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ORIGINAL PAPER

Determination of cocaine on banknotes through an aptamer-based electrochemiluminescence biosensor

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Abstract A novel electrochemiluminescence (ECL) "sandwich" biosensor has been developed to detect cocaine. The sandwich biosensor was fabricated on the basis of the fact that a single aptamer could be split into two fragments and the two dissociated parts could form a folded, associated complex in the presence of targets. One of these (capture probe), which had hexane–thiol at its 5′-terminus, was immobilized on a gold electrode via thiol–gold binding. The other one (detection probe) was labeled with the ECL reagent tris(2,2′-bipyridyl)ruthenium(II)-doped silica nanoparticles (RuSiNPs) at its 3′-terminus. Owing to the weak interaction between the two fragments, the sensor exhibited a low ECL signal in the absence of cocaine. After the target cocaine had been added to the solution, it induced association of the two fragments and stabilized the associated complexes, leading to immobilization of RuSiNPs on the electrode surface, and the ECL detected on the electrode surface was enhanced. The enhanced ECL intensity was directly proportional to the logarithm of the cocaine concentration in the range from 1.0×10^{-9} to1.0×10^{-11} mol/L, with a detection limit of 3.7×10^{-12} mol/L. The biosensor was applied to detect trace amounts of cocaine on banknotes with satisfactory results.

Keywords Electrochemiluminescence · Biosensor · Cocaine · Aptamer · Banknote

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Introduction

Cocaine is a powerfully addictive stimulant drug which plays an important part in stimulating the central nervous system [1]. Repeated use of cocaine can cause a long-term change in the brain's reward system by increasing levels of dopamine. Additionally, abuse of cocaine will lead to a variety of adverse effects on the body, such as increases in body temperature, heart rate, and blood pressure, and increases the spread of human immunodeficiency virus infection and drug-resistant tuberculosis [2, 3].

Banknotes may be directly or indirectly contaminated by the drugs (including cocaine) in general circulation [4] because drug trafficking involves large sums of cash in the trading of drugs, bringing banknotes directly into contact with drugs. Moreover, many drug users use a wrapped banknote to sniff the drug [5]. Banknotes carrying traces of cocaine can be used as a part of the evidence to reveal the relationship between an individual and drugs [6]. So, development of a method for determination of trace amounts of cocaine on banknotes is very important.

The systematic evolution of ligands by exponential enrichment (SELEX) technique was employed to produce an aptamer, which can bind with high affinity and specificity to small molecules, proteins, organic molecules, and so on [7–11]. Aptamers have a lot of advantages, such as easy synthesis, chemical labeling, and good stability over antibodies [7, 10]. Accordingly, aptamers have become novel popular recognition elements and are used for designing aptasensors based on different analytical approaches, including electrochemistry [12–14], fluorescence [15], and colorimetry [16]. The applications of aptamers in biosensors open another door for the develop-

ment of cocaine sensors. For example, Madru et al. [17] proposed an aptamer-based biosensor for determination of cocaine in human plasma and He et al. [18] developed a new fluorescence aptamer-based biosensor for sensitive, selective, and economical detection of cocaine.

Electrochemiluminescence (ECL) is produced by chemiluminescent reactions triggered by electrochemical methods, and the ECL technique has the remarkable feature of high sensitivity [19]. In particular, the combination of the high sensitivity of the ECL technique with the high selectivity of aptamers has led to the emergence of aptamer-based ECL biosensors. ECL biosensors always use $Ru(bpy)_3^{2+}$ (where bpy is 2,2′-bipyridyl) derivatives or $Ru(bpy)_3^{2+}$-doped silica nanoparticles (RuSiNPs) tagged on the aptamers and constructed platforms for biological analysis [20, 21]. In the presence of targets, probes with ECL labels modulate the distance between ECL labels and electrode surfaces by a conformation switch, resulting in ECL signal changes [22–25]. However, these kinds of ECL biosensors always need a large-scale conformational change of the aptamers upon binding of targets, so they generally suffer from a substantial signal. Recently, association of split aptamer fragments was used for development of DNAzymes and aptamer biosensors to circumvent this drawback [26]. This kind of biosensor has been employed for detection of thrombin [27], cocaine [3, 28, 29], and ATP [29]. Such biosensors are designed as electrochemical sandwich assays based on the mechanism of target-induced formation of a supracomplex composed of aptamer units and targets and they do not require binding of multiple aptamers and the aptamers undergoing a conformational change. To the best of our knowledge, there has been no report of an ECL aptamer biosensor based on association of split aptamer fragments for the determination of cocaine.

