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Electrochemiluminescent sensor for the detection of DNA hybridization using stem-loop structure DNA as capture probes

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Abstract An electrochemiluminescent (ECL) sensor is presented for the detection of DNA hybridization using single-strand DNA with stem–loop structure as capture probes. After the probe DNA was immobilized on an gold electrode, labeled with ruthenium complex, and formed a stem–loop structure, this sensor produced a high ECL signal. On hybridization with the complementary target DNA, the ECL intensity significantly decreased. Different from other DNA sensors, in which the probe DNA was labeled in advance with a signalling molecule and then assembled onto an electrode, the approach presented here relies on assembling first, and then labelling with ruthenium complex, without the complicated purifying processes after the probe DNA was labeled. The ECL intensity versus the concentration of the target DNA was linear in the range from 1.0 pM to 0.1 μM with a detection limit of 0.5 pM. The biosensor conceivably can be used as a general approach for the ECL detection of DNA.

Keywords ECL · DNA sensor · Stem-loop structure · Hybridization · Ruthenium complex

In recent years, the detection of DNA hybridization has attracted considerable interest in a wide range of areas including DNA diagnostics, gene analysis, fast detection of biological warfare agents, and forensic applications, etc. [1]. Consequently, a variety of DNA detection systems based on the hybridization between a probe DNA and its complementary target have been described including optical [2–5], electrochemical [6–13], electrochemiluminescent [14–20], acoustic [21] and gravimetric [22] methods. Among these, an optical approach termed molecular beacons with stem-loop structure has attracted significant interest and revealed high sensitivity and selectivity [23, 24].

Recently, Heeger, Plaxco, and others [8–10] developed a series of novel reagentless, sensitive and selective electrochemical DNA sensor that combines the significant advantages of electrochemical detection method with the versatility of surface-attached molecular beacons. This electrochemical DNA sensor employs an electrode-attached, stem–loop structure DNA labeled with an electroactive reporter (e.g., ferrocene or methylene blue) as a capture probe. Before hybridization between the probe DNA and its complementary target, the electroactive label is localized to the electrode surface by means of hybridization of the stem region of the probe DNA, allowing facile electron transfer between the electroactive label and the gold electrode. In the presence of a complementary target, the stem–loop is converted into a rigid, linear double helix and the distance between the label and the electrode is significantly expanded, leading to a large, readily measurable signal change.

ECL technique has many distinct advantages over other detection methods. For example, compared with fluorescence methods, ECL does not involve a light source and avoids the attendant problems of scattered light and impurities luminescent. Moreover, the specificity of the ECL reaction associated with the ECL label and the co-reactant species decreases problems with side reactions and is characterized by good spatial, temporal resolution and

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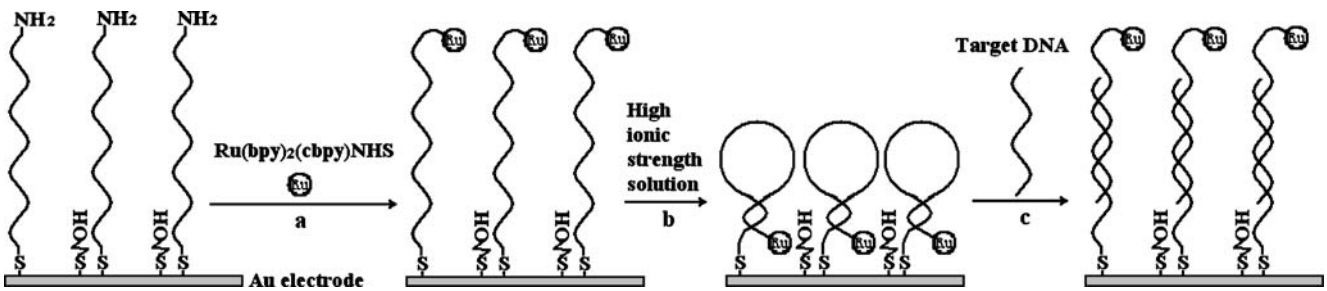
higher sensitivity [16]. Recently, ECL detection method for DNA hybridization has received much attention. Fang and co-workers [14] reported ECL detection for DNA hybridization using *N*-(4-aminobutyl)-*N*-ethylisoluminol (ABEI) as labels. ABEI was used as a marker to label a known single-strand DNA (ss-DNA), which was used as a DNA probe for identifying a target ss-DNA immobilized on a polypyrrole modified electrode. Miao et al. [16] developed an ECL method for the detection of the target ss-DNA tagged with tris(2,2′-bipyridyl)ruthenium(II) as an ECL label through hybridization with the capture ss-DNA which preattached to a gold electrode surface. Zhang and co-workers [17, 18] developed highly sensitive ECL methods for the detection of DNA hybridization based on multiple reporters per hybridization event using gold nanoparticles or single-wall carbon-nanotubes as carriers for ECL labels and ss-DNA. However, all these ECL detection methods for DNA are not reagentless and require post-hybridization treatment with either hybridization indicators or other exogenous signaling molecules. Therefore, it is valuable to develop new ECL detection methods for DNA that is simultaneously sensitive, selective and reagentless.

