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A reusable potassium ion biosensor based on electrochemiluminescence resonance energy transfer†

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A reusable potassium ion biosensor was reported for the first time based on the reversible DNA structural change and the interaction between surface plasmons of Au nanoparticles (NPs) and the ECL emission of CdS nanocrystals (NCs).

K^+ plays an important role in biological systems. The difficulty in monitoring K^+ under physiological conditions comes from the coexistence of Na^+. A G-quadruplex is a four-stranded DNA structure formed by G-rich nucleic acid sequences, which is composed of stacked guanine tetrads connected by Hoogsteen type base pairing.[1] Due to the appropriate size and charge, K^+ ions can be located in the cavity between two adjacent G-tetrads of a G-quadruplex and can reduce the electrostatic repulsion between oxygen atoms of the G-quartet by tightly coordinating with the eight carbonyl oxygen atoms.[2] When K^+ is introduced, the conformation of DNA can transform from an unfolded form to a folded G-quadruplex structure. Monitoring DNA structural changes can reveal the concentration of K^+. And this strategy shows good selectivity to K^+. Recently, many techniques have been reported to monitor DNA conformational changes caused by the introduction of K^+, such as fluorescence,[3–5] colorimetry,[6] resonance scattering analysis,[7] and so on. In practical applications, regeneration is one of the appealing properties for a sensing system.[8,9] However, the existing K^+ sensors can hardly be regenerated. Thus, it is necessary to design a new system which is renewable for K^+ ion detection.

The electrochemiluminescence (ECL) technique is becoming very useful for analytical applications due to its simplicity, high sensitivity, rapidity, easy controllability and low background signal.[10] Recently, ECL research involving semiconductor NCs has been widely studied.[11,12] The unique electrochemical and photophysical properties of luminescent NCs allow them to engage in different types of resonance energy or electron transfer systems. AuNPs, with a high extinction coefficient and a broad absorption spectrum in visible light that is overlapped with the emission wavelength of usual energy donors, play an important role in energy transfer systems.[13] Our group has found that ECL emission from NCs can induce surface plasmon resonance (SPR) of AuNPs and the induced SPR can in turn enhance the ECL response of NCs, and the interaction between them greatly depends on the separation distance.[14,15] Based on this, some biosensors for the ultrasensitive detection of biological macromolecules, *e.g.*, DNA[14,16] and thrombin,[15] have been designed. In those cases, hairpin-DNA or double-stranded DNA with a rigid structure was used to control the distance between AuNPs and NCs.

Here we report a simple and resuable ECL sensor for selective detection of K^+ *via* ECL energy transfer. In this case, a 36-mer oligonucleotide containing a thrombin-binding (TB) sequence was used to control the distance between CdS NCs film on a glassy carbon electrode (GCE) and AuNPs. Benefiting from the cathodic ECL characteristics of CdS NCs film, the negatively charged electric field of the electrode surface in ECL scanning made the negatively-charged oligonucleotide stand upright on the electrode due to the repulsion force. ECL enhancement took place owing to the larger DNA spacer between AuNPs and CdS NCs film. In the presence of potassium ions, the G-rich DNA sequence formed a G-quadruplex and caused the AuNPs to be close to CdS NCs film, which induced ECL decrease. The change of ECL allows us to sensitively detect potassium ions. Moreover, potassium ions could be easily removed by washing with water and the oligonucleotide could stand upright on the electrode again at the negative ECL potential. Thus the biosensor could be reused for detection of potassium ions.

The mechanism of this ECL strategy is illustrated in Scheme 1. A GCE was modified by drop-coating 10 μL of CdS NCs used as ECL emitters. For detecting K^+, the test solution should not contain any K^+. So we chose the tris-HCl as the detecting buffer solution and H_2O_2 as the coreactant. And the CdS NCs film can generate a strong and stable ECL emission.

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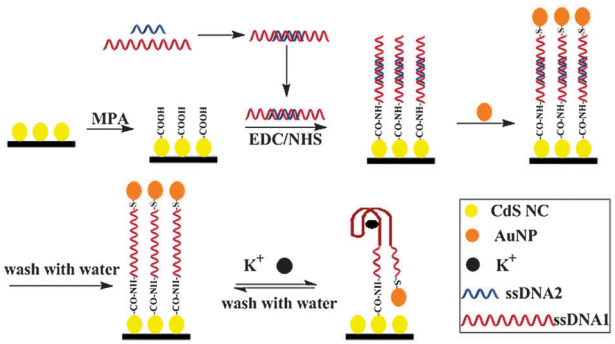

Scheme 1 Preparation of the ECL biosensor for K^+ assay based on energy transfer between CdS NCs and AuNPs.

