Aptamer-based fluorescence sensor for rapid detection of potassium ions in urine†

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We unveil a new homogeneous assay—using OliGreen and an ATP-binding aptamer—for the highly selective and sensitive detection of potassium ions.

Potassium ions (K⁺) are involved in many biological functions, including nerve transmission, regulation of blood pressure and pH, enzyme activation, and the formation of collagen or elastin.1 Abnormal levels of K⁺ ions in biological fluids can cause muscle cramps or weakness, nausea, diarrhoea, frequent urination, dehydration, paralysis, and changes in heart rhythms.1

Potassium ions promote the formation of G-quadruplexed structures of telomeric DNA at the termini of the eukaryotic chromosome. The G-quadruplex of telomeric DNA is an unusual tetraplex conformation that has been demonstrated to directly inhibit telomerase activity in immortalized and most cancer cells.2,3 Thus, the ability to monitor the concentration of K⁺ ions in biological fluids has become a very important challenge. Although many fluorescence indicators—including benzo[15]crown ethers and σ-cyclodextrin fluoroionophores—have been employed for the detection of K⁺ ions, several drawbacks, such as the lack of selectivity against other alkali ions or the need for non-aqueous solutions, limit their clinical applications.4

Oligonucleotides have been used for their highly selective and sensitive detection to improve the accuracy of assays for K⁺ ions.5 Single-stranded (ss) and random-coiled oligonucleotides possessing four GGG sequences form uniquely selectivity K⁺ binding sites in G-quadruplexes,5 presumably because of the similar sizes of the K⁺ ion (1.3 Å) and the cavities created between two G-quadruplexes.5 Fluorescence resonance energy transfer (FRET) imaging has been used in conjunction with G-rich oligonucleotides possessing the human telomere sequence [dG3(T2AG3)3] and the 15-mer thymidin-binding aptamer, labeled with 6-carboxyfluorescein and 6-carboxytetramethylrhodamine, respectively, to monitor K⁺ ions in an excess of Na⁺.5a,b In addition, the K⁺-induced formation of efficiently emitting excimers from dGGTTGGTGTGGTTGG and pyrene has been demonstrated.5c Although cationic conjugated polymer—G-quadruplex assemblies, G-quadruplex-induced aggregation of gold nanoparticles (Au NPs), and an electrochemical aptasensor have been developed for the selective detection of K⁺ over Na⁺,5d,f their applicability to biological systems is limited by the complicated nature of their sample matrices.

In this paper, we report a molecular fluorescence switching method to signal aptamer-K⁺ binding using an ATP-binding aptamer (dACCTGGGGGAGTATTGCGGAGGAAGGT) and OliGreen (OG), a commercially available asymmetrical cyanine dye that is used to label oligonucleotides.6 OG is weakly fluorescent, but exhibits a greater-than-1000-fold enhancement in its fluorescence upon binding to ssDNA (with excitation and emission maxima at 480 and 520 nm, respectively). Scheme 1 outlines the sensing mechanism that we employed in this study. When the ATP-binding aptamer (Apt) in its random-coil (ss) structure is added to an OG solution, an OG-Apt complex is formed immediately, leading to increased fluorescence. After adding K⁺, the conformation of Apt changes from that of a random coil to two stacked G-quadruplexes and two short double-helix stems. Because OG prefers binding to ssDNA, dissociation of the OG-Apt complex occurs to some extent upon this conformational change. As a result, the fluorescence of the system decreases in the presence of K⁺.

Fig. 1 displays the fluorescence spectra that prove this concept. Curve A indicates that the fluorescence of OG (0.025X) in 5 mM Tris-HCl (pH 7.4) is very weak. In this study, we define the concentration of OG obtained from Molecular Probes (Portland, OR) as 100X. The fluorescence intensity at 520 nm of the OG solution (0.025X) increased by greater than 1000-fold after the addition of 50 nM random-coil ATP Apt (curve B). We note that the fluorescence intensity reached a plateau at 50 nM Apt when the concentration of OG was 0.125X (Fig. S1, ESI†). When 10 μM K⁺ was added into a mixture of OG (0.025X) and Apt (50 nM), a rapid

Scheme 1 Schematic representation of a K⁺ sensor that operates based on modulation of the fluorescence of the complex formed between the dye OG and the ATP-binding aptamer. h Planck’s constant; ν frequency of light.

