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Development of an electrochemical sensor based on the catalysis of ferrocene actuated hemin/G-quadruplex enzyme for the detection of potassium ions

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ABSTRACT

Sensitive and selective sensors need to be explored to detect the physiological potassium level due to its important role in the living organisms. In the present system, a novel electron transfer mediator actuated electrocatalytical biosensor was demonstrated to assay K^+ based on the conformational change of DNA. With the hybridization between the complementary bases and the self-folding of guanine-rich nucleic acid sequence, the horseradish peroxidase-mimicking enzyme (HRP-DNAzyme) was formed and brought to approach the ferrocene (Fc) unit on Au nanoparticles (AuNPs). Thus, in the system, Fc unit acted as the relay, stimulating the electrical contact of HRP-DNAzyme with the electrode to obtain the bioelectrocatalyze reduction signal. Under the Fc actuated catalysis of HRP-DNAzyme and amplification of Au nanoparticles, the obtained biosensor exhibited a sensitive detection for K^+. A satisfying result of a wide linear range and low detection limit were obtained with the novel electrocatalytical biosensor which was then applied in real samples.

1. Introduction

As is well-known, potassium ions are familiar in tissues of animals and plants, biological fluids and extracellular spaces (Lippard and Berg, 1994; Bi et al., 2010a,b,c). They play important roles in living organisms, for instance, its known role in regulating blood pressure for reducing the risk of high blood pressure and stoke, balancing the pH of cells, maintaining extracellular osmolarity and muscular strength, controlling the apoptosis of a cell and enzyme activation biological systems, as well as the concentration of other ions in living organisms (Boram et al., 2012; Teresa et al., 2000; He et al., 2003). In biological fluids, normal K^+ levels are between 3.8 and 5.4 mmol/L (Yu et al., 1997). However, abnormal K^+ concentrations are harmful to human health, usually leading to several diseases such as stroke, kidney, adrenal gland diseases, high blood pressure, and Addison's disease (Modesto et al., 2006; Michaud and Strickberger 2001; Teixeira et al., 2009). Therefore, study and development of sensitive and selective sensors for the detection of physiological potassium level are important.

Recently it is reported that the introduction of K^+ would cause the DNA conformational changes, in which an unfolded guanine-rich nucleic acid sequence (Guoc et al., 2009) can stack as G-quadruplex. The reason for this is that K^+ ions can be located in the cavity of the G-quadruplex and tightly coordinate with the eight carbonyl oxygen atoms, which reduces the electrostatic repulsion between oxygen atoms of the G-quadruplex (Sundquist and Klug, 1989). In addition, a G-quadruplex structure can be inserted with hemin, composing a complex of hemin/G-quadruplex horseradish peroxidase-mimicking enzyme (HRP-DNAzyme). HRP-DNAzyme is in possess of peroxidase-like activities to effectively catalyze the H_2O_2-mediated oxidation of luminol (Bi et al., 2010a,b,c), 3, 3′, 5, 5′-tetramethyl-benzidine (TMB) (Zhang et al., 2011; Song et al., 2011), or 2, 2′-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) (Zhou et al., 2010; Jia et al., 2011) etc. On account of the DNA conformational changes caused by the introduction of K^+ and peroxidase-like activities of HRP-DNAzyme, many methods based on HRP-DNAzyme have been reported to develop bioassays for the selective detection of K^+ ions against sodium ions (physiological condition, 135 mM), such as colorimetry (Li et al., 2009), fluorescence (He et al., 2005; Qin et al., 2010), resonance scattering analysis (Cai et al., 2010) and so on. These assays are effective but usually require sophisticated preparation or labor-intensive labeling procedures. Electrochemical methods have been attractive due to

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their fast response, low production cost, high sensitivity, high compatibility, and simple instrumentation (Radi et al., 2005; Wu et al., 2008). Therefore, electrochemical detection of K^+ based on the catalytic effect of HRP-DNAzyme has drawn considerable attention recently (Chen et al., 2013; He et al., 2013).