In this work, we exploited a single cocaine aptamer that was split into two fragments (capture probe and detection probe). Then, a novel ECL "sandwich" biosensor based on target-induced association of split aptamer fragments was developed. The biosensor was composed of three units: cocaine as a model target, RuSiNPs as the ECL label, and cocaine aptamer fragments as molecule recognition elements. Compared with traditional biosensors, the sensor proposed has higher sensitivity. The sensor was applied to detected trace amounts of cocaine on banknotes.

Experimental

Chemicals and materials

Two cocaine aptamer fragments were synthesized by Sangon (Shanghai, China), purified by high-performance liquid chromatography, and used as received. The sequences were as follows:

Capture probe	5′-HS $(CH_2)_6$-AGACAAGGAAAA-3′
Detection probe	5′-TCCTTCAATGAAGTGGGTGG-NH_2-3′

A single cocaine aptamer was also synthesized, the sequence of which was the combination of the former two fragments. Cocaine, 6-mercaptohexanol, and 2-(dibutylamino) ethanol were purchased from Sigma (St. Louis, MO, USA) and used as received. RuSiNPs were synthesized and functionalized as reported in [30] (the diameters of the RuSiNPs were about 55±5 nm [31]). Fifty microliters of detection probe solution was added to 50 μL of a solution of functionalized RuSiNPs and the mixture was stirred for 120 min in a 37 °C water bath. The functionalized RuSiNP surface finally covalently bound with the detection probe and the terminal of the oligonucleotides was labeled. After this, the product was washed, centrifuged, and resuspended in phosphate buffer solutions (PBS), and then stored at 4 °C for the following experiments. All other chemicals were of analytical grade and used without further purification. DNA solution was prepared by dissolving DNA in 0.05 mol/L pH 7.4 PBS. The water used was obtained by purification of distilled water with a Millipore Milli-Q system.

Sensor preparation

The gold electrodes (3 mm in diameter) were polished with alumina slurry on a polishing pad, followed by ultrasonic washing with ethanol and water for 3 min to get a clean surface. Then, they were voltammetrically cycled in 0.50 mol/L H_2SO_4 until they were stable and ideal voltammograms were obtained. Finally, they were washed with distilled water. Capture probe solution was dropped on the cleaned electrode surface for 2 h at room temperature to obtain a thiolated DNA immobilized electrode. Then, the immobilized electrode was passivated in 6-mercaptohexanol solution for 1 h to cover the nonspecific sites and this was followed by washing with distilled water to remove unspecific adsorption. The surface coverage of the gold electrode by the capture probe was about 5.5×10^{11} molecules/cm^2, as calculated by a method reported previously [32]. Then, the RuSiNP-labeled detection probe solution was mixed with cocaine solution for 1 h, and the capture-probe-modified electrode was incubated in the mix solution for 2 h. For stability detection, the resulting electrode was washed with washing buffer and dried prior to use.

Apparatus and detection procedures

The experimental system for ECL measurement consisted of a BPCL ultraweak luminescence analyzer (Institute of

Biophysics, Chinese Academy of Science, Beijing, China) and a CHI 660a electrochemical system (Shanghai Chenhua, Shanghai, China). A glass cell with an optically flat bottom was used as the ECL cell and employed with a three-electrode system consisting of a gold working electrode, an Ag/AgCl reference electrode, and a platinum wire counter electrode. The electrochemical cell was placed directly in front of a photomultiplier (operated at −800 V).

