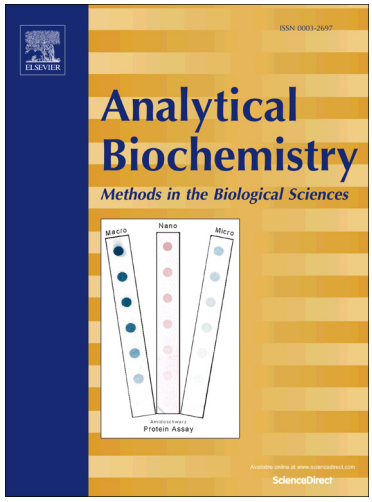

A fluorescent aptasensor for Potassium ion detection based triple-helix molecular switch

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Title: A fluorescent aptasensor for Potassium ion detection based triple-helix molecular switch

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Abstract

Herein, a biosensor based on quadruplex-forming aptamer for the determination of K^+ is presented. The aptamer was used as a molecular recognition element; it was adjacent to two arm fragments and a dual-labeled oligonucleotide serving as a signal transduction probe (STP) which is complementary of arm fragment sequence. In the presence of K^+, the aptamer were displaced from STP, which was accompanied by decrease signal. The quenching percent of fluorescence intensity was proportional to the concentration of K^+ in the range of 0.05 mM to 1.4 mM. A detection limit of 0.014 mM was achieved. Furthermore, other metal ions, such as Na^+, Li^+, NH_4^+, Mg^{2+} and Ca^{2+} caused no notable interference on the detection of K^+.

Keywords: G-quadruplex, Aptamer, Potassium ion, Biosensor, Fluorescence aptasensor.

1. Introduction

Potassium ion (K^+) plays an important role in living organisms. The maintenance of suitable potassium concentration is essential for many physiological activities in living cells, such as nerve transmission, preventing muscle contraction, enzyme activation, balancing the pH, apoptosis, regulation of blood pressure, etc. [1-4]. Unusual K^+ concentrations may cause several diseases such as Addison's, kidney, adrenal gland diseases, heart disease, diabetes, AIDS and cancer [5-7]. Therefore, the determination of K^+ is very critical in clinical diagnosis and nutritional analysis. To date, ion chromatography (IC) [8], ion-selective electrodes (ISE) [9], flame atomic absorption spectrometry [10], electrochemistry [11] and surface plasmon resonance (SPR) [12] schemes have been used in the detection of potassium. However, these conventional

methods are intricate, time-consuming and involve cumbersome laboratory procedures. It is necessary to develop simple and sensitive alternatives for K^+ determination. Recently, aptamers have received much attention as the recognition components in the biosensors design and diagnostic applications due to their advantage compared with the conventional recognition components, antibodies [13-15]. Accordingly, a number of potassium aptasensors have been constructed based on the quadruplex-forming aptamers [2, 16-18]. G-rich oligonucleotides can form the four-stranded helical conformation with stacked arrays of G-quartets connected by Hoogsteen-type base pairing called G-quadruplex [19, 20]. Excellent studies on the use of this structure as an identifier probe have been reported [16, 21-27]. The ionic radius of cation is the parameter that determines how well cation stabilizes G-quadruplex [28]. Lately, several fluorescence aptasensors for K^+ based on the formation of the G-quadruplex have been offered [29-32]. This type of probe, usually called molecular beacon, shows high selectivity and sensitivity for K^+ detection. However, it requires oligonucleotides to be labeled since labeling with different fluorescent and quenching molecules might affect the primary binding affinity and specificity between the aptamers and their target, therefore greatly limiting their applicability [11]. Herein, we reported a new type of potassium aptasensor which is based on a newly reported triple-helix molecular switch strategy [33]. A recently developed 30-base-long insulin-binding aptamer (IBA) was chosen for K^+ detection because it formed an interamolecular parallel G-quadruplex structure [34-36]. Our aptasensor shows excellent selectivity against other common interfering ions. In addition, by separating the molecular recognition element and signal reporter, there is no need to label the original aptamer, which is required in most of the aptasensors to preserve the affinity and specificity of the original aptamer.