As an essential aspect of bioanalysis, amplification has been successfully achieved by employing nanocontainers, nanoparticles or enzymes as amplifiers for sensitive detection of biorecognition events (Pavlov et al., 2004; Bi et al., 2010a,b,c; Rissin et al., 2010). Among the signal amplification strategies, there are some limitations in the application of conventional enzymes (including glucose oxidase, horseradish peroxidise, etc.) such as cumbersome enzyme labeling process, bad thermal stability and complicated synthesis. Comparing with conventional enzymes, HRP-DNAzyme possesses higher thermal stability and less expenditure (Zhang et al., 2010; Willner et al., 2008; Hobartner and Silverman, 2007) and especially without the fussy labeling process. HRP-DNAzyme has been intensively researched (Li et al., 2007; Zheng et al., 2012; Bi et al., 2010a,b,c) for signal amplification and it provided an ideal candidate for the development of a simple and sensitive bioanalytical platform (Pelossof et al., 2010) for the detection of metal ions (Liu et al., 2009; Navani and Li, 2006), DNA (Pelossof et al., 2011; Pelossof et al., 2010), aptamer–substrate complexes (Weizmann et al., 2006; Li et al., 2007), and so on. However, most HRP-DNAzyme based signal amplification strategies only involved bioelectrocatalytic effect of HRP-DNAzyme itself (Willner et al., 2008; Zhou et al., 2010; Zheng et al., 2012). As we know, conventional enzymes including HRP and glucose oxidase have been demonstrated to readily interact electronically with electron transfer mediators such as ferrocene (Tatsuma et al., 1989; Xu et al., 2003), methylene blue (Ye and Baldwin, 1988) and meldola blue (Gorton et al., 1984). These electron transfer mediators show the merits of excellent electrochemical behavior and possess an important role as relay for enzymes in electrochemical biosensors with amplified signal (Piperberg et al., 2009; Xu et al., 2003). Because of the horseradish peroxidase-mimicking properties of the HRP-DNAzyme, we suggest it would have further signal amplification combining the bioelectrocatalytic properties of the HRP-DNAzyme with the excellent electrochemical behavior of the electron transfer mediator (Chen et al., 2011; Zhang et al., 2002).

Inspired by the bioelectrocatalytic functions of the HRP-DNAzyme and the excellent electron mediator property of Fc unit, in the present system we constructed a new electrochemical biosensor for the detection of K^+. The electrical communication between the HRP-DNAzyme and the electrode was realized with Fc as the electrical relay. By the biocatalytic amplification of Fc actuated HRP-DNAzyme and the specific effect of K^+ in the conformational change of HRP-DNAzyme, the system has a sensitive and selective response to K^+.

2. Experimental

2.1. Materials and apparatus

HPLC-purified oligonucleotides (sequences of the used oligonucleotides: substrate probe (S1): 5′-SH-$(CH_2)_6$-TTCCCATCCCGC-3′; capture probe (S2): 5′-SH-$(CH_2)_6$-TAATCCTCTATGGGT-TGGGCGGGATGGG-3′; S3: 5′-Fc-TAGAGGATTA-3′; S4: 5′-TAGAGGATTA-3′), Tris (hydroxymethyl) aminomethane (Tris), and hemin were purchased from Sangon Biotechnology Co. Ltd. (Shanghai, China). All the other reagents used in this experiment were purchased from the Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All solutions were prepared with ultrapure water (18.2 MΩ cm) processed by PSDK2-10-C (Beijing, China). Ultrapure water was used throughout the experiments. The washing buffer (10 mM Tris–HCl, pH 7.4, 100 mM NaCl), incubation buffer and hybridization buffer (10 mM Tris–HCl, pH 7.4) solution were used in the experiments. To test the practicality of the proposed sensor, human serum samples were collected as an example. Human serum samples were kindly obtained from the Yijishan Hospital (Wuhu, China). Before use, human serum samples were centrifuged at 10,000 rpm for 15 min, and then the supernatant was diluted with Tris–HCl.

The pH values of phosphate saline buffer (PBS) and Tris–HCl were measured with a glass electrode connected to a PHS-3C pH meter (Shanghai, China). Centrifugation was carried out by a HERMLEZ 36 HK apparatus (Wehingen, Germany). Electrochemical measurements were carried out on a model CHI660B electrochemical analyzer (ChenHua Instruments Co. Ltd., Shanghai, China) which is controlled by a personal computer with a saturated calomel electrode (SCE) as reference electrode, a (modified) gold electrode (GE, 2 mm diameter) as working electrode and a platinum wire electrode as auxiliary electrode. All the electrochemical experiments were carried out at room temperature. Cyclic voltammetry (CV) was carried out in 0.05 M Tris–HCl buffer (pH 7.4) within the potential range from 0 to −0.4 V using a step potential 0.05 V. Electrochemical impedance spectra (EIS) was carried out in 1/15 M PBS (pH 7.4) containing 5 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ and 0.1 M KCl in the frequency range from 0.1 Hz to 100 kHz with 5 mV as the amplitude at a polarization potential of −0.18 V.