Here, we demonstrate the use of a reagentless ECL sensor with high sensitivity and selectivity for the detection of DNA hybridization by using stem-loop structure DNA as capture probes. The probe DNA used here was tailor-made to have six complementary bases at both ends, at which the 5′-terminal and the 3′-terminal were modified with a thiol group and an amino group, respectively. Firstly, the probe DNA self-assembled onto a gold electrode by means of facile gold-thiol bonding and was passivated with 2-mercaptoethanol (ME) to displace nonspecifically bound oligonucleotides and hold the unassembled surface, as shown in Scheme 1. Then, as step a in Scheme 1, the probe DNA immobilized on Au electrode was labeled with ruthenium complex as an ECL element. After that, the modified electrode was immersed in a high ionic strength solution to form stem-loop structure [8, 10] holding the ECL label in close proximity to the electrode surface and resulting in a high ECL signal (step b in Scheme 1). On hybridization with the complementary target DNA, the probe DNA with stem-loop structure was converted into a rigid, linear double helix DNA and a distinct decrease of the ECL signal is observed presumably because the ECL label is separated from the electrode surface (step c in Scheme 1). Different from other approaches for preparing DNA sensors, in which the probe DNA was in advance labeled with signal molecules, and then purified by a series of steps including ethanol precipitation or filtration through a Sephadex G-25 column, gel electrophoresis or reverse-phase HPLC before assembling onto the surface of the electrode [8, 14, 16], the approach proposed here was assembling first and then labeled with ruthenium complex leaving out the complicated purifying processes after the probe DNA was labeled, which was more simple and convenient.

Experimental

Chemicals and apparatus

Bis-(2,2′-bipyridine)-4′-methyl-4-carboxybipyridine ruthenium(II) *N*-succinimidyl ester bis(hexafluorophosphate) ($Ru(bpy)_2(cbpy)NHS$) was obtained from Fluka (http://www.sigmaaldrich.com). Tripropylamine (TPA) (99%) was obtained from ACROS (http://www.acros.com). Tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) and 2-mercaptoethanol (ME) were purchased from Alfa Aesar China (Tianjin) Co., Ltd. (http://www.alfa.com). The oligonucleotides were synthesized by Shanghai Sangon Biological Engineering Technology and Services Ltd. (China) (http://www.sangon.com). The stem–loop structure DNA with the base sequence of 5′- HS-$(CH_2)_6$-CCT AGC TCC TTC CAC TCG GAT AAG ATG CGC TAG G-$(CH_2)_6$-NH_2-3′ (oligo 1) was designed with six complementary bases at its 5′ and 3′ ends in the hope that the DNA strand will be closed by the base pairs and thus form a stem-loop structure with either end close to the gold surface. The sequences of complementary target DNA and three-mismatch DNA were 5′-GCA TCT TAT CCG AGT GGA AGG A-3′ (oligo 2) and 5′-GCA TCT TAT CCG GTA GGA AGG A-3′ (oligo 3), respectively. All other reagents were of analytical reagent grade and redistilled



Scheme 1 Schematic representation of the procedure of preparing ECL-DNA sensor for the detection of DNA hybridization

water was used throughout. All solutions and water were degassed with pure nitrogen before use.

Electrochemiluminescence studies were performed using a BPCL-2-KIC mode Ultra-Weak Chemiluminescence Analyzer controlled by a personal computer with BPCL program (Institute of Biophysics, Chinese Academy of Science, Beijing, China, http://www.ibp.ac.cn) in conjunction with a CH Instruments model 760c Electrochemical Analyzer (Shanghai Chenhua Instrument Co., China).