2. Experimental

2.1. Apparatus

UV-Vis absorption spectra were recorded in 1 cm path length quartz cuvette on a Shimadzu UV-2550 (Shimadzu Corporation, Kyoto, Japan) double beam spectrophotometer. Fluorescence measurements were performed on a Hitachi F-2700 spectrophotometer (Tokyo, Japan) equipped with a xenon lamp. Fluorescence emission spectra were collected using a bandwidth of 10 nm. Water used in these experiments was purified by using Milli-Q apparatus (Millipore Inc., Billerica, MA). All pH measurements were performed with a WTW pH meter-pH 537.

2.2. Chemical and reagents

HPLC purified DNA oligonuleotide were purchased from Bioneer, Korea. Table1 shows the oligonucleotide used in this study.

Insert Table 1

All sequences were dissolved in highly pure water (sterile Miniporewater, 18.3MΩ) as stock solutions; the concentrations of ordinary oligonucleotides is determined from absorption spectra from the nearest-neighbor model of Cantor et al. [37]. In the case of STP the concentration was determined by measuring the absorbance of the attached Fluorescein moiety at 496 nm using a molar extinction coefficient of 4.1×10^4 $M^{-1}cm^{-1}$ [38]. All chloride salts were of analytical grade and were used without further purification.

2.3. Fluorescence detection procedure of sensing K^+

Aptamers were diluted from 10.0×10^{-5} M stock solution to 100 nM and 140 nM for STP and IBA, respectively, in 5 mM Tris-HCl (pH 7.5) containing 20 mM NaCl and 2 mM $MgCl_2$ and incubated at room tempreture for 20 min to form a triple-helix. The fluorescence intensity of STP (100 nM) was monitored for a few minutes. Then, IBA1 or IBA2 was added to the probe buffer, and the final concentration was 140 nM; the fluorescence intensity was measured at room temperature. After confirming that there was no change of fluorescence with time about 20 min, (Fig. S1), cation was added. Excitation wavelengths all fluorescence spectra were 480 nm for FAM. All measurements were carried out using a 10 mm quartz cell. The same procedures were repeated in the presence of NaCl, $CaCl_2$, LiCl, $MgCl_2$ and NH_4Cl instead of KCl to assess the selectivity against other metal ions. The concentration of K^+ was quantified by the decreased fluorescent intensity at 525 nm.

3. Results and discussion

3.1. Principle of fluorescent assay of K^+ and the analytical performance of the sensor

The idea behind these sensors is brought in view of special metal cation binding properties of oligonucleotides. The most interesting feature of quadruplex structure is the presence of cavity formed by stacking of G-tetrads that can accommodate selectivity certain cations. This consideration provided the motivation to design an aptasensor for the potassium ion based on conformation switch of IBA to G-quadruplex structure. The designed procedure of this aptamer-based fluorescent method for K^+ detection was depicted in Scheme 1.

Insert Scheme 1

A recently developed 30-base-long insulin-binding aptamer (IBA) is used in this study as a probe which can detect potassium ion (Table 1). The IBA sequence is known to form an

interamolecular parallel G-quadruplex structure [34-36]. Conformation switch of IBA from a loose random coil into a compact G-quadruplex in the presence of cation is the base for this aptasensor designing. The triple-helix molecular switch consist of a central, insulin binding aptamer sequence (in red) flanked by two arm segments and a dual-labeled oligonucleotide serving as a signal transduction probe (STP) which is complementary of arm segments sequence (in blue). In the absence of IBA the sequence of STP is fold into the hairpin-shaped structure, thus, bringing the fluorophore (FAM) and quencher (BHQ1) into spacer vicinity. After adding IBA into STP the arm segments of IBA bound with the loop sequence of STP by Watson-Crick and Hoogesteen base pairings and the intensity of fluorescence of FAM increase. Upon the introduction of potassium, the IBA forms G-quadruplex structure and releases the STP. The STP then changes from the linear configuration to the originally loop-shape configuration, leading to decrease signal. We investigated the arm length of the aptamer effect on operation of aptasensor (IBA1 and IBA2) and selected IBA1 as the probe for our aptasensor (Fig. S2). Fig. 1 shows the fluorescence emission spectra of the STP at three stage operation of aptasensor.

