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An aptamer-based electrochemiluminescent biosensor for ATP detection

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ABSTRACT

An aptamer-based electrochemiluminescent (ECL-AB) biosensor for ATP detection with high sensitivity and specificity was developed. The biosensor was assembled based on several steps. First, a complementary DNA (cDNA) of the ATP-binding aptamer, which has six complementary bases at both its ends, was hybridized with the aptamer molecule to form a rigid, linear double-stranded DNA (ds-DNA). The ds-DNA was then labeled with a ruthenium complex at the 3′ terminus of cDNA, followed by the immobilization of this ds-DNA onto Au electrode surface through the 5′-HS on the cDNA. In the presence of ATP, due to the ATP binding to the aptamer, the aptamer molecules dissociated from the ds-DNA complex, which resulted in the formation of stem-loop structure of the single-stranded cDNA and led to the increase of the ECL signal. The increased ECL intensity was found linearly to the logarithm of the concentration of ATP ranging from 0.05 nM to 10 nM with a detection limit of 0.02 nM. Different from other ECL-AB biosensors with aptamers as the probes, this sensing system proposed here is based on the utilization of the cDNA of aptamers as the probes for ECL sensing. Therefore, such sensing system could provide a promising label-free and more readily regenerated model for aptamer-based small-molecules detection.

1. Introduction

Aptamers are DNA or RNA molecules selected from random-sequence nucleic acid libraries using systematic evolution of ligands with exponential enrichment (SELEX) (Ellington and Szostak, 1990; Tuerk and Gold, 1990), which can specifically bind to a variety of target molecules with high affinity, such as drugs, proteins, as well as organic and inorganic molecules, etc. (Hamula et al., 2006; Jayasena, 1999; Kawde et al., 2005; Tombelli et al., 2005). Compared to traditional molecular recognition system such as antibody–antigen interaction, aptamer exhibits many advantages in terms of the simplicity of synthesis, ease of labeling and excellent stability. Due to its unique properties, aptamer has been widely employed as recognition element for biosensing applications, such as optical (Fang et al., 2001; Hamaguchi et al., 2001; Heyduk and Heyduk, 2005; Jhaveri et al., 2000; Liu and Lu, 2004, 2006; Stojanovic and Landry, 2002; Yang et al., 2005), electrochemical (Baker et al., 2006; Feng et al., 2008a,b; Kang et al., 2008; Lai et al., 2007; Lu et al., 2008; Radi et al., 2005, 2006; Shen et al., 2007; Wu et al., 2007; Xu et al., 2005; Xiao et al., 2005a,b; Zayats et al., 2006; Zuo et al., 2007) and electrochemiluminescent (ECL) (Fang et al., 2008; Li et al., 2007, 2008; Wang et al., 2007) biosensors. In particular, the marriage of highly sensitive electrochemical or ECL method with the specificity of aptamer to target has led the emergence of aptamer-based electrochemical (E-AB) biosensors or aptamer-based electrochemiluminescent (ECL-AB) biosensors.

Recently, a series of E-AB biosensors to detect thrombin, cocaine, potassium, and adenosine triphosphate (ATP) (Baker et al., 2006; Lai et al., 2007; Radi et al., 2006; Xiao et al., 2005a,b; Zuo et al., 2007) have been developed based on the conformation change of aptamer induced by target binding. In those works, the aptamers were dual-modified with a redox moiety at their one end such as methylene blue or ferrocene for electrochemical sensing, and a thiol group at the other end for the immobilization of sensing probe onto Au electrode surface. Mao and co-workers reported a new strategy for E-AB biosensor build-up (Lu et al., 2008), where the complementary DNA (cDNA) of aptamer was used as sensing probe. In their work, the probe cDNA was designed as stem-loop structure with several complementary bases at its both ends. In addition, the cDNA was functionalized with an amino and a thiol group at its 5′ and 3′ ends, respectively. After the hybridization of cDNA probe with aptamer to form a rigid, linear double-stranded DNA (ds-DNA) helix, the helix was further labeled with a redox moiety via amino group. Through thiol-Au chemistry, the whole sensing moiety was finally immobilized onto Au electrode surface to construct this E-AB type biosensor. Different from other E-AB biosensors, this type of E-AB biosensor avoided the complicated procedures to regenerate the sensor, presumably due to the strong binding affinity of the aptamer to its target molecule.

