Aptamer conformational switch as sensitive electrochemical biosensor for potassium ion recognition†

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We report the first use of an electrochemical aptasensor for selective potassium recognition, based on a conformational change, affording an electric signal transduced electrochemically by square wave voltammetry or electrochemical impedance spectroscopy.

Aptamers are single-stranded DNA or RNA molecules, isolated from large pools of random-sequence oligonucleotides, that are capable of binding a wide range of chemical or biological entities with high affinity and specificity.[1] Aptamer oligonucleotides that contain single or multiple guanine-rich segments are known to form specific four-stranded helical conformations in solution. The formation and stabilities of these conformations are strongly dependent on the presence of monovalent cations with an extraordinary selectivity for potassium.[2] Aptamers can serve as molecular switches based on the conformational ordering they undergo upon analyte binding.[3] In this context, fluorescent molecular sensors have been designed for potassium sensing in aqueous media, taking advantage of G-rich synthetic oligonucleotide recognition of monovalent cations, especially potassium ions.[4]

Herein, we describe the first use of a signal-on architecture for electronic recognition of potassium ions in aqueous solution. It is known that the K^+ ion can promote the conversion of a G-rich sequence from a loose random coil into a compact G-quadruplex. This consideration provided the motivation to design an aptasensor for the potassium ion using an assay format that could probe the G-quadruplex structure. This approach generated a signal upon recognition of K^+, which was transduced electrochemically by square wave voltammetry (SWV) and electrochemical impedance spectroscopy (EIS), representing a signal-on device and demonstrating for the first time the exploitation of an aptamer to electrochemically sense cations.

The response of the biosensor for different concentrations of K^+ was evaluated with SWV as this technique obviates double-layer effects and typically gives lower detection limits. When the 5′-ferrocene(Fc)–DNA–SH-3′ modified Au electrode is exposed to a solution of K^+ in HEPES buffer, an anodic peak, corresponding to the oxidation of ferrocene is observed, as shown in Fig. 1.

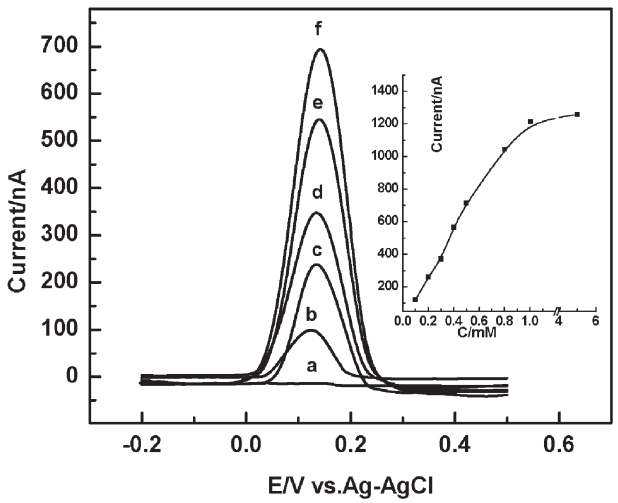
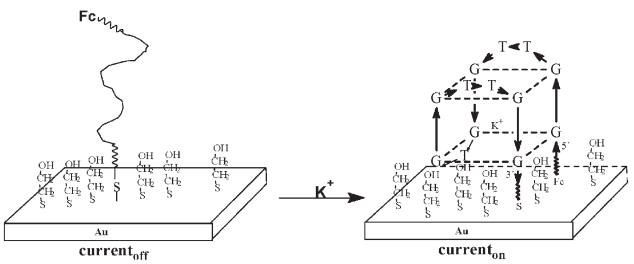


Fig. 1 Baseline-corrected square wave voltammograms in blank HEPES buffer solution (0.01 M, pH 8.0) for (a) 5′-Fc–DNA–SH-3′ modified gold electrode after exposure to increasing concentrations of K^+ (b) 0.1, (c) 0.2, (d) 0.3, (e) 0.4 and (f) 0.5 mM. Step potential of 4 mV, amplitude of 50 mV, and frequency of 120 Hz. Inset: the corresponding calibration plot of the peak current *vs.* the K^+ concentration.

A possible explanation for these electrochemical results is shown in Scheme 1. In the absence of potassium, the aptamer that contains multiple guanine-rich segments, adopts a random-coil structure. Exposure to K^+ solution displaces the equilibrium in favor of the quadruplex form; the G-quadruplex being a conformation of guanine-rich DNA resulting from the association of sets of four guanine residues into planar arrays. This results in the localization of the 5′-end attached ferrocene in close proximity to the gold surface, switching the electronic path for current flow



Scheme 1 Mechanism of the structural transition of a G-rich aptamer from random coil to G-quadruplex induced by K^+. Solid and dashed lines indicate covalent and hydrogen bonding connections, respectively. Arrows indicate the strand directions.