Insert Fig. 1

The free STP is quenched owing to proximity of BHQ1 and FAM in hairpin-shape. Then, the formation of a triple-helix structure separates these labels and the intensity of fluorescence increases. After adding K+ the IBA fold to G-quadruplex and releases the STP, leading to decrease the intensity.

For quantitative analysis of K+, the fluorescent signal of solution triple-helix in the presence of KCl was measured. Fig. 2 shows the changes in the fluorescence intensity of the sensor with the addition of different concentrations of potassium. The experiments were performed in triplicate.

Insert Fig. 2

Fig. 2A indicated that the fluorescent signal at 520 nm decreased with the addition of K^+; a very good linear relationship was observed between fluorescent signal at 520 nm and the K^+

concentration. The plot in Fig. 2B demonstrates useful concentration range for quantitative analysis of K^+. The linear dynamic range gave the limit of K^+ detection (LOD) as low as 0.014 mM and an extremely wide linear range between 0.05–1.4 mM. Here, IBA shows the ability to be used as a new sequence which can detect K^+ in the concentration range between 0.05 to 1.4 mM.

1.3.2. The specificity of the aptasensor

In order to evaluate the selectivity of the proposed sensor for the detection of K^+, experiments were thus conducted by using Na^+, Li^+, NH_4^+, Ca^{2+} or Mg^{2+} as the potential interference ions (Fig. 3), the concentrations of cations are 2 mM. As shown in Fig. 3A, for these ions, only small current signals can be obtained. This indicates that the designed aptasensor has an adequate specificity for K^+, and is also able to discriminate K^+ from its analogs in complex samples. As shown in Fig. 3B, the fluorescence spectra for the mixture of the different metal ions (Ca^{2+}, Mg^{2+}, Na^+, Li^+ and NH_4^+, all at 2 mM) other than K^+ ions (2 mM) had a little change compared with the background due to the low binding affinity of the aptamer to these ions and little tendency to form a G-quadruplex. The results show that only K^+ caused a marked response on the fluorescent intensity at 520 nm and other metal ions has no obvious interference, suggesting that this proposed sensing system is specific for the detection of K^+.

Insert Fig. 3

4. Conclusion

Herein, we describe a simple and sensitive fluorescence aptasensor for the detection of K^+ based on G-quadruplex structures. Recently, several fluorescence aptasensors for K^+ based on the formation of the G-quadruplex have been reported. Most of them require labeling of the

oligonucleotides to be labeled and the labeling with different molecules might affect the original binding affinity and specificity between the aptamers and their targets to some extents, therefore greatly limiting their applicability. Fortunately, in our aptasensor the complementary oligonucleotide was modified by Fluorophore labels to ensure the affinity and specificity of aptamer with K^+. The method demonstrated a linear relationship between 0.05 mM to 1.4 mM of K^+, with a detection limit of 0.014 mM. Moreover, in the presence of other metal ions, this method was able to detect K^+ with high selectivity.

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Scheme 1. Schematic illustration of a K^+ sensor that operates based on IBA folding to G-quadruplex.

Fig. 1. Fluorescence emission spectra of 100 nM STP (a), (a) + 140 nM IBA1 (b), and (b) + 1.4 mM K^+ (c). λex = 480 nm

Fig. 2. (A) The Fluorescence emission spectra of aptasensor over the K^+ concentration of 0.014 mM–5 mM, (B) the peak absorbance change at 520 nm as a function of K^+ concentration. Inset: the peak absorbance change of 520 nm is linear with K^+ concentration over the range from 0.05 mM to 1.4 mM.