ECL offers great potentials compared to other detection methods in many aspects such as simplicity, high sensitivity, rapid response

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and ease of control. The unique combination of highly sensitive ECL technique with specific binding aptamer emerged as ECL-AB biosensors in recent years, such as for the determination of cocaine and thrombin (Fang et al., 2008; Li et al., 2007, 2008; Wang et al., 2007). Li et al. reported an ECL-AB biosensor for cocaine detection (Li et al., 2007). In their work, they used aptamer as the probe DNA, which was labeled with a ruthenium complex as an ECL element. The biosensor was fabricated by the aptamer self-assembling onto Au electrode surface *via* thiol-Au interaction. In another report, Wang et al. developed an ECL-AB biosensor for thrombin detection (Wang et al., 2007). The mechanism of the thrombin sensor was based on the displacement of probe DNA by thrombin. The probe DNA was first tagged with $Ru(bpy)_3^{2+}$-doped silica nanoparticles, and then hybridized with the aptamer, which was pre-confined on the Au electrode surface through Au–S bond. More recently, another type of ECL-AB sensor for the detection of thrombin (Fang et al., 2008) was proposed by adopting an aptamer-based sandwich format. Although the above-mentioned biosensors were quite sensitive and selective, they all suffer a common problem, the difficulty to regenerate the sensor due to the tight binding of aptamers toward targets. To the best of our knowledge, ECL-AB biosensors using the cDNA of aptamer as sensing probe have not yet been reported.

In the present work, another kind of ECL-AB biosensor for target detection was developed by using aptamer cDNA as probes for ECL sensing, which was different from other ECL-AB biosensors with aptamers as the sensing probes. As a model system, we choose ATP as the analyte. After the cDNA hybridized with the ATP-bonding aptamer to form a rigid, linear ds-DNA, which was then labeled with a ruthenium complex at the 3′ terminus of cDNA, the ECL-AB biosensor was fabricated by the cDNA-aptamer complex self-assembling onto the Au electrode surface through the 5′-HS on the cDNA. The as-prepared ECL-AB biosensor can detect ATP specifically as low as 0.05 nM, which was more sensitive than other ATP biosensors, and can be regenerated for several cycles through the incubation of the sensor surface in an ATP-binding aptamer solution. Therefore, such sensing system could provide a promising label-free and more readily regenerated model for aptamer-based small-molecules detection.

2. Experimental

2.1. Chemicals and apparatus

The ATP-binding aptamer and its complementary DNA (cDNA), both designed according to the literatures (Lu et al., 2008; Shen et al., 2007; Zayats et al., 2006; Zuo et al., 2007) with some modifications (shown in supplementary material), and adenosine triphosphate (ATP), cytidine triphosphate (CTP), guanosine triphosphate (GTP) were all purchased from Shanghai Sangon Biological Engineering Technology and Services Ltd. (China). The sequence of cDNA was 5′-HS-$(CH_2)_6$-GCA CCT TCC TCC GCA ATA CTC CCC CAG GTG C-$(CH_2)_6$-NH_2-3′. The six bases at its both 5′ and 3′ ends are complementary so that the cDNA can form a stem-loop structure. The sequence of ATP-binding aptamer was 5′-GCA CCT GGG GGA GTA TTG CGG AGG AAG GT-3′.

Bis-(2,2′-bipyridine)-4′-methyl-4-carboxybipyridine ruthenium(II) *N*-succinimidyl ester bis(hexafluorophosphate) ($Ru(bpy)_2$(cbpy)NHS) was obtained from Fluka. Tripropylamine (TPA) (99%) was obtained from ACROS. Tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) and 2-mercaptoethanol (ME) were purchased from Alfa Aesar China (Tianjin) Co., Ltd. All other reagents were of analytical reagent grade and were used without further purification. 0.1 M phosphate buffer solution (PBS, pH 7.4) was used as a stock buffer solution and for preparing 10 mM PBS (pH 7.4). Redistilled water was used throughout the experiments. All solutions and water were degassed with pure nitrogen before use.

Electrochemiluminescence studies were performed on a BPCL-2-KIC mode Ultra-Weak Chemiluminescence Analyzer controlled by BPCL program (Institute of Biophysics, Chinese Academy of Science, Beijing, China) in conjunction with a CH Instruments model 760c Electrochemical Analyzer (Shanghai Chenhua Instrument Co., China). The UV–vis spectra were recorded on a UV–vis spectrophotometer (Hitachi U-3010, Japan).