2.2. Substrate DNA (S1) self-assembled on gold electrode

Prior to use, these oligonucleotides were dissolved in 0.05 M Tris–HCl buffer solution (pH 7.4) and persevered at 4 °C for one night. Before modification, the oligonucleotides solutions were heated to 80 °C and then slowly cooled to room temperature and meanwhile the gold electrode was pretreated freshly by the following steps: first, the gold electrode was polished with 0.3 and 0.05 μm alumina powder respectively. Then the electrode was sonicated in ethanol and ultrapure water for 5 min each. Finally, in order to remove any remaining impurities via electrochemical cleaning, the gold electrode was scanned in 0.1 M H_2SO_4 between −0.2 and 1.55 V at 100 mV/s until a steady-state redox wave was observed. The immobilization of the substrate probe (S1) was carried out by dropping 30 μL of 10 mM Tris–HCl buffer (pH7.4) containing 1.0 μM S1 probe and 100 mM NaCl on the cleaned gold electrode (bare GE) in a humidified chamber. The self-assembly was proceeded 10 h through the covalent bond with thiol of S1 and Au atom. Then the gold electrode was rinsed with water and incubated in 2 mM 6-mercaptohexanol in Tris–HCl buffer for 2 h to eliminate the nonspecific-bonded DNA. After being rinsed thoroughly with the washing buffer, the S1 probe modified gold electrode (S1/GE) was stored at 4 °C in the washing buffer for further use.

2.3. Preparation of capture DNA (S2) and Au nanoparticles bioconjugates (S2-AuNPs)

The 13 nm gold nanoparticles (AuNPs) were prepared using the method of citrate reduction with some modification (Kimling et al., 2006; Jana et al., 2001). Their functionalization with S2 was synthesized on the basis of the published protocol (Jin et al., 2003). In brief, 100 mL 0.01% (w/v) $HAuCl_4$ aqueous solution was added into a round-bottom flask and heated to boil under stirring. The color of the solution became wine red with rapidly 3.2 mL 1% trisodium citrate added into the boiling solution. After stirring the boiling solution for another 15 min, the mixture was cooled down to the room temperature. Then it was stored in the refrigerator (4 °C) for further use. Based on the standard surface modification protocol and the well-known gold-thiol chemistry (Prashant et al., 2006), S2-modified AuNPs were prepared. In brief, the synthesized

AuNPs solution (600 μL, ~13 nm) was mixed with thiol modified oligonucleotides, S2 (280 μL, 6.6 μM), with incubation for 12 h at room temperature in darkness. With the addition of NaCl aqueous solution (90 μL, 1 M) and Tris–HCl buffer (10 μL, 20 mM, pH 7.4), the resultant mixture was incubated for another 10 h at room temperature. Finally, the solution was separated by a centrifuge at 7000 rpm for 10 min. After that, the precipitated S2-AuNPs were washed with ultrapure water twice and dissolved with buffer solution for further use.

2.4. Preparation of Fc and HRP-DNAzyme modified S1/GE (Fc/HRP-DNAzyme-S1/GE)

The Fc and HRP-DNAzyme modified gold electrode was fabricated as follows: first, the S1/GE was incubated with the previous prepared S2-AuNPs bioconjugates (500 μL) for 2 h in a hybridization buffer solution. Then the gold electrode was modified with S1 and S2-AuNPs bioconjugates, which was named as S2-AuNPs-S1/GE. The S2-AuNPs/S1/GE was immersed sequentially in the incubation buffer solution containing 3 μM S3 DNA sequences for 2 h, forming S3/S2-AuNPs/S1/GE. Because S3 was designed with Fc labeled on its 5′ end, the modified electrode was also named as Fc/S2-AuNPs/S1/GE for easy comparison in results and discussion. And then, the S3/S2-AuNPs/S1/GE was incubated in the incubation buffer solution containing 100 μM K^+ and 6 μM hemin for further 2 h. After that, the HRP-DNAzyme structure was formed on the modified electrode. Thus, the Fc and HRP-DNAzyme were assembled on the surface of S1/GE. The modified electrode obtained was named as Fc/HRP-DNAzyme/S1/GE. To confirm the catalysis of HRP-DNAzyme with Fc actuated, a number of control experiments were investigated by comparing the prepared electrodes as follows: S4 containing the same sequence with S3 but without Fc label was hybridized with S2/AuNPs-S1/GE under the same conditions. The resulting electrode was named as S4/HRP-DNAzyme/S1/GE. When S1/GE was incubated with S2 without AuNPs modification and S3 under the same conditions, it was named as S3/S2/S1/GE.