Preparation of samples on the banknotes

Fifty mimic new banknotes were obtained from the Bank of China. The banknotes were exposed to cocaine to simulate banknotes which had been contaminated in general circulation. The simulation process was proposed in a previous report [33]. Briefly, latex surgical gloves were contaminated with cocaine worn by the operator and the banknotes were swabbed with two different levels of cocaine (level 1 1.0 μg and level 2 2.0 μg) as they were counted one by one. Then, the banknotes were placed in random environments for a few days. Finally, cocaine was extracted from the banknotes using 0.01 mmol/L acetic acid (300 mL) to dissolve the drugs on them. Then, 10 mL of the extracts was diluted 20 times by the buffer solution, and the final two different spiked concentrations of cocaine were about 4.85×10^{-10} and 9.70×10^{-10} mol/L. In addition, a 'blank" solution also prepared using the same quantity of banknotes without illicit cocaine contamination was prepared via the same procedure. All the solutions were stored at 4 °C in a refrigerator.

ECL detection procedure

To detect cocaine or other small molecules, the sensor was immersed in a detection cell containing 2 mL of PBS (pH 7.4) and 10 μL of 1.0 mol/L (dibutylamino)ethanol (working as an ECL coreactant [34]). Cyclic voltammetry (CV) in the range from 0.8 to 1.6 V with a scan rate of 0.05 V/s was performed and the ECL signal was recorded simultaneously. The intensity of peak ECL signal was selected for quantitative detection.

Results and discussion

Principle of the biosensor

To fabricate the "sandwich" aptasensor, the 32-base cocaine aptamer was split into a 12-base fragment and a 20-base fragment, which worked as a capture probe and a detection probe, respectively. The cutting site has to be such that it does not impede target binding by circular permutation [29]. A schematic diagram of the proposed ECL aptasensor is shown in Fig. 1. The capture probe with a thiol group at the 5′-terminal was attached to the electrode surface by the self-assembled monolayer technique. The detection probe had a group covalently labeled with RuSiNPs at the 3′-terminal. In the absence of cocaine, there was weak interaction between the two fragments, so we observed a weak ECL signal. In the presence of cocaine, the target molecules induced the detection probe to combine with the capture probe to form a three-way junction to bind cocaine, and stabilized the associated complexes. In turn, allowing RuSiNPs to be immobilized on the electrode and close to the electrode surface led to a greatly increased ECL signal.

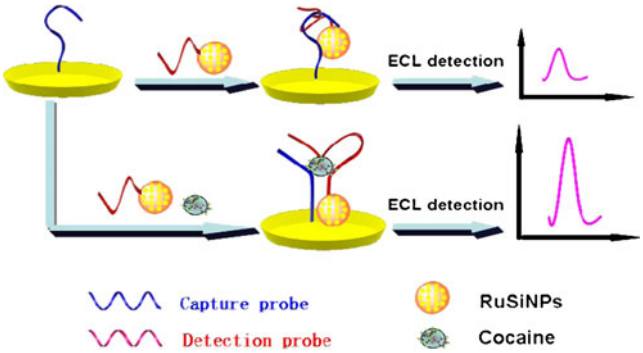

Fig. 1 The electrochemiluminescence (*ECL*) aptasensor for cocaine detection in a sandwich manner. Note that each part is not according to the proportion. *RuSiNPs* $Ru(bpy)_3^{2+}$-doped silica nanoparticles

Characterization of the electrodes

The process of immobilization of the oligonucleotide on the electrode surface was characterized by faradic electrochemical impedance spectroscopy using electroactive $Fe(CN)_6^{3-/4-}$ ions in the test solution containing 0.50 mol/L KCl. An equivalent circuit (Fig. 2, inset) as the model of the working electrode was developed to fit the electrochemical impedance