The procedure of preparing the ECL-DNA sensor

The surface of the Au electrode (2 mm diameter) was polished with alumina slurry (0.3 and 0.5 μm) and then washed with redistilled water and anhydrous ethanol in an ultrasonic bath for 5 min, respectively. The Au electrode was further treated electrochemically in 0.5 M H_2SO_4 by the scanning potential from −0.2 to 1.7 V at a scan rate of 0.1 Vs^{-1} until an ideal voltammogram was observed. Finally, it was washed with redistilled water.

Oligo 1 was dissolved in 10 mM PBS (0.1 M NaCl, 1 mM TCEP, pH=7.4) solution 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 oligo 1 to chemisorb on the Au electrode surface through Au–S binding, and then washed with 0.1 M PBS (pH=7.4) to remove the unbinding probe DNA on the surface of the gold electrode, which was denoted as Au-oligo 1 electrode. The electrode was subsequently passivated by being immersed in a 2 mM ME solution (10 mM PBS, pH=7.4) for 30 min to displace nonspecifically bound oligonucleotides and hold the unassembled surface. Then, the probe DNA immobilized on Au electrode was labeled with a ruthenium complex as an ECL element through the reaction of the amino group at the 3′-terminal of the oligo 1 with the $Ru(bpy)_2(cbpy)NHS$ by immersing the electrode in a 500 μL of 20 μM $Ru(bpy)_2(cbpy)NHS$ solution (0.1 M sodium tetraborate, pH=8.5) [26] for overnight at room temperature with slightly shaking. The modified electrode in this stage was denoted as Au-oligo 1-$Ru(bpy)_2(cbpy)$-1. After vigorously rinsed in 70% ethanol and 0.1 M PBS solution (pH=7.4) with agitation for 5 min [25, 26], respectively, the electrode was immersed in 1 M sodium chloride solution (0.1 M PBS, pH=7.4) for 1 h to form stem-loop structure holding the ECL label in close proximity to the electrode surface and resulting in a high ECL signal [8, 10]. However, $Ru(bpy)_2(cbpy)NHS$ is positively charged, so it may nonspecifically bind to the negatively charged DNA. But, the rinsing process with 70% ethanol and 0.1 M PBS solution [25, 26] followed by incubation in a high ionic strength solution (1 M NaCl, 0.1 M PBS, pH=7.4) could leaving out the nonspecifically binding $Ru(bpy)_2(cbpy)NHS$, because the cations in the high ionic strength solution can compete with $Ru(bpy)_2(cbpy)NHS$ for binding to the anionic DNA backbone through the electrostatic attraction [27, 28]. In reference 28, the $Ru(NH_3)_6^{3+}$ bonding to the DNA phosphate sites through the electrostatic interaction was almost displaced by Na^+ and the pair of redox peaks of $Ru(NH_3)_6^{3+}$ in cyclic voltammogram almost disappeared when the sensor was immersed in 0.1 M sodium chloride solution. At this stage, the electrode was denoted as Au-oligo 1-$Ru(bpy)_2(cbpy)$-2. After washed, the modified electrode was used later as an ECL-DNA sensor.

ECL detection for target DNA

The ECL-DNA sensor was incubated with the complementary target DNA by being immersed in 500 μL of oligo 2 solution with different concentrations in 10 mM PBS (0.1 M NaCl, 5 mM $MgCl_2$, pH=7.4) for 40 min at 37°C (step c in Scheme 1). As a result, the probe DNA with stem-loop structure was converted into a rigid, linear double helix DNA and a distinct decrease of the ECL signal is observed presumably because the ECL label is separated from the electrode surface.

The ECL measurement for the determination of complementary target DNA was performed using cyclic voltammogram (CV) at a potential range of 0.0 V to 0.8 V at a scan rate of 100 $mV{\cdot}s^{-1}$ in 2.0 mL of 0.10 M PBS (pH=7.4) containing 0.10 M TPA with a conventional three-electrode system being used as the electrolytic system, which was composed of an ECL-DNA sensor as working electrode, a platinum wire as counter electrode and an Ag/AgCl (saturate KCl) electrode as reference electrode. A commercial cylindroid's glass cell was used as an ECL cell, and it was placed directly in front of the photomultiplier tube which was biased at 900 V.