Fig. 3. Specificity assays. (A) The relative response of the sensing system to different ions. The quenching percent changes for other interferences, compared to K^+, are very small, indicating excellent selectivity. The concentrations of cations are 2 mM. (B) The Fluorescence spectra of triple-helix solution in the presence of different ions with various concentrations (a) 0 mM K^+, (b) Na^+, Li^+,Ca^{2+}, Mg^{2+} and NH_4^+ (all at 2 mM), (c) K^+, Na^+, Li^+,Ca^{2+}, Mg^{2+} and NH_4^+ (all at 2 mM).

Scheme 1.

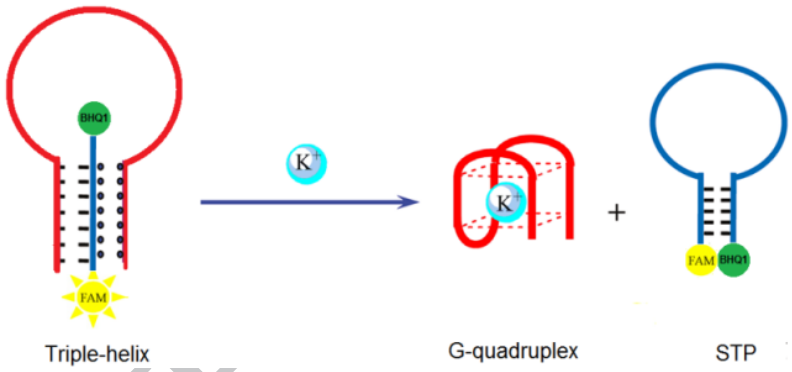


Figure 1

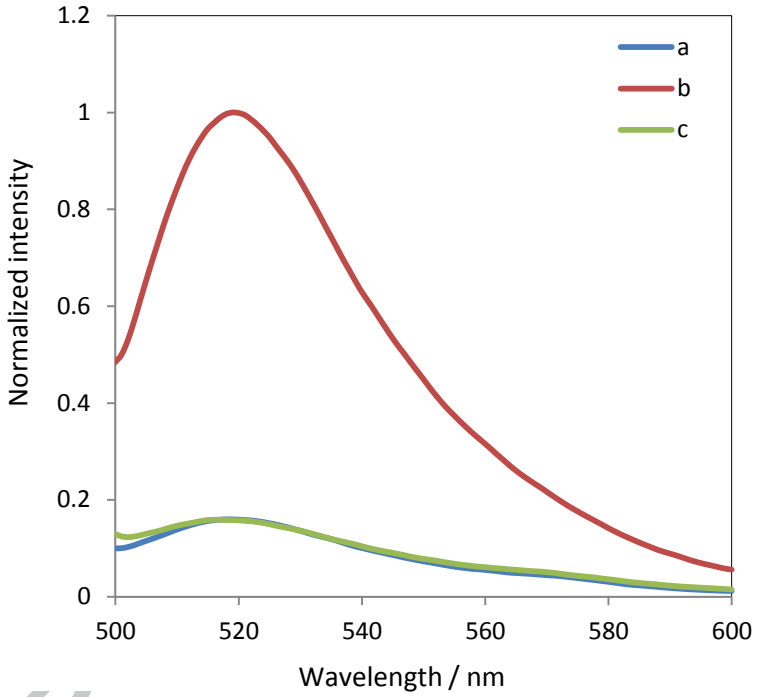


Figure 2

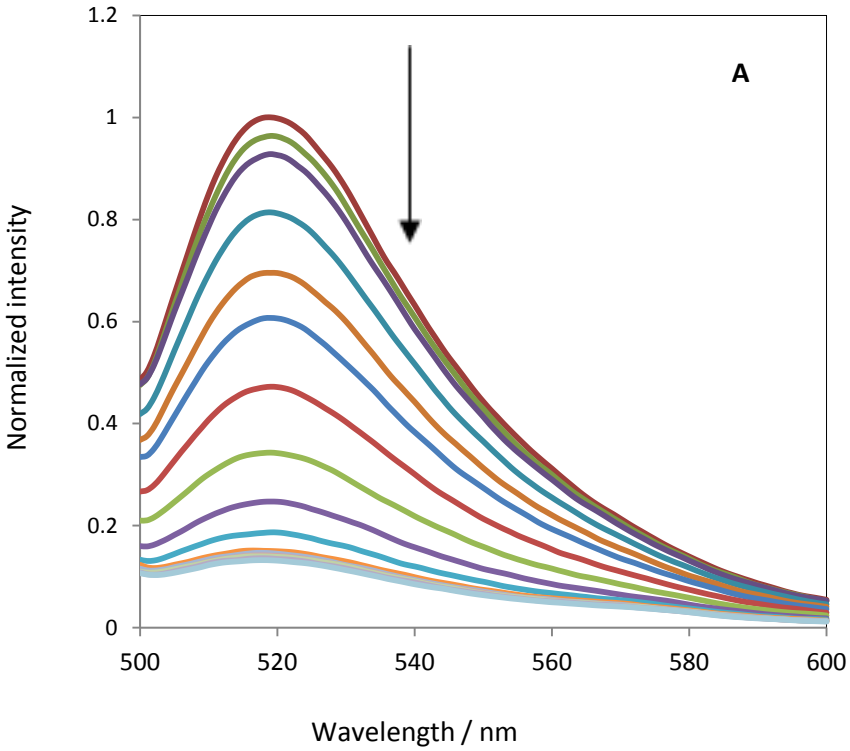


Figure 2

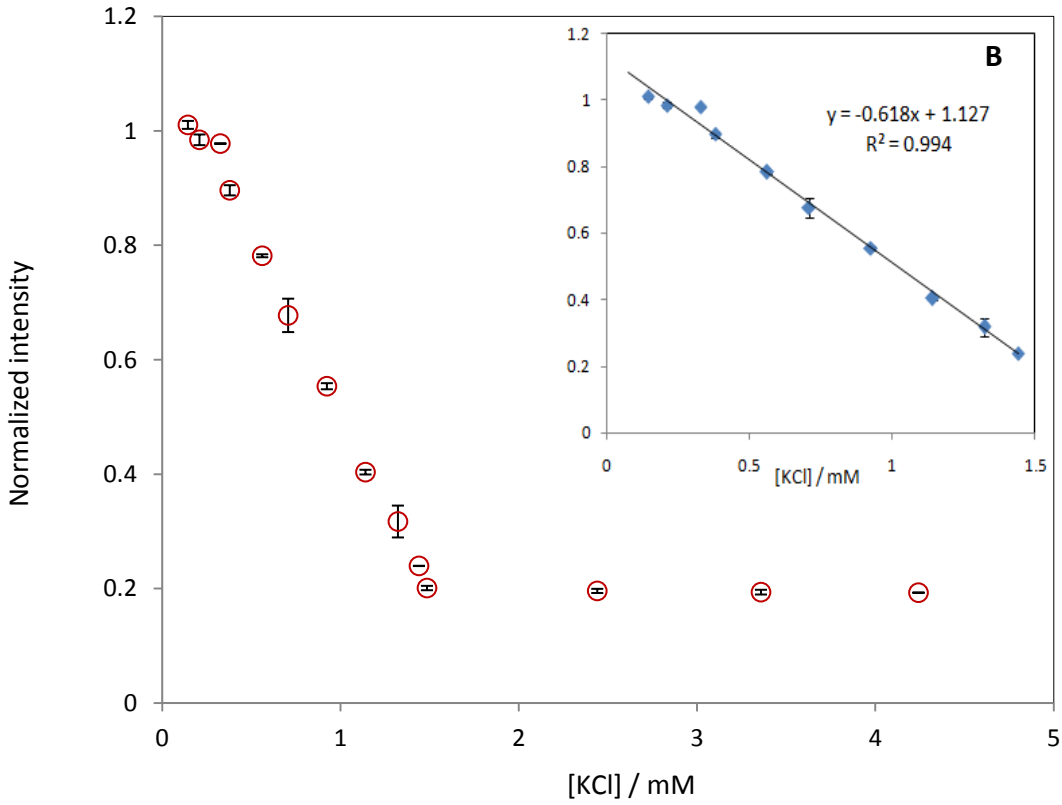
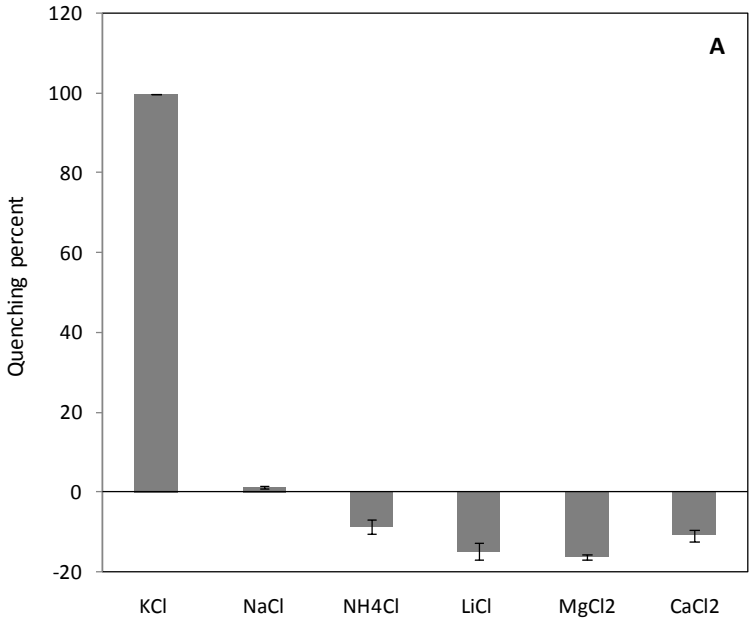