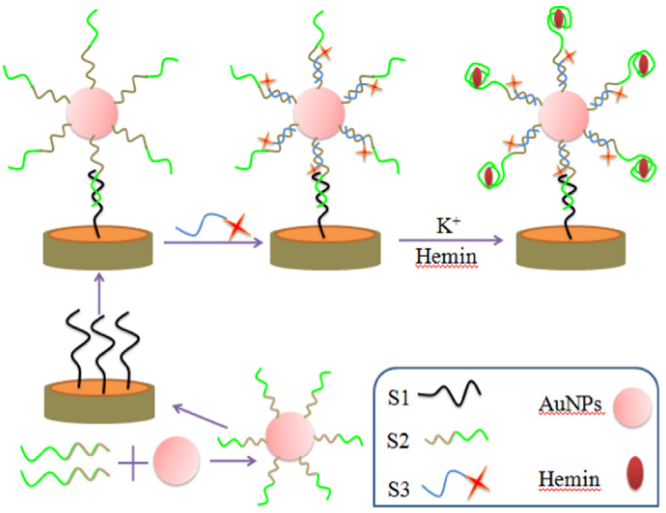
2.5. The electrochemical experimental process

In the whole experiment, electrochemical experiments were performed at room temperature. Cyclic voltammetry (CV) was carried out in 10 mM Tris–HCl (pH 7.4) within the potential range from 0 to −0.4 V using a step potential of 0.05 V at the scan rate of 50 mV/s. Electrochemical impedance spectra (EIS) was carried out in 1/15 M PBS (pH 7.4) containing 5 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ and 0.1 M KCl in the frequency range from 0.1 Hz to 100 kHz with 5 mV as the amplitude at a polarization potential of −0.18 V. Amperometric current was also conducted at −0.4 V in 10 mM Tris–HCl (pH 7.4) with the addition of H_2O_2 as the solution rotated at 3000 rpm for optimum sensitivity.

3. Results and discussion

3.1. Design of the sensor

The fabrication of the present biosensor for the detection of K^+, which is based on the Fc actuated catalysis of the HRP-DNAzyme and amplification of Au nanoparticles, is depicted as shown in Scheme 1. The two thiolated nucleic acid sequences were designed as follows: S1 (black part in Scheme 1) is a substrate probe for the organization of the DNAzyme catalytic system; S2 was designed with a guanine-rich sequence (green part of S2 in Scheme 1) and 10 bases complementary with S1 (black part of S1 in Scheme 1). First, S1 was self-assembled on the gold electrode surface by Au–S covalent bond. Because gold nanoparticles (AuNPs) possess high surface area and good biocompatibility with nucleic acids (Li et al., 2010), they motivated us to apply in the sensing platform for signal amplification (Tang et al., 2011). AuNPs was modified with S2 to form S2-AuNPs bioconjugates. Then by the hybridization of S1 and S2-AuNPs bioconjugates, the S2/AuNPs-S1/GE was formed. Fc labeled S3 was specially designed containing Fc at 5′ end (red part of S3 in Scheme 1) and 10 complementary bases with S2 (gray parts of of S2 in Scheme 1) at 3′ end (blue part of S3 in Scheme 1). Because of the complementary base pairs between S2 and S3, S3 was hybridized with the S2/AuNPs-S1/GE forming S3/S2/AuNPs-S1/GE (Fc/S2-AuNPs/S1/GE). In the presence of K^+ and hemin, the guanine base-rich sequence of S2 on the S3-S2-AuNPs-S1/GE folded into G-quadruplex to form HRP-DNAzyme fabricating Fc/HRP-DNAzyme/S1/GE which brought the Fc approaching to HRP-DNAzyme. With the electron transfer between the HRP-DNAzyme and Fc, bioelectrocatalytic reduction of the HRP-DNAzyme with Fc actuated was realized. Owing to the amount of K^+ ions relative to the formation of HRP-DNAzyme, it was suggested that the resulting bioelectrocatalytic responses correlated with the concentration of K^+. This provides us a potential strategy to detect K^+.