2.2. Synthesis of the probe ds-DNA-$Ru(bpy)_2$(cbpy) and cDNA-$Ru(bpy)_2$(cbpy)

The hybridization of synthetic cDNA with its corresponding ATP-binding aptamer was carried out by mixing 35 μL, 100 μM aptamer, 37 μL, 100 μM cDNA and 8 μL, 0.1 M PBS (0.5 M NaCl, pH 7.4) on a PCR system (Bio-radiCycler, USA). The resulting mixture was heated to 95 °C for 10 min and gradually cooled down from 95 °C to 25 °C at a rate of 1 °C min^{-1} to allow the formation of ds-DNA.

The ECL reagent, $Ru(bpy)_2$(cbpy)NHS, was covalently conjugated to the 3′-terminus of the c-DNA in as formed ds-DNA, which was denoted as ds-DNA-$Ru(bpy)_2$(cbpy). The ds-DNA-$Ru(bpy)_2$(cbpy) was synthesized according to the published methods (URL: http://www.probes.com/media/pis/mp00143.pdf; Yang et al., 2002) with some modifications. 0.25 mg of $Ru(bpy)_2$(cbpy)NHS was dissolved in 15 μL DMSO and then added to 55 μL 0.1 M PBS (pH 7.4). To the resulting mixture, 70 μL of the above-mentioned ds-DNA solution was added. The mixed solution was incubated with gentle stirring at room temperature for 12 h. Finally, 50 μL of 3.0 M sodium acetate and 1.0 mL of cold, absolute ethanol were sequentially added to the mixed solution. The resulting solution was chilled for 24 h at −18 °C and then centrifuged for 30 min at 12000 r min^{-1}. The pellet was washed with 0.5 mL of cold 70% ethanol solution for three times to remove any free $Ru(bpy)_2$(cbpy)NHS. The obtained ds-DNA-$Ru(bpy)_2$(cbpy) was dried in air and re-dissolved in 36 μL of redistilled water. The solution was stored at −18° C in refrigerator for later use.

Similar to the synthesis of ds-DNA-$Ru(bpy)_2$(cbpy), the cDNA was also labeled with $Ru(bpy)_2$(cbpy)NHS, which was denoted as cDNA-$Ru(bpy)_2$(cbpy). The detailed synthesis process was also similar to that of ds-DNA-$Ru(bpy)_2$(cbpy) and was omitted.

2.3. Preparation of the ECL-AB biosensor

A gold electrode was polished sequentially with 0.3 and 0.05 μm alumina slurry followed by ultrasonic cleaning with distilled water, absolute ethanol, and distilled water for 5 min each. Then the gold electrode was electrochemically pretreated by consecutively cycling the potential between −0.2 and 1.7 V at a scan rate of 0.1 V s^{-1} in 0.5 M H_2SO_4 solution until a cyclic voltammogram characteristic of a clean Au electrode was obtained. Finally, it was washed with redistilled water and dried in a nitrogen stream.

The ds-DNA-$Ru(bpy)_2$(cbpy) was dissolved in appropriate volume of 10 mM PBS solution (0.1 M NaCl, 5 mM $MgCl_2$, 1 mM TCEP, pH 7.4) to a final concentration of 1 μM. The cleaned electrode was quickly immersed in 500 μL of this solution for 18 h at room temperature to allow the ds-DNA-$Ru(bpy)_2$(cbpy) to chemisorb on the surface of Au electrode. The electrode was then washed with 10 mM PBS (pH 7.4) and subsequently immersed in a 2 mM ME solution (10 mM PBS, 1 M NaCl, pH 7.4) for 1 h to displace the nonspecifically bound oligonucleotides. The resulting electrode was again washed with 10 mM PBS (pH 7.4) and used as ECL-AB biosensor, which was characterized using cyclic voltammogram (CV) and electrochemical impedance spectroscopy (EIS).

2.4. ECL detection of ATP

The ECL-AB biosensor was incubated in 500 μL of ATP solution (10 mM PBS, 0.1 M NaCl, pH 7.4) with different concentrations for 30 min at 37 °C. After washed with 10 mM PBS (pH 7.4), the ECL-AB biosensor was immersed in a high ionic strength solution (1 M NaCl, 10 mM PBS, pH 7.4) containing 5 mM $MgCl_2$ for 30 min at room temperature, inducing the formation of stem-loop structure of cDNA on Au electrode surface (Fan et al., 2003; Liu et al., 2008).