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between the redox label and the electrode surface *i.e.*, the conformational transition is transduced into an electric based "off–on" electrochemical switch. As K^+ is added, the surface loading of bound K^+ increases, boosting the Faradaic current until a levelling off is obtained at a K^+ concentration of $\sim$1.0 mM (Fig. 1. inset), as a consequence of the full occupation of all the primary binding sites in the film by one potassium per DNA and the formation of an intermediate 1:1 complex. At concentrations higher than 1.0 mM K^+, a second potassium is accommodated by the secondary binding sites to form a potassium-saturated, 2:1 complex. The structure of the DNA aptamer in the presence of one potassium is, however, very similar in the most respect to that in the presence of two potassiums per DNA.[5] The aptasensor showed a linear response of anodic signal to the increase of K^+ concentration in the range of 0.1–1.0 mM. The limit of detection (LOD) was 0.015 mM, estimated as $3 \times S_b/m$, where S_b is the standard deviation of the measurement signal for the blank and m the slope of the analytical curve.[6]

Then, a test of the aptasensor for the determination of K^+ in physiological conditions was achieved (physiological concentrations: Na^+, 136–158 mM; K^+, 3.61–4.85 mM; Ca^{2+}, 3.04–5.27 mM; Mg^{2+}, 1.01–2.12 mM).[7] The biosensor response was evaluated for an assayed solution with mole fractions similar to those found in physiological solutions of the four cations. The electrode responded significantly to K^+ over Mg^{2+}, Na^+ and Ca^{2+} cations. The aptasensor generated anodic signals of values 715, 140, 60 and 35 nA for K^+, Na^+, Mg^{2+} and Ca^{2+} at concentrations of 0.5, 70.0, 0.15 and 0.5 mM, respectively. The stabilization of the G-quadruplex structure in the presence of Ca^{2+} cations was reported.[8] The use of ethylene glycol-bis(2-aminoethyl ether)-*N*,*N*,*N*',*N*'-tetraacetic acid (EGTA) to control the concentration of calcium is a common practice with calcium determinations.[9] In the absence of EGTA, background levels of Ca^{2+} limit the detection ability of the aptasensor for potassium. As expected, addition of the chelating agent EGTA diminished the anodic signal due to the reduction in available Ca^{2+} and no signal could be detected for equimolar concentration of EGTA. In a separate experiment, the addition of the chelating agent EGTA to a mixture of K^+ and Ca^{2+}, afforded the same anodic signal, which corresponds to the presence of potassium alone in the absence of added calcium. The addition of K^+ in the presence of 70.0 mM Na^+ and 0.15 mM Mg^{2+} leads to a linear increase in anodic signal intensity from 0.3 to 1.0 mM with a detection limit of 0.035 mM. Although the measurement range and detection limit of the aptasensor were shifted to higher concentrations with increasing initial Na^+ or Mg^{2+} concentration, the aptasensor is still sensitive enough for determining physiological levels of K^+ after sample dilution.

For the aptasensor to be useful as a biosensor, the transitions between the random coil and quadruplex forms should be relatively dynamic. The aptamer could be regenerated in 0.1 M HCl (within 60 s). Subsequent addition of K^+ rapidly resulted in the re-formation of the G-quartet and the aptasensor was operated continuously for 15 regeneration/association/measurement cycles in the same day with no significant change in electrochemical signal, with a RSD of 7.0%.

EIS was used to further probe ion recognition at the interface. The impedance response for a DNA–SH-3′/2ME modified gold electrode was measured in the presence of a $[Fe(CN)_6]^{3-/4-}$ redox probe with increasing K^+ concentration and the data are presented in Fig. 2, as a Nyquist plot (Z_{im} *vs.* Z_{re}). The impedance spectra of the DNA–SH-3′ SAMs are best described by the Randles equivalent circuit and include a semicircle portion. The immobilization of DNA–SH-3′on the electrode surface generates a negatively charged interface that electrostatically repels the $[Fe(CN)_6]^{3-/4-}$ anionic redox probe, introducing a barrier for electron transfer at the electrode surface, increasing the electron transfer resistance R_{et}. The recognition event results in an increase in the electron transfer resistance R_{et}. Because the dominant interaction between $[Fe(CN)_6]^{3-/4-}$ and DNA is electrostatic in nature, 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 and that of more condensed G-quadruplex DNA is only about 1.1 nm,[10] while they possess the same charge numbers. Accordingly, formation of the quadruplex enhances the repulsion of the redox probe and results in a further increase in the electron transfer resistance at the electrode. The dependence of R_{et} on the concentration of potassium ions showed an initially linear increase of R_{et} at low concentrations of K^+ that levels off at concentrations above 1.0 mM, and finally reaches a plateau value (Fig. 2, inset).