Scheme 1. The fabrication process of the sensor based on the ferrocene actuated bioelectrocatalysis of the hemin/G-quadruplexes enzyme.

3.2. Electrochemical impedance spectroscopy of the modifying procedure

As electrochemical impedance is a powerful method for studying the characteristics of surface-modified electrodes, the stepwise modification processes of the present electrode were confirmed by electrochemical impedance spectroscopy and the matching results are shown in Fig. 1. In the Nyquist plots, the semicircle diameter of EIS, which is equal to the value of Ret, indicates the surface electron transfer resistance of the electrode. The bare gold electrode shows a very small semicircle (curve a of Fig. 1), suggesting a low electron transfer resistance. The Ret gives an obvious rise (curve b of Fig. 1) after S1 was immobilized on the electrode surface, owing to the fact that the nucleic acid sequence has a severely obstructive effect on the electron transfer toward the electrode surface, which is also in accordance with the literature (Liu et al., 2005; Keighley et al., 2008). After the S2-AuNPs bioconjugates were hybridized with S1/GE and the successive assembling of S3, the diameters of the semicircles continue to increase (curves c and d of Fig. 1). This may be because more nucleic acid sequences on the electrode lead to the increase of the

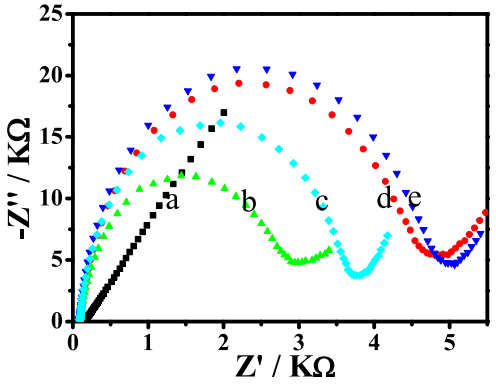


Fig. 1. EIS of the electrodes in different procedures: bare GE (curve a); S1/GE (curve b); S2/AuNPs-S1/GE (curve c); Fc/S2-AuNPs/S1/GE (curve d); and Fc/HRP-DNAzyme/S1/GE (curve e).

electron transfer resistance. Then the diameter of the semicircle exhibits a continued increase (curve e of Fig. 1) after the S3/S2-AuNPs/S1/GE was incubated with K^+ and hemin. We suggested it result from that hemin inserted in the G-quadruplex near the surface of electrode. This demonstrates that the process may be in accordance with what we projected before.

3.3. Response of the Fc/HRP-DNAzyme based system toward H_2O_2

To investigate our proposal, the cyclic voltammogram responses of different modified electrodes were studied and the corresponding results are shown in Fig. 2. As Fig. 2A shows, the cyclic voltammetries of the Fc/HRP-DNAzyme-S1/GE were obtained in Tris–HCl buffer with (curve b of Fig. 2A) and without (curve a of Fig. 2A) the addition of H_2O_2. It is obvious that the bioelectrocatalytic reduction currents on the Fc/HRP-DNAzyme/S1/GE are generated in the presence of H_2O_2. Fig. 2B–E shows the cyclic voltammetry of the control experiments to further validate the bioelectrocatalytic system. When S1/GE was studied with and without the addition of H_2O_2, there is no obvious bioelectrocatalytic reduction on S1/GE (Fig. 2B). This implies that the DNA sequence does not possess the character of bioelectrocatalytic reduction of H_2O_2. As Fig. 2C shows, a small response is recorded on the Fc/S2-AuNPs/S1/GE. We suggest the small response may result from the effect of AuNPs and Fc (Jena and Raj, 2006; Maye et al., 2000). This also indicates that the formation of HRP-DNAzyme which has a catalysis on the reduction of H_2O_2 (Pelossof et al., 2010; Xu et al., 2003) is important and indispensable in the system. When S4/HRP-DNAzyme/S1/GE without Fc unit was examined with the addition of H_2O_2, the bioelectrocatalytic current was also blocked as shown in Fig. 2D, illustrating that the electrically contacted enzyme system may not be assembled without the ferrocene unit. According to the literature (Tatsuma et al., 1989; Xu et al., 2003), we suggest that in the system Fc as a relay stimulates the biocatalytic functions of HRP-DNAzyme with the electrode, promoting the bioelectrocatalytic efficiency of the HRP-DNAzyme on the electrode. This proves that the assembly of HRP-DNAzyme and Fc are essential to promote the bioelectrocatalytic reduction of H_2O_2. In addition, the signal amplification efficiency of AuNPs was investigated by comparing the biocatalytic responses with AuNPs labeled DNA strand (Fig. 2A) or without (Fig. 2E). Obviously, the formed S3/S2/S1/GE without AuNPs (Fig. 2E) displayed remarkably weaker response than that shown in Fig. 2A. This is attributed to the 10 complementary bases of S2 matched with S1 blocked the guanine base-rich region to fold into G-quadruplex in the absence of AuNPs. In the system, it is demonstrated that AuNPs indeed possesses good capability to amplify the electrochemical signal with more Fc/HRP-DNAzyme units fabricated on the electrode.