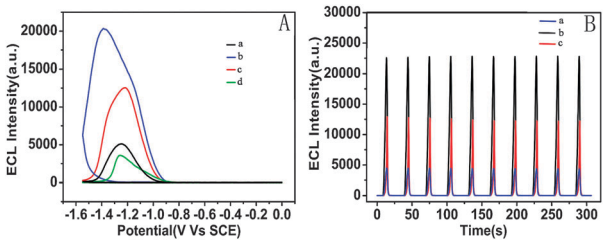

Fig. 1 (A) Cyclic ECL curves of (a) CdS NCs film on a GCE; (b) GCE/CdS NCs/DNA/Au NPs and washed with water (c) after being reacted with 10 mM K^+ ions. (d) After being reacted with 200 mM K^+. (B) Stabilization of ECL emission under continuous cyclic potential scan for 10 cycles from (a) CdS NCs film; (b) Au NPs/DNA/CdS NCs film and washed with water (c) after incubation in 10 mM K^+ solution. ECL detection buffer: 50 mM tris-HCl (pH 8.0) + 15 mM H_2O_2; for the detection of K^+, the detection buffer contained 10 mM KCl. Scan rate, 100 mV s^{-1}.

In such a case, upon the potential scan with an initial negative direction, the CdS NCs immobilized on the electrode were reduced to nanocrystal species ($CdS^{-\bullet}$) by charge injection, while the coreactant H_2O_2 was reduced to the strong oxidant $^\bullet OH$. And then $^\bullet OH$ reacted with the negatively charged $CdS^{-\bullet}$ by injecting a hole into the highest occupied molecular orbital to produce an excited state of CdS NCs (CdS*), and emitted light. Or H_2O_2 directly oxidized $CdS^{-\bullet}$ to its excited state, and then emitted light. The corresponding ECL processes of CdS NCs are as follows:[12,17]

$$CdS + e^- \rightarrow CdS^{-\bullet} \quad (1)$$

$$H_2O_2 + e^- \rightarrow OH^- + {}^\bullet OH \quad (2)$$

$$CdS^{-\bullet} + {}^\bullet OH \rightarrow CdS^* + OH^- \quad (3)$$

$$\text{or } 2CdS^{-\bullet} + H_2O_2 \rightarrow 2CdS^* + 2OH^- \quad (4)$$

$$CdS^* \rightarrow CdS + h\nu \quad (5)$$

Under physiological conditions for detection of potassium ions, the appropriate DNA sequences should be selected for use, and some factors should be considered: first, the binding constant of the potassium complex should be suitable; then, the G-quadruplex sequences should be sensitive to K^+ compared with that to Na^+.[3] The 15-mer TB sequence d(GGTTGGTGTGGTTGG) can form a chair-type quadruplex structure when K^+ is present even in the presence of an excess of sodium, and this quadruplex incorporates K^+ with a 1 : 1 stoichiometry.[18,19] So the 36-mer ssDNA 1 which was modified with the –SH linker at the 3′-end and amine at the 5′-end and contained the TB sequence was used in this work. After the preparation of CdS NCs film, the ssDNA 1 was first incubated with ssDNA 2 to form dsDNA to avoid the adsorption of negatively charged AuNPs used later on the long-chain ssDNA 1 and to make the –SH exposed on the surface of the electrode for the easy combination of AuNPs. Through the crossing-linking of EDC and NHS, the dsDNA was attached to the MPA modified CdS NCs on the surface of the GCE. It has been reported that thiols are among the most successful chemicals employed for attachment to metals *via* forming a strong metal–sulfur chemical bond. When the electrode was continuously incubated in the colloidal AuNPs solution, AuNPs were attached to the thiol modified DNA through sulfur–gold chemistry. In order to detect K^+, we should eliminate the interfering ions (*e.g.* Na^+, Mg^{2+}) used before. So the electrode was washed with water thoroughly, and ECL signals were recorded (Fig. 1A, curve b). Obviously, we could find that the ECL signal was 4-fold higher than the emission of the naked CdS NCs (Fig. 1A, curve a). DNA molecules have negative charges due to the phosphates in the sugar–phosphate backbone. While washing with water, in the case of the absence of inorganic cations, the mutual repulsion between the negatively charged phosphate groups of the DNA strand caused the double-stranded DNA to melt.[20] Subsequently when scanning the electrode at a negative potential of 0 to −1.6 V (*vs.* SCE), the surface of the electrode was negatively charged, which resulted in the negatively-charged DNA-linked AuNPs to move away from the electrode due to the repulsion force[21] and caused the ssDNA 1 to stand upright on the electrode. Owing to the DNA spacer, the AuNPs were far away from CdS NCs on the electrode. The perfect overlap between surface plasmon absorption of AuNPs and the ECL emission of CdS NCs (see Fig. S1 in ESI†) and the long-distance interaction between them led to the enhancement of the ECL response. The ECL emission was very stable upon continuous potential scan for 10 cycles (Fig. 1B, curve b). After incubating in 10 mM K^+ solution, a large decrease of the ECL intensity was observed (Fig. 1A, curve c). When the concentration of K^+ was increased to 200 mM, the ECL intensity (Fig. 1A, curve d) was lower than the emission of the naked CdS NCs. In this situation, the TB section of the ssDNA 1 sequence changed into a hairpin-like G-quartet structure upon binding K^+ ions, which caused the AuNPs to be close to CdS NCs film, and efficient Förster resonance energy transfer induced quenching[14] was observed. And the biosensor also showed good stability in response to 10 mM K^+ ions (Fig. 1B, curve c). Because of this, it was very suitable for the detection of K^+.

