties arranged face-to-face. The spectra also showed a slight, but steady

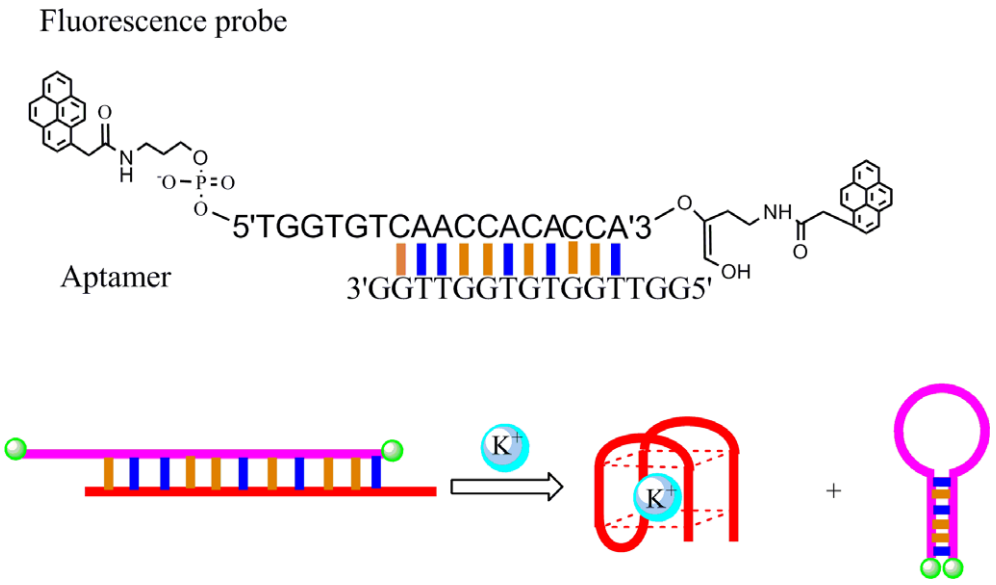

Fig. 1. General strategy for DNA/aptamer-based optical biosensors: chemical structure of the pyrene-labeled molecular beacon and the expected hairpin structure formation induced by K^+ ions.

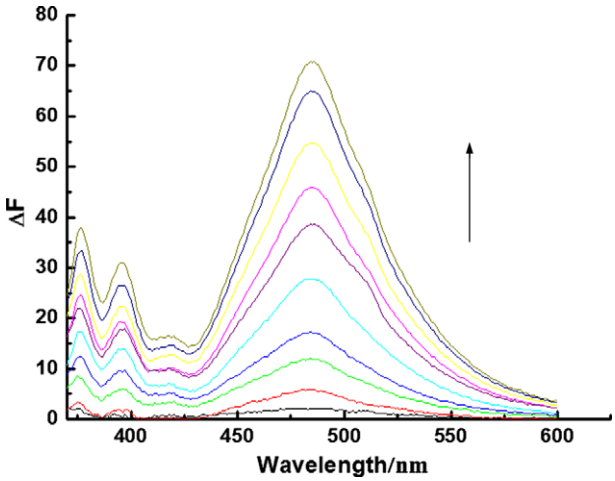

Fig. 2. Fluorescence changes of 0.4 μM heteroduplex of aptamer and complementary oligonucleotide on addition of various amounts of K^+ ions (0, 0.1, 0.6, 1, 2, 4, 8, 10, 20, 40 mM) in 5 mM Tris–HCl (pH 7.2) containing 20 mM NaCl and 2 mM $MgCl_2$; λ_{ex} = 340 nm.

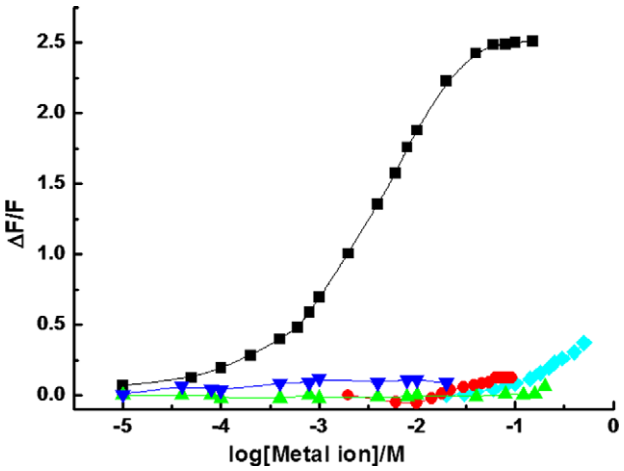

Fig. 3. Plots of the relative fluorescence intensities of heteroduplex of aptamer and complementary oligonucleotide (0.4 μM) against different concentrations of KCl (■), NaCl (♦), $MgCl_2$ (●), $CaCl_2$ (▼), and NH_4Cl (▲).

increase in the emission peaks at 380 and 395 nm, which was due to the fluorescence of the pyrene monomer fraction simultaneously present when the free complementary oligonucleotide gave a self-structure in hairpin configuration. It was also reported that the fluorescence of the pyrene monomer coexisted when two pyrene moieties were labeled at the same end of the oligonucleotide [19].