Based on the result of cyclic voltammograms shown in Fig. 2A, the electrochemical catalysis of the system toward different concentrations of H_2O_2 was investigated. As Fig. 3A shows, the bioelectrocatalytic currents enlarge as the concentration of H_2O_2 increased. Fig. 3B shows the calibration curve corresponding to the CV responses of Fc/HRP-DNAzyme-S1/GE towards different concentrations of H_2O_2 in the range of 5–100 μM. The detection limit was 0.5 μM evaluated by $3n/s$ in which n was the standard deviation of the intercept and s was the slope of the calibration curve.

3.4. Detection of K^+ based on the bioelectrocatalytic system

Under the optimized conditions discussed in Supplementary information, different concentrations of K^+ were further investigated in the Fc/HRP-DNAzyme based electrocatalytic system. Fig. 4A shows the CV responses of the present system toward different concentrations of K^+. A series of dynamically increased electrocatalytic currents enlarge along with the concentration of K^+ increasing. The amperometric currents also increase linearly with K^+ concentration between 5 and 200 μM shown as Fig. 4B with a good correlation coefficient of 0.9964. The resulting calibration curve is shown in Fig. 4C with a detection limit of 1.6 μM evaluated using a signal three-fold the background noise. The linear range and detection limit are compared with the reported methods shown as Table S1 in Supplementary information and the results are satisfying.

3.5. Specificity and practicality of the proposed biosensor

The biosensor not only needs to be sensitive to different concentrations of K^+, it also must be specific. We studied the specificity of this K^+ sensor by challenging it with other metal ions as shown in Fig. 5. As expected, under the same experimental conditions, the bioelectrocatalytic reduction response of the biosensor has scarcely any changes after incubation in 0.5 mM Na^+, Mg^{2+}, Ca^{2+}, Al^{3+}, Zn^{2+} or Cu^{2+} solutions, respectively, comparing with that obtained in 100 μM K^+ solution. This demonstrates that the sensor shows a good selectivity for K^+ over other tested ions which is mainly attributed to the fact that the capture sequence forms stable G-quadruplex complexes with K^+ ion. In addition, the practicality of the proposed strategy was also investigated by detecting K^+ in 100-fold diluted human serum samples under the same detection procedure as that described in the aforementioned experiment. As can be seen from Table S2 in Supplementary information, the experimental results confirmed the effectiveness of this method for the determination of K^+ content in real samples. Moreover, the present electrochemical K^+ detection system has other advantages over some recently developed K^+ sensors (shown as Table S1), such as easy operation, high sensitivity and ideal selectivity.

4. Conclusions

In summary, this work describes a novel Fc/HRP-DNAzyme based electrocatalytic system for the determination of K^+ with the amplification of Au nanoparticles. Fc unit was used as a relay to electrically contact HRP-DNAzyme with the electrode for the bioelectrocatalytic signal. The interaction of HRP-DNAzyme and ferrocene unit is dependent on the complementary bases. Considering this, the combination of HRP-DNAzyme with ferrocene unit can also be used for alternative sensing devices envisaged to detect some metal ions based on the DNA-metal base pairs.