The ECL measurement for ATP detection was performed with cyclic voltammogram at a potential ranging from 0.0 V to 0.75 V at a scan rate of 100 mV s^{-1} in 2.0 mL of 0.10 M PBS (pH 7.4) solution containing 0.10 M TPA with a conventional three-electrode system. The ECL-AB biosensor, a platinum wire and an Ag/AgCl (saturate KCl) electrode were used as working electrode, counter electrode and reference electrode, respectively. A commercial cylindroid's glass cell was used as an ECL cell, which was placed directly in front of the photomultiplier tube (PMT) which was biased at −900 V.

The ECL-AB biosensor was regenerated by immersing the ATP-challenged Au electrode into a 500 μL 1 μM ATP-binding aptamer solution (10 mM PBS, 0.1 M NaCl, 5 mM $MgCl_2$, pH 7.4) for 60 min at 37° C. After washed with 10 mM PBS (pH 7.4), the regenerated electrode became available for another assay.

3. Results and discussion

3.1. Principle of the ECL-AB biosensor

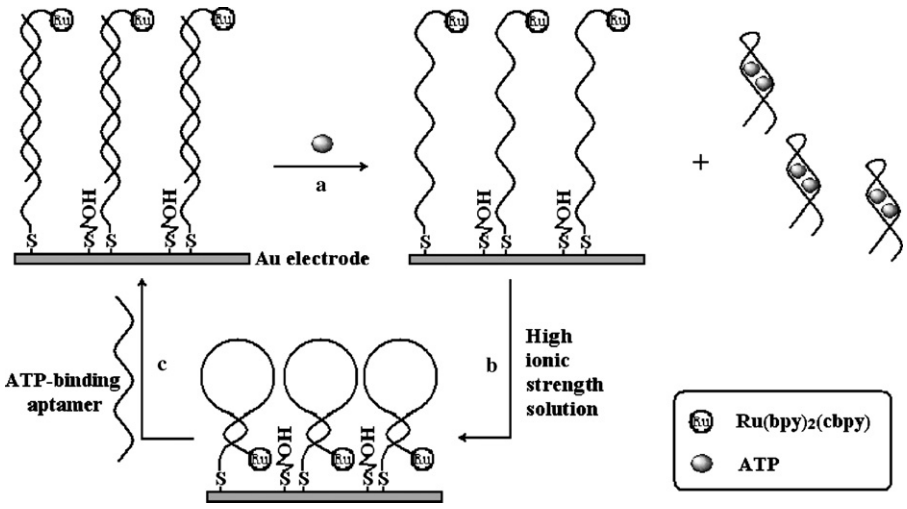
In the present study, the design concept of the ECL-AB biosensor was based on the specific interaction between ATP-binding aptamers and ATP molecules with high affinity. After hybridizing with an ATP-binding aptamer, the probe cDNA having a thiol group at 5′ terminus and an amine group at 3′ terminus formed a rigid, linear ds-DNA helix. Then, the ds-DNA-Ru$(bpy)_2$(cbpy) was synthesized by the reaction of 3′-NH_2 on the cDNA of the as-formed ds-DNA with Ru$(bpy)_2$(cbpy)NHS, in which the Ru$(bpy)_2$(cbpy)NHS was used as the ECL elements. Subsequently, the ds-DNA-Ru$(bpy)_2$(cbpy) was immobilized onto the cleaned surface of a gold electrode through the Au–S bonding. The modified gold electrode was lastly passivated in a ME solution to remove the nonspecifically bound oligonucleotides and deactivate the unassembled surface as shown in Scheme 1.