Fig. 1 Schematic representation of a K⁺ sensor that operates based on modulation of the fluorescence of the complex formed between the dye OG and the ATP-binding aptamer. h Planck’s constant; ν frequency of light.
In this case we did not observe any K⁺ (10⁻⁶ M), but it decreased at higher concentrations (Fig. S3, ESI†). Bromide (EtBr), YOYO-3, TOTO-3, methylene blue, acridin orange, and DMDAP all have weaker binding affinities toward DNA (Kₐ = 10⁴–10⁶ M⁻¹) than does OG,⁹ their sensitivity for the detection of K⁺ was lower than that of OG in the presence of Apt. We conclude that, among the complexes we tested, the OG-Apt complex was the most sensitive toward K⁺ ions.

Fig. 2(a) depicts the changes in fluorescence of the OG-Apt complex upon the addition of K⁺ (0–5.0 µM) under the optimum conditions (0.01X OG, 50 nM ATP Apt, 10 mM Tris-HCl, pH 7.4). The OG-Apt sensor exhibited a linear increase in its value of (I₀ – I)/I₀ upon increasing the K⁺ concentration from 100 to 1000 nM. The limit of detection (LOD) was found to be 75 nM K⁺, based on a signal-to-noise ratio (S/N) of 3. Thus, our approach pushes the sensitivity lower, by one order of magnitude, relative to those of other reported G-quadruplex-mediated K⁺ sensors.⁵ Although the linear range of our probe is narrow, it can be tuned by changing the concentrations of OliGreen and Apt. When using higher concentrations of OliGreen and Apt, we can achieve a wider linear range despite having a lower sensitivity. For example, we can achieve a linear range 0.25–5.0 µM (LOD 0.22 µM) when using 0.05X OliGreen and 250 nM Apt. The binding constant (Kₘ) for the interaction between the OG-Apt complex and K⁺ ions, calculated (by fitting experimental data to an equation derived for a complex having 1 : 1 stoichiometry) from the plot provided in the inset to Fig. 2, was 2.5 × 10⁶ M⁻¹ (Fig. S5, ESI†). The ultralow background fluorescence of OG and the high affinity of Apt toward K⁺ are two factors contributing to this system’s greater sensitivity relative to that of traditional sensors.⁷ The K⁺ binding oligonucleotide possessing the human telomere sequence [dG₃(T₂A₂G₃)]₃ was also tested for K⁺ sensing (Fig. S6, ESI†). The linear relationship exhibited increases in the value of (I₀ – I)/I₀ upon increasing the K⁺ concentration over the range 10–250 µM. The limit of detection (LOD) was 8.5 µM. However,
its sensitivity is lower than that on using ATP aptamer, mainly because the formation constant for the K⁺–aptamer complex is smaller than that of K⁺–ATP aptamer complex.5

We investigated the selectivity of our new sensor toward K⁺ over other ions, namely Na⁺, Li⁺, NH₄⁺, Mg²⁺, and Ca²⁺, under the optimum conditions. When we plotted the values of (I_F0 – I_F)/I_F0 for the OG-Apt complex against the concentration of the tested ions (Fig. 2b), we found that only K⁺ provided a significant positive response; only when the concentrations of NH₄⁺, Mg²⁺, Ca²⁺, and Na⁺ exceeded 0.01, 0.01, 0.10, and 1.00 mM, respectively, did these ions induce slight dissociations of the OG-Apt complex. Generally, the ionic radius is the parameter that determines how well G-quadruplex structures are stabilized by various cations.2,5 The stabilities of alkali and alkalai earth metal ions in G-quadruplex structures decrease in the order K⁺ > Na⁺ > Rb⁺ > Cs⁺ > Li⁺ and Sr²⁺ > Ba²⁺ > Ca²⁺ > Mg²⁺, respectively.5 The selectivity of our OG-Apt complex sensor toward K⁺ over Na⁺ was greater than 10 000-fold, which is much higher than the values obtained for the potassium-binding benzo[1,5]dioxophosphate (1.5) and coumarin diacid cryptand [2.2.2] (3.4) and is comparable to that of the best reported G-quadruplex-based K⁺ sensor.4,5 Note, however, that our simple method does not require covalent labeling of Apt prior to analysis.