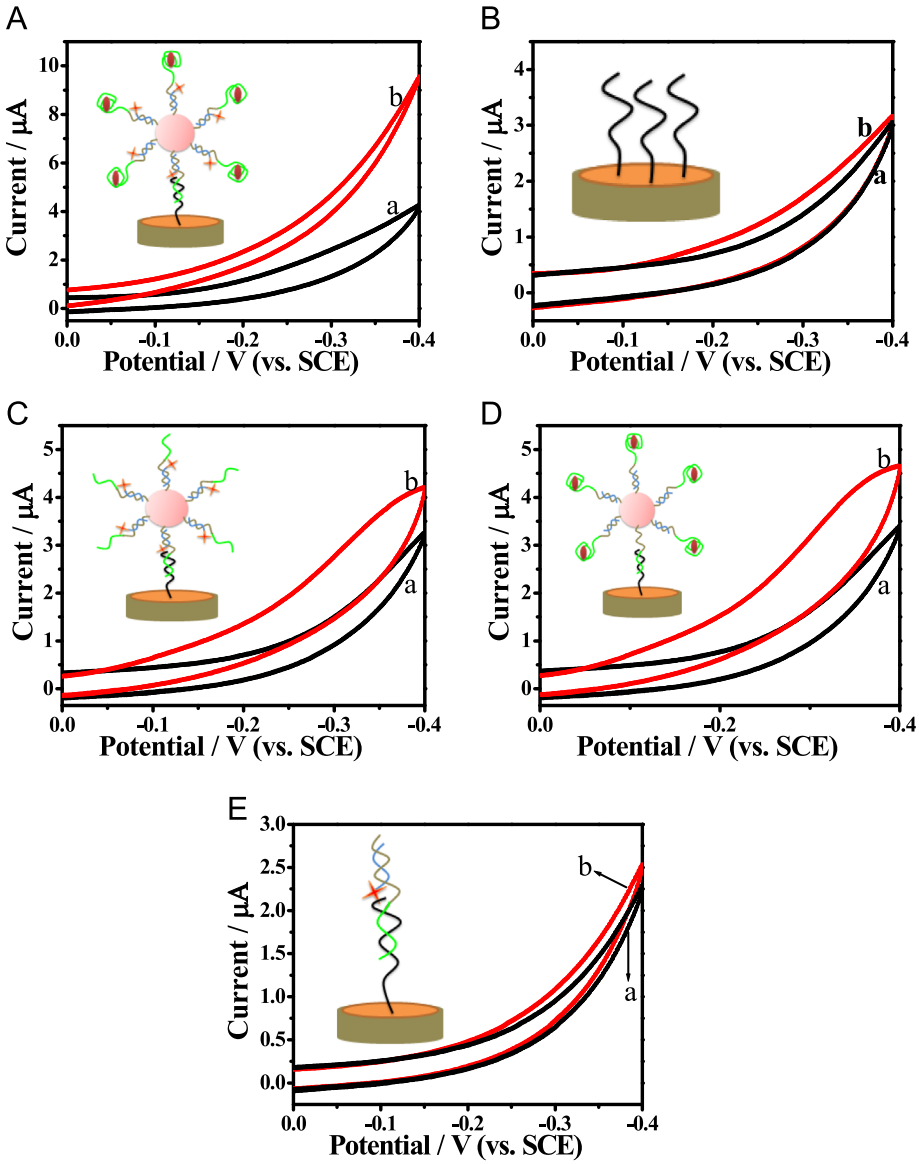


Fig. 2. Cyclic voltammograms of different modified electrodes in the absence (curve a) and presence of 100 μM H_2O_2 (curve b) in Tris–HCl. (A) Fc/HRP-DNAzyme/S1/GE; (B) S1/GE; (C) Fc/S2-AuNPs/S1/GE; (D) S4/HRP-DNAzyme/S1/GE; and (E) S3/S2/S1/GE.

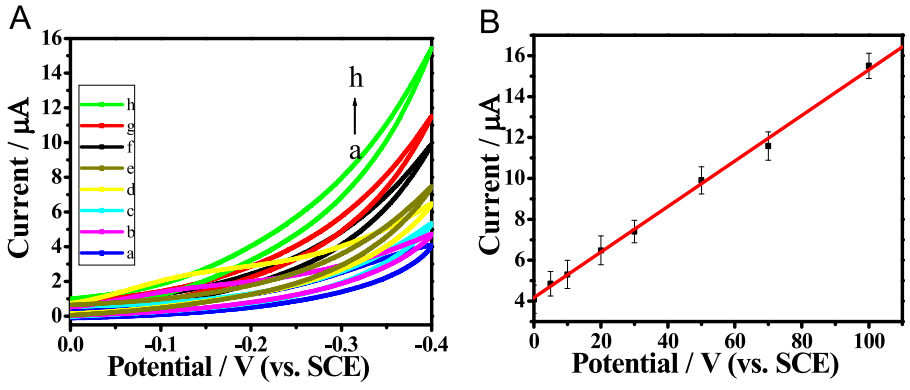


Fig. 3. (A) Cyclic voltammograms of bioelectrocatalytic reduction of H_2O_2 with various concentrations: 0, 5, 10, 20, 30, 50, 70, and 100 μM (a–h) by the Fc/HRP-DNAzyme based electrocatalytic system in Tris–HCl; (B) corresponding calibration curve of current vs. the concentration of H_2O_2.