In the absence of ATP, the Ru$(bpy)_2$(cbpy)-labeled ds-DNA maintained a rigid, linear double helix conformation, which led to a weak ECL signal, probably due to the separation of the ECL elements from the electrode surface. In contrast, when the ECL-AB biosensor was immersed in an ATP solution, the ATP-binding aptamer favored to form ATP-aptamer complex in lieu of aptamer-cDNA duplex. As a result, the ATP-binding aptamer entered the solution phase but the single-stranded cDNA was still bound to the electrode surface (step a in Scheme 1), resulting in a slight increase of the ECL intensity. After the resulting electrode was incubated in a high ionic strength solution containing 5 mM $MgCl_2$, the cDNA formed stem-loop structure thus bringing the ECL elements in close proximity to the electrode surface and resulting in a stronger ECL intensity (step b in Scheme 1). The ECL intensity was linear to the logarithm of ATP concentration. Therefore, this platform could be used as a sensor for ATP detection. One significant advantage of this ECL-AB biosensor is that the sensor can be regenerated after hybridizing the stem-looped cDNA with the ATP-binding aptamer (step c in Scheme 1).

3.2. Characterization of the ECL-AB biosensor

The ECL probe ds-DNA-Ru$(bpy)_2$(cbpy) was characterized by UV–vis spectrophotometry. Fig. S1 of the supplementary material shows the UV–vis spectra of ds-DNA (curve a), Ru$(bpy)_2$(cbpy)NHS (curve b) and ds-DNA-Ru$(bpy)_2$(cbpy) (curve c) in 10 mM PBS (pH 7.4) with the inset showing the magnification of curve c in the range from 480 nm to 380 nm. The absorption peaks of ds-DNA-Ru$(bpy)_2$(cbpy) at 446 nm and 258 nm, corresponded to the metal-to-ligand charge transfer band of Ru$(bpy)_2$(cbpy)NHS and the characteristic absorption of ds-DNA, respectively, indicating that the Ru$(bpy)_2$(cbpy)NHS was labeled to the ds-DNA. The concentration of ds-DNA-Ru$(bpy)_2$(cbpy) solution was calculated to be 1 μM according to the absorption value of Ru$(bpy)_2$(dcbpy)NHS at 450 nm (Miao and Bard, 2003). The cDNA-Ru$(bpy)_2$(cbpy) was also characterized by UV–vis spectrophotometry as shown in Fig. S2 of supplementary material. From Fig. S2, we can conclude that the Ru$(bpy)_2$(cbpy)NHS was also labeled to the cDNA.

The cyclic voltammogram (CV) and electrochemical impedance spectrum (EIS) of $Fe(CN)_6^{4-/3-}$ were used to monitor the hybridization procedure of cDNA with ATP-binding aptamer and the interaction of the aptamer with ATP. Fig. 1A shows CVs of $Fe(CN)_6^{4-/3-}$ on the Au electrode at different stages. When the bare gold electrode was modified with 1 μM cDNA solution (10 mM PBS, 0.1 M NaCl, 5 mM $MgCl_2$, 1 mM TCEP, pH 7.4) for 18 h at room temperature and passivated in a 2 mM ME solution for 1 h, the peak currents of $Fe(CN)_6^{4-/3-}$ decreased and the peak-to-peak potential separation (ΔE) increased noticeably compared with those of



Scheme 1. Schematic illustration of the ECL-AB biosensor for ATP detection and its regeneration.