Figure 3



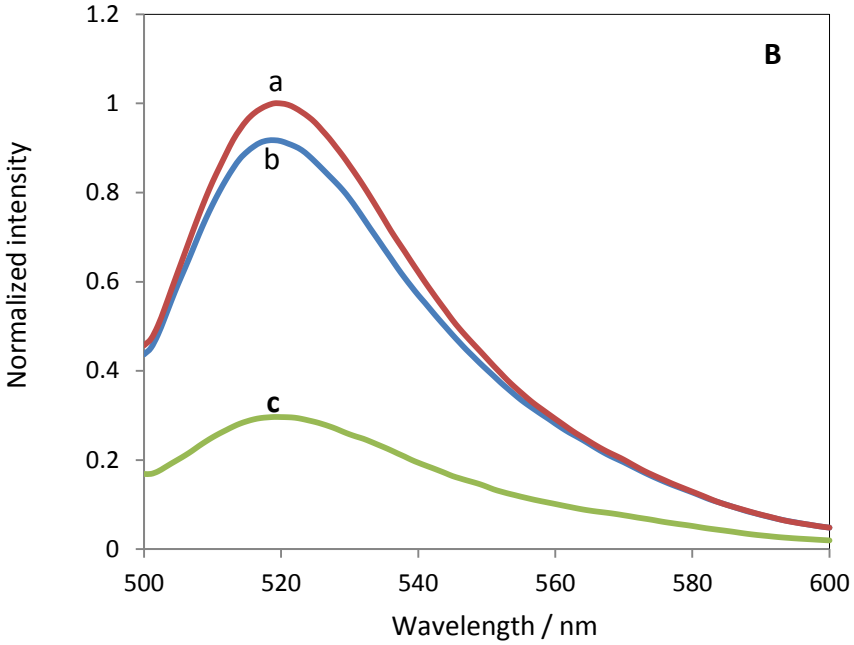


Table 1
Oligonucleotide sequences used in this work

Abbreviation	sequence
IBA1	CTCTCTGGTGGTGGGGGGGGTTGGTAGGGTGTCTTCTCTCTC
IBA2	CTCTCTCGGTGGTGGGGGGGGTTGGTAGGGTGTCTTCCCTCTCTC
STP	FAM-GAGGAGAGAGAGAGATCCTC-BHQ1