Results and discussion

Characterization of the ECL-DNA sensor

The cyclic voltammogram (CV) and electrochemical impedance spectrum (EIS) of $Fe(CN)_6^{4-/3-}$ were used to monitor the modification processes of the electrode and the hybridization procedure [29]. Figure 1a showed CVs of $Fe(CN)_6^{4-/3-}$ at the Au electrode in different stages. It can be seen that when the bare gold electrode was modified with the oligo 1 (Au-oligo 1 electrode), the peak currents of $Fe(CN)_6^{4-/3-}$ decreased and the peak-to-peak potential separation (ΔE) increased (ΔE=81 mV) (curve b), compared with that of the bare gold electrode (ΔE=67 mV) (curve a) because of the electrostatic repulsion between the probe DNA with a negatively charged backbone and the anionic redox probe [29]. After oligo 1 modified Au electrode was

passivated in a 2 mM ME solution (10 mM PBS, pH=7.4), the peak currents of $Fe(CN)_6^{4-/3-}$ increased and the potential separation (ΔE) decreased to 76 mV (curve c). This is due to the decrease of negative charges on the surface of Au electrode since the nonspecifically adsorbed oligonucleotides are displaced by ME. With the probe DNA confined on the Au electrode surface being labeled with Ru$(bpy)_2$(cbpy)NHS (Au-oligo 1-Ru$(bpy)_2$(cbpy)-1 electrode) and forming a stem-loop structure (Au-oligo 1-Ru$(bpy)_2$(cbpy)-2 electrode), the peak currents of $Fe(CN)_6^{4-/3-}$ further increased and the potential separation (ΔE) also decreased to 64 mV (curve d) and 63 mV (curve e), rexspecrespectivelytively, which might be owing to the electrostatic attracting between the ECL elements with positive charges and the anionic redox probe. After the labeled probe DNA with stem-loop structure hybridized with the complementary target DNA, the peak currents of $Fe(CN)_6^{4-/3-}$ decreased and ΔE (ΔE=111 mV) increased further (curve f). The reason for this was that with the probe DNA hybridizing with the complementary target DNA, the negative charges on the surface of Au electrode increased again, which further blocked the electrochemical reaction of the anionic redox probe.

Electrochemical impedance spectroscopy is one of the most powerful and sensitive techniques for investigating the features of surface-modified electrodes. The impedance spectra consisted of a semicircle at high frequency and a line at low frequency. 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 [29]. Figure 1b showed the changes of the impedance spectra of the electrode in each step. The changes of the R_{ct} for the different steps were in agreement with the results of the CV. The results of CV and EIS both confirmed that the ECL probe DNA was immobilized on the gold electrode surface, the stem-loop structure of the probe DNA was formed, and the hybridization between the probe DNA and the complementary target DNA occurred.

The ECL was further used to monitor the modification processes of the electrode and the hybridization procedure. Figure 2 showed the ECL profiles of Au-oligo 1 electrode after passivation (curve a) before step a in Scheme 1, Au-oligo 1-Ru$(bpy)_2$(cbpy)-2 electrode (curve b) after step b and the Au-oligo 1-Ru$(bpy)_2$(cbpy)-2 electrode after hybridized with the complementary target DNA (curve c) after step c in Scheme 1 in 0.10 M PBS (pH=7.4) solution containing 0.10 M TPA. Au-oligo 1 electrode after passivation did not produce ECL, but after the probe DNA being labeled with Ru$(bpy)_2$(cbpy)NHS and forming a stem-loop structure, a high ECL signal was observed. With the probe DNA hybridizing with the complementary target DNA, the ECL obviously decreased. These results further indicated that the ECL elements were labeled to the