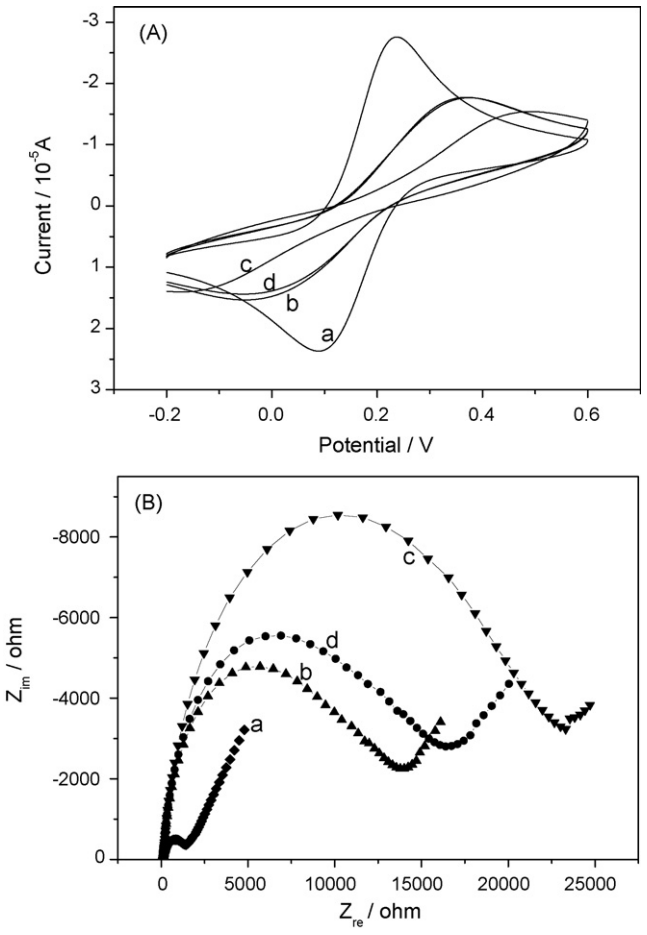


Fig. 1. CV (A) and EIS (B) of the modified electrode at different stages in 10 mM PBS (pH 7.4) containing 2.5 mM $Fe(CN)_6^{3-}$/$Fe(CN)_6^{4-}$ and 0.1 M KCl. (a)bare Au electrode; (b)cDNA modified Au electrode; (c)ds-DNA modified Au electrode; (d)ds-DNA modified Au electrode after immersed into an ATP solution and followed by incubation in 1 M NaCl solution (10 mM PBS, pH 7.4) containing 5 mM $MgCl_2$. The frequency changed from 0.05 Hz to 100 kHz and the amplitude was 5 mV.

the bare gold electrode. This phenomena could be accounted for the electrostatic repulsion between the negatively-charged cDNA and the anionic redox probe (Fig. 1A, curve a and b). The peak currents of $Fe(CN)_6^{4-/3-}$ decreased and ΔE further increased after the hybridization of cDNA with ATP-binding aptamer by immersing the cDNA-confined Au electrode into a 1 μM ATP-binding aptamer solution for 1 h at 37° C (Fig. 1A, curve c). The reason for this change probably was due to the increased negative charges on the Au electrode surface as cDNA hybridizing with aptamer, which blocked the electrochemical reaction of the anionic redox probe. The peak currents of $Fe(CN)_6^{4-/3-}$ and ΔE were almost restored to the original value of the cDNA-modified electrode after the ds-DNA-modified electrode was immersed in a 10 nM ATP solution (10 mM PBS, 0.1 M NaCl, pH 7.4) for 30 min at 37° C, followed by the incubation in a high ionic strength solution containing 5 mM $MgCl_2$ for 30 min. This may indicate the dissociation of the ATP-binding aptamer from the electrode surface upon the presence of specific target and the formation of stem-loop structure of the cDNA (Fig. 1A, curve d).

Electrochemical impedance spectroscopy is one of the most powerful and sensitive techniques for investigating the features of surface-modified electrodes. The impedance spectra consists of a semicircle at high frequencies corresponding to the electron transfer limiting process and a line at low frequencies resulting from the diffusion limiting step of the electrochemical process. Since the diameter of the semicircle corresponds to the charge-transfer resistance (R_{ct}), the smaller the diameter of the semicircle, the smaller the R_{ct} is (Bard and Faulkner, 2001). Fig. 1B shows the changes of the impedance spectra of the electrode in each step. After the bare Au electrode was modified with cDNA, the R_{ct} increased (Fig. 1B, curve a and b). The R_{ct} further increased upon the hybridization of cDNA with the ATP-binding aptamer (Fig. 1B, curve c). However, when the electrode with ds-DNA formed was incubated in ATP solution, the R_{ct} value decreased (Fig. 1B, curve d). The changes of R_{ct} for the different steps were in agreement with the results as seen in the CV, which further confirmed the successful immobilization of cDNA on the gold electrode surface, the hybridization between the cDNA and the ATP-binding aptamer, the dissociation of ATP-binding aptamer from the electrode surface upon specific ATP binding and the formation of stem-loop structure of the cDNA.