We tested our OG-Apt complex sensor for the determination of K⁺ ions under physiological conditions. Aliquots of K⁺ (0–20 mM) were spiked into synthetic solutions (145 mM Na⁺, 1.5 mM Mg²⁺, and 2.5 mM Ca²⁺) and then the mixtures were diluted with 10 mM Tris-HCl (pH 7.4). The value of (I_F0 – I_F)/I_F0 of the OG-Apt complex increased linearly upon increasing the concentration of spiked K⁺ ions from 0 to 10 mM (Fig. S7, ESI†); the recovery of K⁺ was 93–98%. Next, we tested the practicality of this present approach for the analysis of K⁺ ions in urine samples. The level of K⁺ in urine is indicative of certain kidney diseases.10 The normal range of K⁺ excreted from a healthy human being on a regular diet is 25–125 mmol L⁻¹ per 24 h. We filtered urine samples from seven healthy volunteers through 0.2 μm membranes, diluted the samples 100 000-fold using 10 mM Tris-HCl (pH 7.4), and then analyzed them using the 50 nM OG-Apt complex. Table 1 lists the results obtained using both our sensor and inductively coupled plasma mass spectrometry (ICP-MS). On the basis of an F-test, the results from our present approach are in good agreement with those obtained using ICP-MS. Note that, when using our OG-Apt complex sensor, the sample preparation and analysis time was less than 15 min; this assay also provides the advantages of simplicity and cost-effectiveness, and it is amenable to high throughput.

In conclusion, we have developed a homogenous K⁺ assay using ATP Apt and OG. Upon binding to K⁺ ions, the OG-Apt complex having a linear structure converts into a G-quadruplex structure. As a result, some of the OG units dissociate from the complex, leading to a decrease in fluorescence. The selectivity of this system for K⁺ over other ions in aqueous solution is remarkably high (>10 000-fold over Na⁺) and its LOD is 75 nM. The present approach has several advantages over other assays; it is rapid (<15 min), label-free, simple, and cost-effective. This sensing strategy is not limited to probing the formation of G-quadruplexes and the detection of K⁺; it can be applied to investigations of the conformational changes that occur to most biomolecules upon binding to their targets.

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Table 1 Concentrations of K⁺ in urine samples from seven healthy volunteers measured using the OG-Apt complex fluorescence sensor† and ICP-MS

<table>
<thead>
<tr>
<th>Urine sample</th>
<th>OG-Apt sensor</th>
<th>ICP-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD (mM (n = 5))</td>
<td>Mean ± SD (mM (n = 5))</td>
</tr>
<tr>
<td>A</td>
<td>15.0 ± 1.5</td>
<td>16.0 ± 1.8</td>
</tr>
<tr>
<td>B</td>
<td>44.4 ± 2.6</td>
<td>45.3 ± 3.2</td>
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<tr>
<td>C</td>
<td>53.1 ± 2.6</td>
<td>52.6 ± 4.3</td>
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<tr>
<td>D</td>
<td>15.4 ± 1.9</td>
<td>16.5 ± 2.1</td>
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<tr>
<td>E</td>
<td>20.6 ± 2.0</td>
<td>21.5 ± 1.2</td>
</tr>
<tr>
<td>F</td>
<td>48.3 ± 2.5</td>
<td>51.3 ± 3.2</td>
</tr>
<tr>
<td>G</td>
<td>29.3 ± 2.3</td>
<td>32.3 ± 1.3</td>
</tr>
</tbody>
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F-test value is 6.39 at a 95% confidence level.

Notes and references