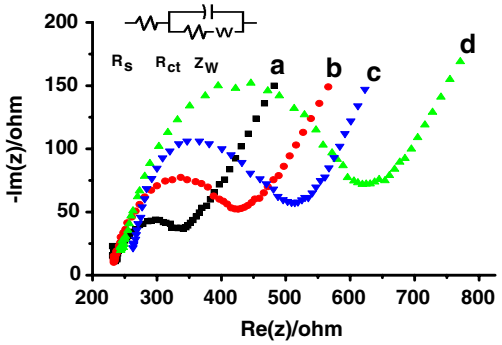

Fig. 2 Impedance spectra of different electrodes: *a* bare gold electrode, *b* single-stranded DNA electrode, *c* double-stranded DNA electrode, and *d* double-stranded DNA–cocaine electrode. All measurements were performed in a solution of 5.0 mmol/L $[Fe(CN)_6]^{3-/4-}$ containing 0.1 mol/L KCL. Biased potential 0.218 V, applying amplitude 5 mV in the frequency range 1–10 kHz. The *inset* shows the equivalent circuit

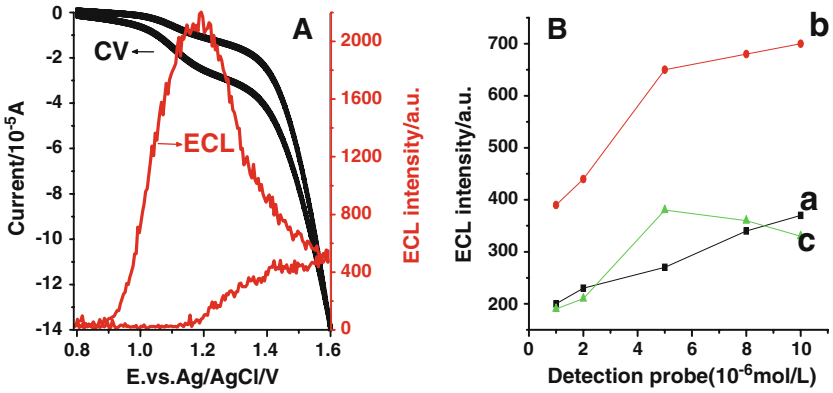

Fig. 3 **a** The cyclic voltammetry (*CV*) curve from the biosensor and the corresponding ECL potential curve. **b** The effect of the detection probe concentration on the enhanced ECL intensities: *a* the background ECL intensity in the absence of cocaine, *b* the ECL intensity at the presence of 1×10^{-11} mol/L cocaine, and *c* the enhanced ECL intensity

spectroscopy data. The charge transfer resistance R_{ct} reflects the electron transfer kinetics of the surface of the different electrode, and R_s, Z_w, and C_d are the electrolyte solution resistance, the Warburg impedance, and the double-layer capacitance, respectively [35]. As shown in Fig. 2, the bare gold electrode (curve a) exhibits a very small semicircular domain at high frequencies, suggesting the electroactive ion of $Fe(CN)_6^{3-/4-}$ transfers electrons easily to the electrode surface. When the capture probe was immobilized on the electrode surface, R_{ct} increased (curve b). This is because the single-stranded acid with a negatively charged phosphate backbone self-assembled on the electrode surface, which increased the distance for electron transfer through the film. When the detection probe was added to the solution in the absence of cocaine, the capture probe interacted weakly with the detection probe through physical adsorption and some of the detection probes were captured on the electrode surface, so the value of R_{ct} of curve c was larger than that of curve b. After the addition of cocaine, both cocaine and the detection probe were captured on the electrode surface, leading to enhanced R_{ct} (curve d). This was attributed to the fact that the detection probe and small molecules produced a nearly insulating layer on the electrode surface which prohibited the electroactive species in the solution from transferring electrons to the electrode surface.