3.3. ECL behavior of the ECL-AB biosensor

Fig. 2 shows the ECL profiles of the ECL-AB biosensor before and after interaction with ATP. The bare gold electrode did not generate ECL (Fig. 2a). However, when an extensively cleaned gold electrode was modified with 1 μM cDNA-Ru(bpy)$_2$(cbpy) solution (10 mM PBS, 0.1 M NaCl, 5 mM $MgCl_2$, 1 mM TCEP, pH 7.4) for 18 h at room temperature and followed by a passivation step to the electrode in the ME solution for 1 h, a high ECL signal was observed (Fig. 2b). After the cDNA hybridized with the aptamer, the ECL intensity greatly decreased (Fig. 2c), which was due to the formation of rigid, linear ds-DNA to bring the ECL element away from the electrode surface. When the sensing interface was immersed into ATP solution, the ATP molecules competed the aptamers off the electrode surface due to the specific ATP-aptamer interaction. As a result, the ECL intensity increased (Fig. 2d) probably due to the partial bending of the cDNA. Then, after the biosensor was incubated in a high ionic strength solution containing 5 mM $MgCl_2$ (Fan et al., 2003; Liu et al., 2008), the ECL intensity further increased and approached to that of the cDNA-Ru(bpy)$_2$(cbpy) firstly modified electrode (Fig. 2b and e), which may be attributed to the formation of stem-loop structure of the cDNA to bring the ECL elements proximity to the electrode surface.

3.4. Optimization of analytical conditions

The applied potential to the ECL-AB biosensor is important because it determines the sensitivity and reproducibility of the ECL-

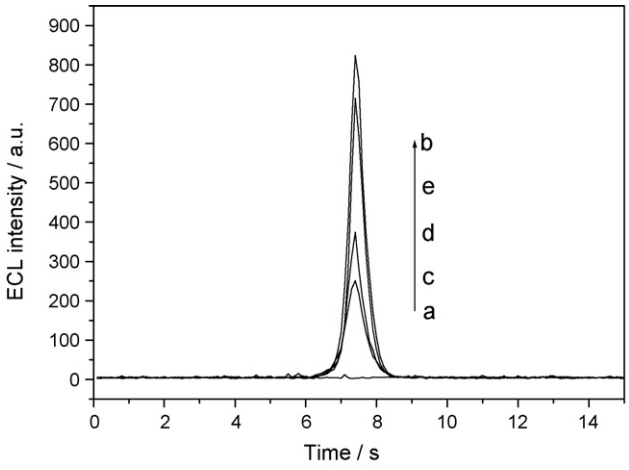


Fig. 2. The ECL profiles of the ECL-AB biosensor at different stages. (a)bare Au electrode; (b)cDNA-Ru(bpy)$_2$(cbpy) modified electrode; (c)ds-DNA-Ru(bpy)$_2$(cbpy) modified electrode; (d)ds-DNA-Ru(bpy)$_2$(cbpy) modified electrode after immersed into an ATP solution; (e)the resulting electrode incubated in 1 M NaCl solution (10 mM PBS, pH 7.4) containing 5 mM $MgCl_2$.

AB biosensor. We have also investigated the dependence of both ECL intensity and reproducibility for ECL-AB biosensor on applied potential. Results showed that the ECL intensities of ECL-AB biosensor increased with the increase of the high potential from +0.60 to +0.75 V in cyclic voltammogram. When the high potential exceeded +0.80 V, the ECL-AB biosensor became irreproducible along with decreased ECL intensity. This could be attributed to the oxidative desorption of the Au-thiol bond on the gold electrode at the potential higher than 0.80 V (Bertolino et al., 2005; Zu and Bard, 2001). Therefore, the optimal potential range is from 0 to +0.75 V.

Different interaction time of the ECL-AB biosensor with ATP might cause noticeable change of ECL intensity. Therefore, the relationship between the interaction time, i.e. the time of immersing the ECL-AB biosensor in ATP solution, and the ECL intensity was investigated to optimize the interaction time. A series of ECL-AB biosensors were immersed in 10 nM ATP solution at 37° C for different incubation time, the ECL signals were thus recorded in 0.10 M PBS (pH 7.4) containing 0.10 M TPA. The results showed that the ECL intensity increased with the increase of hybridization time from 10 to 30 min and then plateaued. This suggested that 30 min incubation was enough for the system to equilibrate. Therefore, we chose 30 min as the optimal interaction time through the experiments.