The response of the DNA–SH-3′ SAMs was determined for the presence of K^+, Na^+, Mg^{2+} and Ca^{2+} at concentrations of 0.5, 70.0, 0.15 and 0.5 mM, respectively (Fig. 3). It is clear that potassium gives the largest response. Besides potassium, Ca^{2+} cations also cause an obvious increase of the charge-transfer resistance. On the other hand, addition of Mg^{2+} has less effect on the charge-transfer resistance. Sodium also gives rise to an increase in charge-transfer resistance. As previously, EGTA was added to complex interfering Ca^{2+} cations. In the presence of these concentrations of cations it is possible to detect potassium concentrations down to 0.1 mM, and the results can be reproduced repeatedly over the course of days with a useful range of 0.3–1.0 mM K^+.

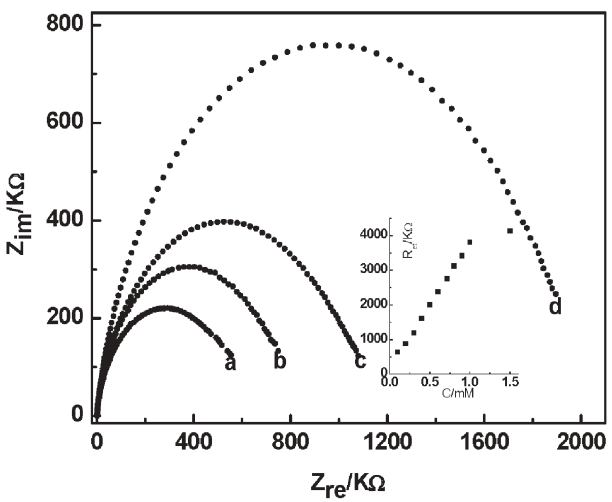

Fig. 2 Typical Nyquist plots recorded at a DNA–SH-3′ modified Au electrode in the presence of increasing concentrations of K^+: (a) 0.1, (b) 0.2, (c) 0.3 and (d) 0.5 mM, along with the corresponding calibration plot (inset). The impedance spectra were recorded in a 1 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) in 0.1 HEPES buffer (pH 8.0) solution, using a frequency range of 100 kHz–10 mHz, a bias potential of +0.160 V and an AC amplitude of 5 mV.

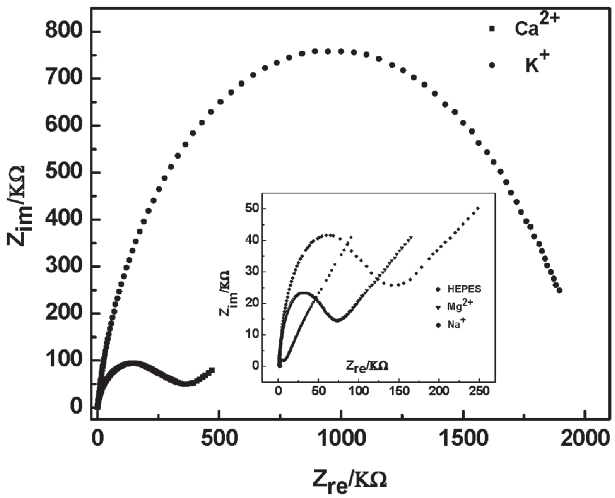

Fig. 3 Nyquist plots recorded at a DNA–SH-3′ modified Au electrode in the presence of K^+, Na^+, Mg^{2+} and Ca^{2+} at concentrations of 0.5, 70.0, 0.15 and 0.5 mM, respectively. Other conditions as in Fig. 2.

In summary, we have shown that it is possible to controllably immobilize a G-rich DNA aptamer onto a gold surface to produce well-defined, highly reversible conformational changes in response to potassium. and this conformational transition can be transduced into an electric "off–on" switch. The rational design of molecular sensing systems by recognition-induced conformational changes is a powerful tool for enhanced biosensing capabilities.

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