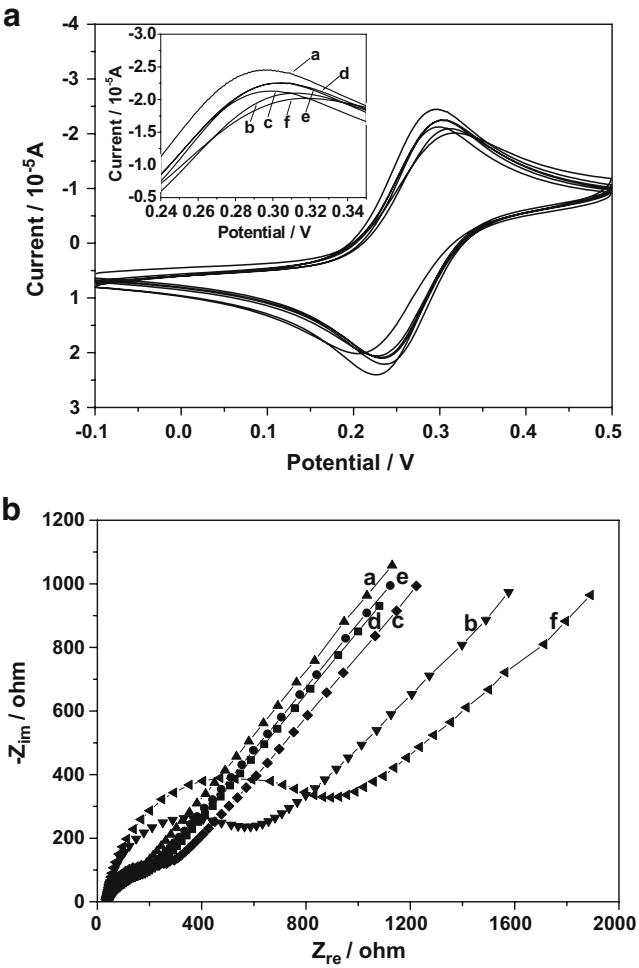


Fig. 1 **a** CVs and **b** EIS spectra of 2.5 mM $Fe(CN)_6^{4-/3-}$ in 0.10 M KCl on different electrodes. **a** bare gold electrode, **b** Au-oligo 1 electrode, **c** Au-oligo 1 electrode after passivation, **d** Au-oligo 1-Ru$(bpy)_2$(cbpy)-1 electrode, **e** Au-oligo 1-Ru$(bpy)_2$(cbpy)-2 electrode, **f** Au-oligo 1-Ru$(bpy)_2$(cbpy)-2 electrode after hybridization with 0.1 nM complementary target DNA. Inset: magnification of curves a-f of Fig. 1a in the range from 0.24 to 0.35 V. Bias potential, 0.260 V vs. Ag/AgCl, frequency range 1.0 Hz–100 kHz, alternate voltage 5 mV

oligo 1, the stem-loop structure was formed, and the hybridization between the probe DNA and the complementary target DNA occurred.

Optimization of analytical conditions

The applied potential to the ECL-DNA sensor designed is an important parameter because it decides the sensitivity and reproducibility of the ECL-DNA sensors. The dependence of the ECL intensity of ECL-DNA sensor on applied potential was checked. The results showed that ECL intensities of ECL-DNA sensor increased with increasing the high potential from +0.60 to +0.8 V in cyclic voltammogram and reached a maximum at about +0.8 V owing to more excited-state molecules being produced. But

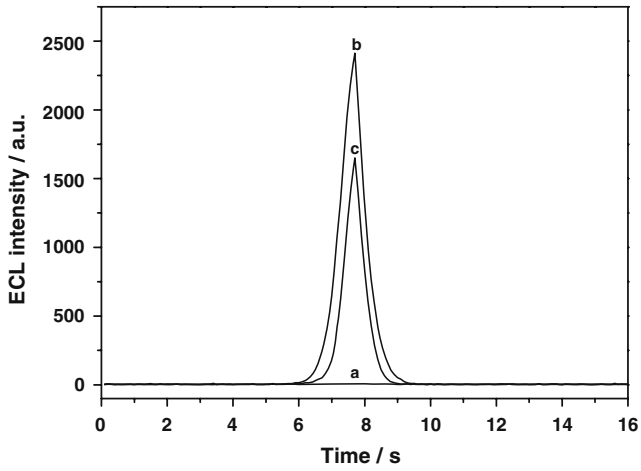


Fig. 2 ECL profiles of **a** Au-oligo 1 electrode after passivation, **b** Au-oligo 1-Ru(bpy)$_2$(cbpy)-2 electrode, **c** Au-oligo 1-Ru(bpy)$_2$(cbpy)-2 electrode after hybridized with complementary target DNA (1 pM) in 10 mM PBS (0.1 M NaCl, 5 mM MgCl, pH=7.4)

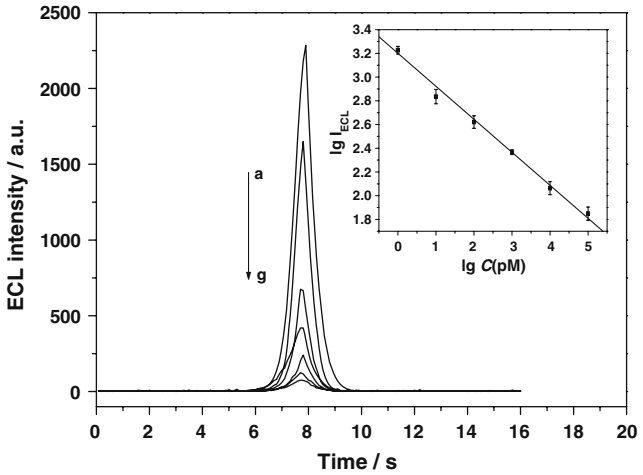


Fig. 3 ECL profiles for the ECL-DNA sensors after incubation in complementary target DNA solution with different concentrations. The concentration of complementary target DNA **a** 0 pM, **b** 1 pM, **c** 10 pM, **d** 100 pM, **e** 1 nM, **f** 10 nM, **g** 100 nM. Inset: The calibration curve for the detection of complementary target DNA concentration