3.5. ECL detection of ATP

The presence of ATP at different concentrations led different ECL intensity, depending on the amount of the released aptamer in the solution. In order to evaluate the performance of the present ECL-AB biosensor, a series of ATP samples from 0.05 nM to 100 nM were tested. The difference in ECL intensity was used to evaluate the ECL response to ATP. The ECL intensity increased with the increase of ATP concentration until reached a maximal value at 10 nM ATP. As shown in Fig. 3, with all the optimized running conditions discussed before, the biosensor showed linear relationship between the ECL intensity and the logarithm of ATP concentration in the range from 0.05 nM to 10 nM. The linear regression equation was $I_{ECL} = 470.6 + 151\ \lg C$ (C is in nM) with a correlation coefficient of 0.994. Three repetitions of each standard ATP solution were carried out to evaluate the reproducibility and precision of the method. The results were reflected by the error bars in inset of Fig. 3. The detection limit was 0.02 nM based on a signal-to-noise ratio of 3, which is lower than that in the previous reports (Wang et al., 2005; Zuo et al., 2007; Lu et al., 2008; Du et al., 2008) as shown in Table S1 of the supplementary material. This indicates that the ECL-AB sensor was highly sensitive and reproductive.

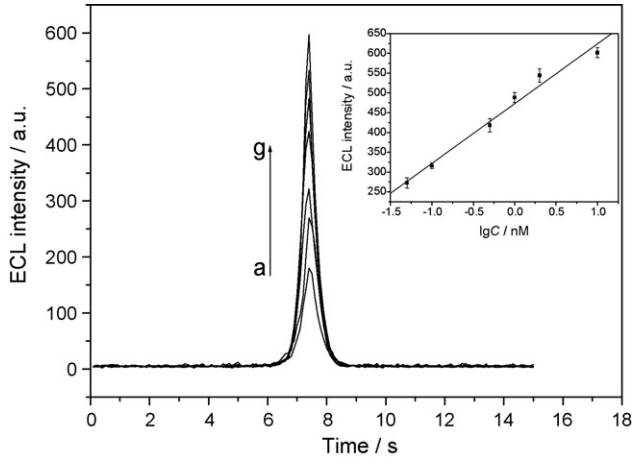


Fig. 3. ECL profiles for the ECL-AB biosensors after incubated in ATP solutions with different concentrations. Inset: The calibration curve of the dependence of ECL intensity on the logarithm of different concentrations of target ATP. The concentrations of ATP were (from a to g) 0 nM, 0.05 nM, 0.1 nM, 0.5 nM, 1.0 nM, 2.0 nM, 10 nM.

3.6. Selectivity and regeneration of the ECL-AB sensor

In order to investigate the specific response of the ECL-AB biosensor to ATP, control experiments were performed by incubating the ECL-AB biosensors in several aqueous solutions containing 10 nM ATP, CTP and GTP, respectively. A water solution was used as blank. It was shown in Fig. S3 of the supplementary material that only ATP samples gave obvious ECL intensity changes, while CTP and GTP samples delivered the same ECL intensity as blank sample. This proves that the ATP-binding aptamer sequence is very specific to ATP. The cross-sensitivity of the sensor in a mixture with three different nucleosides containing ATP was also examined. The signal obtained from the complex was similar to that obtained from ATP only, which further indicated that the ECL-AB biosensor was very specific for ATP determination with negligible response to CTP and GTP.

The current ECL-AB biosensor can be readily regenerated after the completion of ATP test by immersing the biosensor in a 1 μM ATP-binding aptamer solution for 1 h at 37 °C. Also unlike previously reported sensors, the present biosensor did not need high temperature for regeneration. High temperature could cause the loss of probe molecules from the gold electrode surface (Du et al., 2005; Zhang et al., 2008). We can see from Fig. S4 of the supplementary material that after three cycles of regeneration process, the average ECL intensity recovered up to 90% of its original ECL intensity, which indicates the possibility of the reported ECL-AB biosensor to be reused. The reusability is particularly important for a practical biosensor application. The current work provides a relatively milder, more convenient and more readily reusable way over the other aptamer-based ECL sensors, which offers great potential for further development of this type of biosensor.

4. Conclusions

An ECL aptamer-based biosensor with cDNA oligonucleotide as sensing probe and aptamer as recognition element was developed with high sensitivity and specificity for a small molecule as an example of ATP. The ECL-AB biosensor, combined both highly selective ATP-binding aptamer and highly sensitive ECL technique, has demonstrated the success and robustness in the detection of trace amount of ATP. The present ECL-AB biosensor design may offer several unique features such as cost-effectiveness, practical reusability, high sensitivity and high selectivity. In addition, the proposed sensor format may provide a general alternative strategy for the detection of aptamer-based targets in real samples.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2009.04.016.

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