Sensitivity and selectivity for detecting target K^+ ions

The K^+ ions were detected and quantified according to the protocols described above. A correlation curve between the relative fluorescence intensity and the amount of K^+ ions was constructed (Fig. S1), and therefore, a quantitative analysis of an unknown K^+ ion concentration is also achievable. The increase in excimer emission of oligonucleotide probe was observed on the addition of K^+ ions in the concentration range of 0–150 mM with a linear dependence up to 20 mM K^+ ions. The slope of the linear plot was +11.6% per 1 mM K^+ ions and the detection limit for the 5 min of incubation was 0.4 mM, which showed good sensitivity because the serum concentration of K^+ ions is in the range of 3.5–5.3 mM. Therefore, our methodology represented an excellent sensitivity for K^+ ions detection.

Changes in excimer fluorescence of oligonucleotide probes were also monitored in the presence of Na^+, Mg^{2+}, Ca^{2+}, and NH_4^+ ions. The relative fluorescence intensities of excimer emissions were plotted against the concentrations of K^+ ions (Fig. 3). With increases in concentrations of different cation ions, only the excimer emission of K^+ ions increased dramatically, except that excimer emission was slightly detectable for the concentration of Na^+ ions exceeding 100 mM. For Na^+, Ca^{2+}, Mg^{2+}, and NH_4^+ ions, excimer emission intensities were very weak due to their weak binding affinity of thrombin-binding aptamer [26,27]. The results showed high selectivity of the proposed method for K^+ ions over Na^+, Mg^{2+}, Ca^{2+}, and NH_4^+ ions at millimolar concentrations.

K^+ under extracellular conditions comes from the coexistence of an excess of Na^+ ions. To date, numerous studies have been reported concerning K^+ ions sensors. Their selectivities for K^+ against Na^+ ions were rather low, and thus the monitoring of K^+ ions under extracellular condition was very difficult [28,29]. To study the interference of Na^+ ions, the dependence of the excimer emission of the oligonucleotide probe was examined at different concentrations of Na^+ and K^+ ions. Fig. S2 shows that the excimer emission intensities gave a value similar to 2 mM KCl in the presence and absence of 100 mM NaCl. The results indicated that the proposed method could detect 2 mM KCl in the presence of 100 mM NaCl. The minor interference from Na^+ ions clearly showed that our method could be used to detect K^+ ions with high selectivity.

Conclusions

In conclusion, a generic strategy of DNA/aptamer-based optical biosensor was developed, which could be potentially exploited for the monitoring of any target such as proteins, DNA, and RNA by the switch of the fluorescence emission from pyrene monomer to pyrene excimer. The switch of pyrene monomer to excimer was signal-on detection, which significantly reduced background signal from the probe itself. The signal-on detection is usually more sensitive than the corresponding signal-off detection [30]. Moreover, the complementary oligonucleotide was modified by pyrene moieties to ensure the affinity and specificity of aptamer with K^+. Based on these two special features, the methodology presented here represented an excellent sensitivity and specificity for K^+ detection. The strategy demonstrated a wide linear relationship with the concentration of K^+ ions ranging from 6.0×10^{-4} to 2.0×10^{-2} M, and a detection limit of 4.0×10^{-4} M of target K^+ ions. Moreover, in the presence of other cations of biological fluids, this method was able to detect K^+ with high selectivity. Especially, the interference from Na^+ ions was very minor, which is an important reason that most K^+ ion sensors could not be used in a biological environment.

On the other hand, the pyrene excimer has a much longer fluorescence lifetime (40 ns) than that of the biological background (5 ns), and time-resolved measurements can be used to eliminate the biological background [17,31]. Therefore, our strategy with oligonucleotide biocompatibility might be applicable to detect K^+ ions in serum and living cells by time-resolved fluorescence measurements. In brief, our strategy has great promise in applications, especially in a biological environment because of its simplicity, sensitivity, and specificity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ab.2009.12.034.

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