the reproducibility of the ECL-DNA sensors was dwindled and the ECL intentsity decreased with the potential higher than +0.85 V, attributed to the oxidative desorption of thiol of the ECL probe on the gold electrode [30, 33]. Therefore, the potential range from 0 to +0.80 V was chosen in following experiments.

A different hybridization time of the probe DNA with the complementary target DNA might cause a visible difference in the change of ECL intensity. Therefore, the dependence of hybridization time, namely the time of immersing the ECL-DNA sensors in the target DNA solution, on the ECL intensity was investigated to obtain the optimum hybridization time of target DNA. When a series of the ECL-DNA sensors fabricated was incubated in 1.0 nM complementary target DNA solution at 37°C for different time, the ECL signals were recorded in 0.10 M PBS (pH=7.4) solution containing 0.10 M TPA. The results showed that the ECL intensity decreased with increasing the hybridization time from 10 to 40 min and then reached a plateau. This suggested that 40 min was enough for the system to reach equilibrium. Therefore, 40 min was chosen as hybridization time in following experiments.

ECL detection of target DNA

Figure 3 showed the ECL profiles of the ECL-DNA sensors after hybridization with different concentrations of target DNA under the optimized conditions. From Fig. 3, it can be seen that the ECL intensity decreased with increasing of the concentration of complementary target DNA. Under the optimal conditions, the logarithm of ECL intensity decreased linearly with the logarithm of target DNA concentration in the range from 1.0 pM to 0.1 μM. The linear regression equation was $\lg I_{ECL}=3.2-0.278\ \lg C$ (unit of C was pM) with a correlation coefficient of 0.9978 (Inset of Fig. 3). Similar logarithmic signal-versus-logarithm of target DNA concentration relationships have been reported for other solid-state sensors [31, 32]. Three repetitions of each target DNA 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 for the target DNA was 0.5 pM based on a signal-to-noise ratio of 3, indicating that the ECL-DNA sensor was highly sensitive and reproductive. The precision was estimated for seven successive measurements of 1.0 nM complementary target DNA that yielded reproducible ECL intensity with a relative standard deviation of 4.3 %.

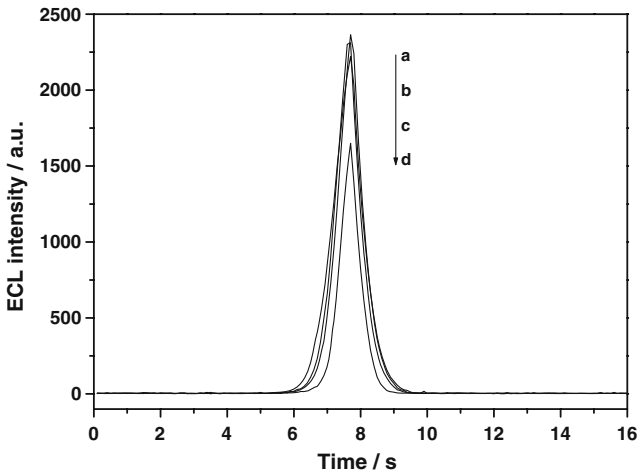


Fig. 4 ECL profiles for the ECL-DNA sensors before (**a**) and after incubation in (**b**) the blank, **c** the three-mismatch DNA and **d** the complementary target DNA solution

Selectivity and regeneration of the ECL-DNA sensor

To confirm the specific response of the ECL-DNA sensor to the complementary target DNA, control experiments were performed through using the blank, three-mismatch DNA and complementary target DNA, respectively, in which the blank was a hybridization buffer without complementary target DNA. The ECL-DNA sensors were incubated in the blank, 1 μM three-mismatch DNA and 1 pM complementary target DNA solutions in 10 mM PBS (0.1 M NaCl, 5 mM $MgCl_2$, pH=7.4) for 40 min at 37°C, respectively. From Fig. 4, it was clearly that negligible ECL signal changes were observed for the ECL-DNA sensor incubated in target DNA-free hybridization buffer (curve b) or in the presence of the three-base mismatch DNA (curve c) at the concentration of 1 μM compared with that before incubation (curve a). But the ECL intensity decreased clearly after the ECL-DNA sensor incubated in the complementary target DNA solution (curve d). This indicated that the selectivity of the sensor between the complementary target DNA and the three-base mismatch DNA is in excess of 10^6, proving that the developed ECL-DNA sensors exhibit high selectivity and high sensitivity.