Since the quantitative determination of K^+ was based on the structural change of the DNA and the interaction between the ECL emission of CdS NCs and the SPR of AuNPs, the immobilization time of AuNPs and the immobilization amount of the DNA on the CdS NCs-electrode surface were studied to establish the optimal conditions for K^+ detection. The results are shown in Fig. S2 (ESI†). Under the optimized experimental conditions (6 h of immobilization time of AuNPs and 0.2 μM ssDNA 1), the relationship between the ECL intensity and the concentration of

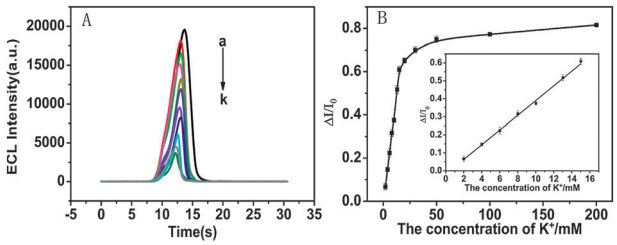

Fig. 2 (A) ECL signals of the biosensor incubated with different concentrations of K^+ at 25 °C for 20 min (from top to down, 0, 2, 4, 6, 8, 10, 13, 15, 30, 100 and 200 mM, respectively). (B) Relationship between $\Delta I/I_0$ and the K^+ concentration, three measurements for each point. Inset: the linear curve for K^+.

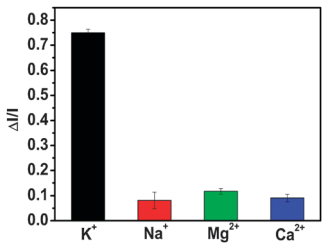

Fig. 3 Selectivity of the ECL biosensor to K^+ against other common metal ions. K^+, Na^+, Ca^{2+}, Mg^{2+} were all used at 50 mM.

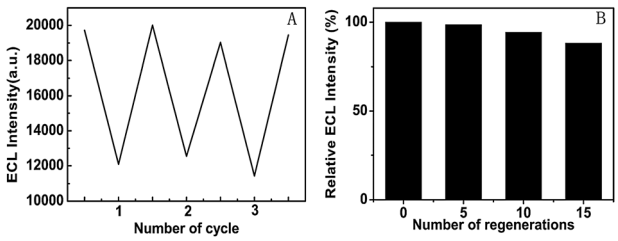

Fig. 4 The reusability of this ECL biosensor. (A) Plot of ECL intensity *versus* regeneration cycles. (B) Plot of relative ECL intensity *versus* the number of regeneration cycles. The original response is assigned the value of 100%.

K^+ was investigated (Fig. 2A). The ECL quenching efficiency ($\Delta I/I_0$, $\Delta I = I_0 - I$) could be used for K^+ detection. The relationship between $\Delta I/I_0$ and K^+ concentration in the range from 0 to 200 mM is shown in Fig. 2B. As the concentration of K^+ increased, the ECL quenching efficiency increased sharply, then slight changes in $\Delta I/I_0$ were observed at a higher concentration than 20 mM. The $\Delta I/I_0$ was found to be related to the K^+ concentration in the range of 2.0–15 mM (R = 0.996, shown as an inset in Fig. 2B).

We also studied the specificity of this K^+ sensor. The ECL quenching efficiency after incubation in K^+, Na^+, Ca^{2+} and Mg^{2+} solutions, respectively, under the same experimental conditions and at the same concentration of 50 mM was compared (Fig. 3). The sensor showed a good selectivity for K^+ over other tested ions. This is mainly attributed to the fact that the TB sequence forms weaker complexes with Na^+, Mg^{2+} and Ca^{2+} ions than with the K^+ ion.

The designed ECL biosensor could be regenerated easily by washing with water and resuable for K^+ detection. Fig. 4A shows the ECL intensity recorded in solution without any alkali metal ion and under the condition of 10 mM K^+. We can clearly see that the sensor can regenerate well. This is because after mild washing the G-quadruplex could be destroyed and the ssDNA 1 could re-react with K^+. The G-rich sequence could change its conformation reversibly from a G-quartet structure to a single-stranded upright structure at a negative potential. Then, the number of regeneration cycles was studied (Fig. 4B). After washing 10 times, the ECL intensity recovered up to ~94% of the original ECL intensity under the same conditions. After washing 15 times, the ECL intensity still remained 88%.

In summary, a simple and resuable ECL biosensor has been constructed for the detection of K^+ ions based on DNA structural changes and the interaction between surface plasmons of AuNPs and ECL emission of CdS NCs. At the negative ECL potential, negatively-charged ssDNA stood upright on the electrode. The reversible conformational change of DNA with K^+ removal and introduction resulted in AuNPs to move away from the CdS NCs on the electrode, leading to the SPR-induced ECL enhancement changing to Förster energy transfer-induced ECL quenching. From all of this, we can realize the sensitive detection of K^+. This smart NCs-based ECL detection strategy may open a new way for developing reusable biosensors.

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