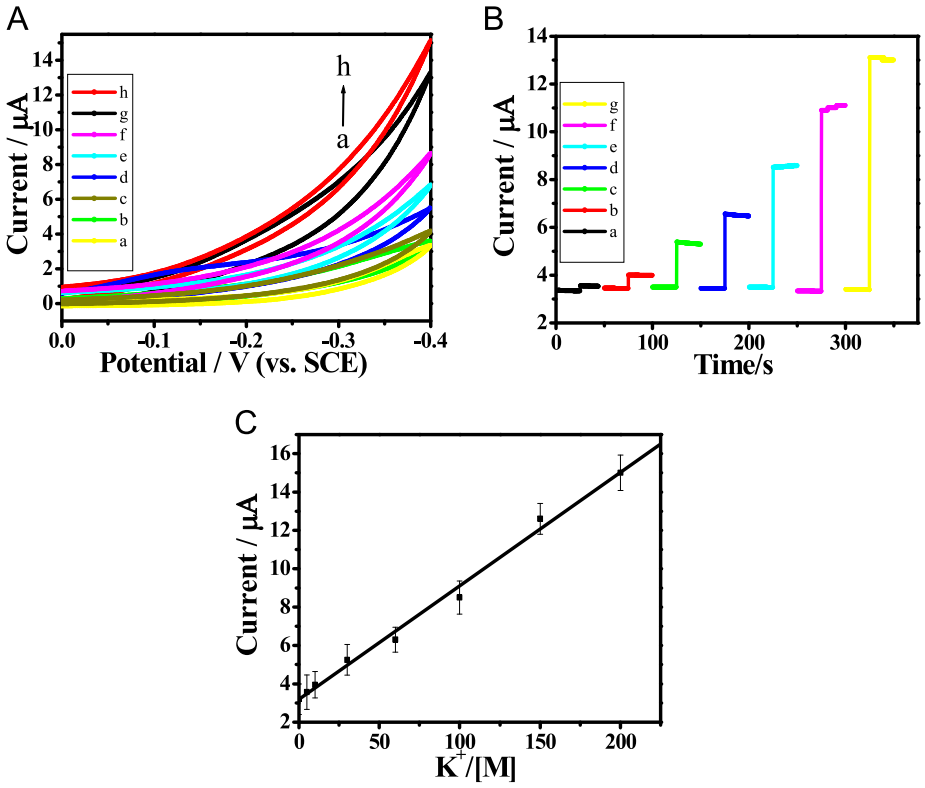


Fig. 4. (A) CV and (B) steady state amperometric current at −0.4 V on Fc/HRP-DNAzyme/S1/GE in the presence of various concentrations of K^+ (a–h): 0, 5, 10, 30, 60, 100, 150, and 200 μM in Tris–HCl (pH 7.4) with 100 μM H_2O_2. (C) Corresponding calibration curve of current vs. the concentration of K^+.

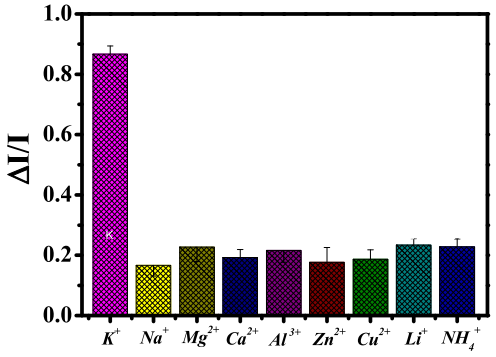


Fig. 5. Selectivity of the biosensor to K^+ against other common metal ions. Na^+, Mg^{2+}, Ca^{2+}, Al^{3+}, Zn^{2+}, Cu^{2+}, Li^+ and NH_4^+ were all used at 0.5 mM and K^+ was at 100 μM.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2014.05.041.

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