In addition, the reusability of the ECL-DNA sensor was investigated. After hybridized with complementary target DNA (0.1 nM), the modified Au electrode was regenerated by immersing in double distilled water at 90°C for 10 min, and then incubating in 1 M sodium chloride solution (10 mM PBS, pH=7.4) for 1 h after washed with 0.1 M cold PBS (pH=7.4), and 80% of the original signal was recovered. The minor signal loss during recovery might arise from the loss of the probe molecules during the heating process in high temperature [34]. This problem may be resolved by improving the bonding strength of the probe DNA with the electrode or changing the regeneration method, which were being investigated in our lab.

Conclusions

An ECL-DNA sensor for the detection of DNA hybridization using stem–loop structure DNA as capture probes was investigated. The modification processes of the electrode and the hybridization procedure were characterized by CV, EIS and ECL. Different from other approaches for preparing DNA sensors, the approach proposed here left out the complex purifying processes after the probe DNA was labeled. The ECL-DNA sensor described here was impressive sensitive, selective and reagentless, and may provide an alternative approach for the ECL detection of DNA in the fields of diagnostics and gene analysis.

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References

1. Sassolas A, Leca-Bouvier BD, Blum LJ (2008) DNA biosensors and microarrays. Chem Rev 108:109–139
2. Zhou X, Zhou J (2004) Improving the signal sensitivity and photostability of DNA hybridizations on microarrays by using dye-doped core-shell silica nanoparticles. Anal Chem 76:5302–5312
3. Liu XJ, Tan WH (1999) A fiber-optic evanescent wave DNA biosensor based on novel molecular beacon. Anal Chem 71:5054–5059
4. Mallard F, Marchand G, Ginot F, Campagnolo R (2005) Optoelectronic DNA chip: high performance chip reading with an all-electric interface. Biosens Bioelectron 20:1813–1820
5. Yao X, Li X, Toledo F, Zurita-Lopez C, Gutova M, Momand J, Zhou FM (2006) Sub-attomole oligonucleotide and p53 cDNA determinations via a high-resolution surface plasmon resonance combined with oligonucleotide-capped gold nanoparticle signal amplification. Anal Biochem 354:220–228
6. Xie H, Zhang CY, Gao ZQ (2004) Amperometric detection of nucleic acid at femtomolar levels with a nucleic acid/electrochemical activator bilayer on gold electrode. Anal Chem 76:1611–1617
7. Wang J, Li JH, Baca AJ, Hu JB, Zhou FM, Yan W, Pang DW (2003) Amplified voltammetric detection of DNA hybridization via oxidation of ferrocene caps on gold nanoparticle/streptavidin conjugates. Anal Chem 75:3941–3945
8. Fan CH, Plaxco KW, Heeger AJ (2003) Electrochemical interrogation of conformational changes as a reagentless method for the sequence-specific detection of DNA. Proc Natl Acad Sci USA 100:9134–9137
9. Lai RY, Lagally ET, Lee SH, Soh HT, Plaxco KW, Heeger AJ (2006) Rapid, sequence-specific detection of unpurified PCR amplicons via a reusable, electrochemical sensor. Proc Natl Acad Sci USA 203:4017–4021
10. Liu G, Wan Y, Gau V, Zhang J, Wang LH, Song SP, Fan CH (2008) An enzyme-based E-DNA sensor for sequence-specific detection of femtomolar DNA targets. J Am Chem Soc 130:6820–6825
11. Jin Y, Yao X, Liu Q, Li J (2007) Hairpin DNA probe based electrochemical biosensor using methylene blue as hybridization indicator. Biosens Bioelectron 22:1126–1130
12. Peng H, Soeller C, Vigar NA, Caprio V, Travas-Sejdic J (2007) Label-free detection of DNA hybridization based on a novel functionalized conducting polymer. Biosens Bioelectron 22:1868–1873
13. Ricci F, Plaxco KW (2008) E-DNA sensors for convenient, label-free electrochemical detection of hybridization. Microchim Acta 163:149–155
14. Yang ML, Liu CZ, Qian KJ, He PG, Fang YZ (2002) Study on the electrochemiluminescence behavior of ABEI and its application in DNA hybridization analysis. Analyst 127:1267–1271
15. Miao WJ, Bard AJ (2004) Electrogenerated chemiluminescence. 77. DNA hybridization detection at high amplification with $[Ru(bpy)_3]^{2+}$-containing microspheres. Anal Chem 76:5379–5386
16. Miao WJ, Bard AJ (2003) Electrogenerated chemiluminescence. 72. Determination of immobilized DNA and c-reactive protein on Au(111) electrodes using tris(2, 2′-bipyridyl) ruthenium(II) labels. Anal Chem 75:5825–5834