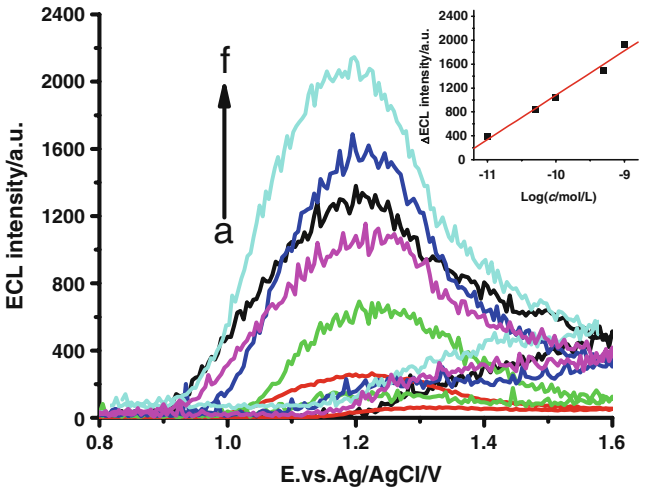

Fig. 4 Change of the ECL intensity of the functionalized electrode with different concentrations of cocaine: *a*–*f*: 0, 1.0×10^{-11}, 5.0×10^{-11}, 1.0×10^{-10}, 5.0×10^{-10}, and 1.0×10^{-9} mol/L. The *inset* shows the linear relationship between the ECL intensity and the logarithm of cocaine concentration

Optimization of the analytical conditions

The CV curves and the ECL potential curves from the biosensor were studied first. As shown in Fig. 3a, there is an oxidation peak in the CV curve at about 1.20 V, which corresponds to the oxidation of $Ru(bpy)_3^{2+}$. The onset of the ECL signal is at about 0.95 V, and it reaches its peak at about 1.20 V. So in this study, the ECL intensity at this potential was chosen for quantitative analysis.

The ECL intensities at different probe concentration in the absence (curve a) and in the presence (curve b) of cocaine are shown in Fig. 3b, and the enhanced ECL intensity ($\triangle I$) is shown as curve c. In the lower concentration range, $\triangle I$ will increase with the probe concentration; however, if the probe concentration becomes too high, more detection probe will interact with the capture

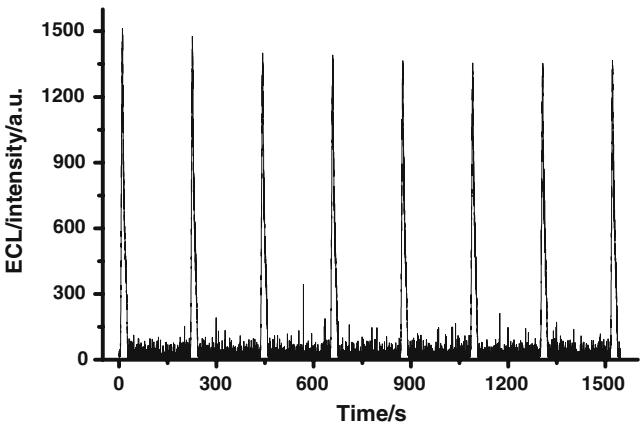

Fig. 5 Stability of the aptamer-based biosensor after reaction with 1.0×10^{-10} mol/L cocaine under continuous CV scanning in the range from 0.8 to 1.6 V at a scan rate of 50 mV/s

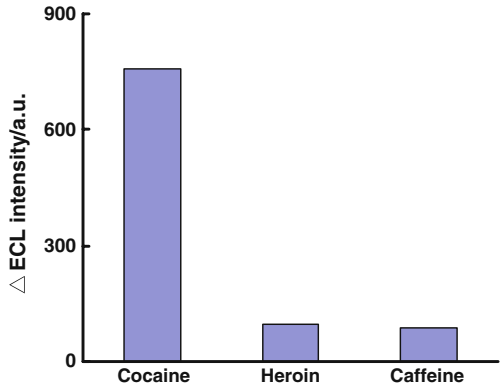

Fig. 6 Selectivity of the biosensor for cocaine, heroin, and caffeine. The concentration of cocaine was 5.0×10^{-11} mol/L and the concentration of heroin and caffeine was 5.0×10^{-9} mol/L

probe through physical adsorption and this will cause an increase of the background intensity and $\triangle I$ will decrease. We found 5.0×10^{-6} mol/L leads to the greatest change of the ECL intensity of the sensor, and thus 5.0×10^{-6} mol/L detection probe was chosen for the following experiment.

The effects of the time of interaction between cocaine and the two split DNAs on the ECL intensities detected were investigated. At first, the ECL intensity increased gradually with the reaction time, and it finally reached a constant value when the time was longer than 120 min. So 120 min was chosen as the optimized time in this study.