17. Wang H, Zhang CX, Li Y, Qi HL (2006) Electrogenerated chemiluminescence detection for deoxyribonucleic acid hybridization based on gold nanoparticles carrying multiple probes. Anal Chim Acta 575:205–211
18. Li Y, Qi HL, Fang F, Zhang CX (2007) Ultrasensitive electrogenerated chemiluminescence detection of DNA hybridization using carbon-nanotubes loaded with tris(2, 2′-bipyridyl) ruthenium derivative tags. Talanta 72:1704–1709
19. Zhang J, Qi HL, Li Y, Yang J, Gao Q, Zhang CX (2008) Electrogenerated chemiluminescence DNA biosensor based on hairpin DNA probe labeled with ruthemium complex. Anal Chem 80:2888–2894
20. Li Y, Qi HL, Yang J, Zhang CX (2009) Detection of DNA immobilized on bare gold electrodes and gold nanoparticle-modified electrodes via electrogenerated chemiluminescence using a ruthenium complex as a tag. Microchim Acta 164:69–76
21. Hook F, Ray A, Norden B, Kasemo B (2001) Characterization of PNA and DNA immobilization and subsequent hybridization with DNA using acoustic-shear-wave attenuation measurements. Langmuir 17:8305–8312
22. Su XD, Robelek R, Wu YJ, Wang GY, Knoll W (2004) Detection of point mutation and insertion mutations in DNA using QCM and MutS, a mutation binding protein. Anal Chem 76:489–494
23. Tyagi S, Kramer FR (1996) Molecular beacons: probes that fluorescence upon hybridization. Nat Biotechnol 14:303–308
24. Tyagi S, Kramer FR (1999) hermodynamic basis of the enhanced specificity of molecular beacons. Proc Natl Acad Sci USA 96:6171–6176
25. Kang Y, Feng KJ, Chen JW, Jiang JH, Shen GL, Yu RQ (2008) Electrochemical detection of thrombin by sandwich approach using antibody and aptamer. Bioelectrochemistry 73:76–81
26. URL: http://www.probes.com/media/pis/mp00143.pdf.
27. Steel AB, Herne TM, Tarlov MJ (1998) Electrochemical quantitation of DNA immobilized on gold. Anal Chem 70:4670–4677
28. Shen L, Chen Z, Li YH, Jing P, Xie SB, He SL, He PL, Shao YH (2007) A chronocoulometric aptamer sensor for adenosine monophosphate. Chem Comm 21:2169–2171
29. Bard AJ, Faulkner LR (2001) Electrochemical methods: Fundamentals and applications, 2nd edn. Wiley, New York
30. Bertolino C, MacSweeney M, Tobin J, O'Neill B, Sheehanb MM, Coluccia S, Berney H (2005) A monolithic silicon based integrated signal generation and detection system for monitoring DNA hybridization. Biosens Bioelectron 21:565–573
31. Wang HY, Zhang XL, Tan ZA, Yao W, Wang L (2008) Enhanced electrogenerated chemiluminescence of $Ru(bpy)_3^{2+}$/TPrA system on CdS nanocrystals film. Electrochem Commun 10:170–174
32. Zhang LH, Dong SJ (2006) Electrogenerated chemiluminescence sensors using $Ru(bpy)_3^{2+}$doped in silica nanoparticles. Anal Chem 78:5119–5123
33. Zu YB, Bard AJ (2001) Electrogenerated chemiluminescence. 67. Dependence of light emission of the tris(2, 2′-bipyridyl) ruthenium(II)/tripropylamine system on electrode surface hydrophobicity. Anal Chem 73:3960–3964
34. Du H, Strobhsahl CM, Camera J, Miller BL, Krauss TD (2005) Sensitivity and specificity of metal surface-immobilized 'molecular beacon' biosensors. J Am Chem Soc 127:7932–7940