ECL behavior of the biosensor and the calibration curve

Figure 4 shows the changes of the ECL intensity upon analyzing different concentrations of cocaine with the biosensor. In the absence of the cocaine, the background ECL signal was about 270 a.u. When a single aptamer was applied instead of the split aptamers, the background ECL signal was about 580 a.u. This result indicates the proposed method has a lower background ECL signal, and this can lead to the lower detection limits. The ECL intensity increased with the concentration of cocaine, which could be due to the formation of the three-way junction, leading to more of the ECL tag approaching the electrode surface. The change of ECL intensity exhibited excellent correlation with the logarithm of the concentration of cocaine in the range from 1.0×10^{-11} to 1.0×10^{-9} mol/L. The linear regression equation was

$$\triangle I/\text{a.u.} = 742.5\log C(\text{cocaine}) + 8505.2,$$

where the correlation coefficient was R=0.9916 and the detection limit was 3.7×10^{-12} mol/L (signal-to-noise ratio of 3). Compared with the target-induced conformational change based ECL aptasensor [22], the biosensor had better sensitivity and has a low detection limit. In addition, the detection limit is more sensitive than that of some established methods for detection of cocaine such as the solid-phase extraction method [17] and fluorescence assay [18]. At the same time, the detection limit obtained using this biosensor is tenfold lower than that of the reported electrochemical biosensor based on the same principle [29].

Stability and selectivity of the biosensor

To assess the long-stern stability of the biosensor, the storage stability of the biosensor was investigated. We repeated continuous potential scanning for eight cycles in 1.0×10^{-10} mol/L cocaine (as shown in Fig. 5). We found that the relative standard deviation of the ECL response was 6.5%. When the biosensor was stored at 4 °C over 10 days, the ECL intensity did not change significantly. These results indicate that the biosensor had good stability. It has been reported that gold/thiol is not stable at a potential higher than 1.00 V (vs. Ag/AgCl) because of the oxidative desorption of thiol on the gold electrode [22, 36, 37], but it has also been reported that it can be stable even at 1.20 V (vs. Ag/AgCl) [38]. In this study, we found that the ECL from the biosensor only decreased a little after continuous scanning eight times, so the biosensor was quite stable. The reason may be that only a little gold/thiol was destroyed during the CV scanning, so the electrode seems quite stable under anodic potential cycling.

The specific response of the aptasensor was determined by incubating the functional electrode in 5.0×10^{-11} mol/L cocaine, 5.0×10^{-9} mol/L heroin, and 5.0×10^{-9} mol/L caffeine. Compared with the other two samples, there was a significant increase of ECL intensity induced by the biosensor interacting with cocaine (as shown in Fig. 6), which further proved that the aptasensor could be used for cocaine determination, without interference from other small molecules.

Determination of cocaine on banknotes

To validate the possibility of the proposed method in a real application, the banknote samples were spiked with two

Table 1 Recoveries of cocaine extracted from the banknotes spiked with cocaine

Sample	Calculated (mol/L)	Found (mol/L)	Recovery (%)	RSD (%)
Blank	0	1.1×10^{-10}		4.4
Level 1	4.85×10^{-10}	4.30×10^{-10}	89	4.4
Level 2	9.70×10^{-10}	8.95×10^{-10}	92	5.2

RSD relative standard deviation

different concentrations of cocaine (levels 1 and 2). The efficiency and reproducibility of the extraction procedure are shown in Table 1. The detected concentration of level 2 is about 2 times that of level 1 (same as calculated). The recoveries at the two different concentrations for cocaine were 89% and 92%, respectively, and the relative standard deviations were 4.7% and 5.2% (n=3). These results indicated that the proposed method can be applied in real applications.

Conclusions

In summary, we demonstrated a novel ECL aptamer-based biosensor based on single aptamer sequences for highly sensitive detection of cocaine. Ruthenium nanoparticles were employed as ECL tags to increase the sensitivity of the biosensor and aptamer fragments as recognition aptamers do not need to undergo a conformational change. Additionally, the biosensor has some distinct advantages, such as convenience, ease of control, and a low dose of material. Moreover, the biosensor was applied to analyze cocaine on banknotes with satisfactory results, and it could be used in the analysis of illicit drugs in complex matrices.

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