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Ultrasensitive and Signal-on Electrochemiluminescence Aptasensor Using the Multi-tris(bipyridine)ruthenium(II)-β-cyclodextrin Complexes

Yingying Zhao, Hong Chen, Qiong Chen, Yantao Qi, Fan Yang, Jie Tang, Pingang He, and Fan Zhang*

Department of Chemistry, East China Normal University, Shanghai 200241, China

An ultrasensitive and signal-on electrochemiluminescence (ECL) aptasensor to detect target protein (thrombin or lysozyme) was developed using the host-guest recognition between a metallocyclodextrin complex and single-stranded DNA (ss-DNA). The aptasensor uses both the photoactive properties of the metallocyclodextrins named multi-tris(bipyridine)ruthenium(II)-β-cyclodextrin complexes and their specific recognition with ss-DNA, which amplified the ECL signal without luminophore labeling. After investigating the ECL performance of different multi-tris(bipyridine)ruthenium(II)-β-cyclodextrin (multi-Ru-β-CD) complexes, tris-tris(bipyridine)-ruthenium(II)-β-cyclodextrin (tris(bpyRu)-β-CD) was selected as a suitable host molecule to construct an atasensor. First, double-stranded DNA (ds-DNA) formed by hybridization of the aptamer and its target DNA was attached to a glassy carbon electrode via coupling interaction, which showed low ECL intensity with 2-(dibutylamino) ethanol (DBAE) as coreactant, because of the weak recognition between ds-DNA and tris(bpyRu)-β-CD. Upon addition of the corresponding protein, the ECL intensity increased when target ss-DNA was released because of the higher stability of the aptamer-protein complex than the aptamer-DNA one. A linear relationship was observed in the range of 0.01 pmol/L to 100 pmol/L between ECL intensity and the logarithm of thrombin concentrations with a limited detection of 8.5 fmol/L (S/N＝3). Meanwhile, the measured concentration of lysozyme was from 0.05 pmol/L to 500 pmol/L and the detection limit was 33 fmol/L (S/N＝3). The investigations of proteins in human serum samples were also performed to demonstrate the validity of detection in real clinical samples. The simplicity, high sensitivity and specificity of this aptasensor show great promise for practical applications in protein monitoring and disease diagnosis.

Keywords aptasensor, host-guest recognition, electrochemiluminescence, metallocyclodextrin

Introduction

Electrochemiluminescence (ECL) results from the electron transfer reaction between electrochemically generated ion radicals on the surface of an electrode causing the emission of light.[1] As an efficacious combination of chemiluminescence and electrochemistry (EC), ECL possesses advantages including high sensitivity, low background, good specificity and controllability. Therefore, ECL has been widely used in biochemical applications such as immunoassays[2-4] and DNA detection.[5-7] Among diverse ECL systems, tris(bipyridine)ruthenium(II) ($Ru(bpy)_3^{2+}$) and its derivatives are the most common ECL reagents used to detect biomolecules.[8-11] Recently, a number of methods using nanoparticles,[12-14] films,[5,15] polymers,[16-18] surfactant addition[19] and chemical-labeled biomolecules[20] for signal amplification have been proposed to improve the sensitivity of $Ru(bpy)_3^{2+}$-based ECL biosensors. In particular, introducing a signal molecule to raise the efficiency of ECL is an important step in the construction of biosensors. Therefore, alternative signaling agents with a high ECL emission efficiency like multi-luminophore materials[21-23] are required.

Aptamers possess a reputation for high affinity and specificity in biotechnology, medical diagnostics and therapeutics.[24] In aptasensors, aptamers have commonly served as the capture molecules to detect proteins.[25,26] Among different methods of fluorescence,[27] EC,[28] ECL[29] and colorimetry,[30,31] ECL has attracted substantial attention in the development of aptasensors because it is highly sensitive and inexpensive, and uses simple instruments. Traditionally, ECL aptasensors have used diverse luminophore-labeled aptamers,[32-34] which are difficult to obtain. Therefore, it is necessary to develop a simple and efficient method to fabricate

* E-mail: fzhang@chem.ecnu.edu.cn; Tel/Fax: 0086-021-54340049
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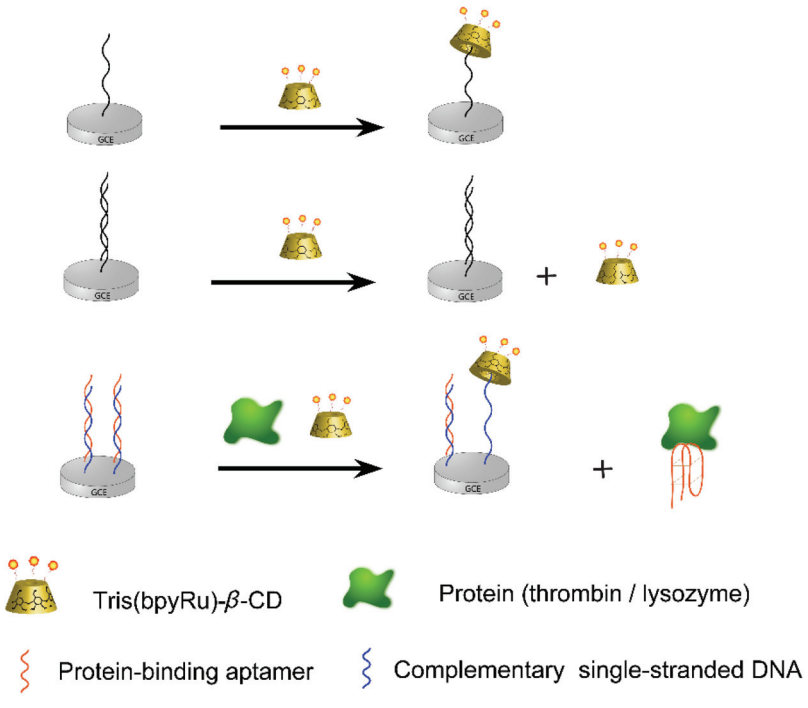
aptasensors.

Polynuclear metallocyclodextrins are effective signal molecules with excellent electronic and photoactive characteristics, so they have aroused growing interest for application in molecular sensing devices.[35,36] To date, metallocyclodextrins have generally been used in metal ion sensors,[37,38] small molecule detectors[39] and electron transport wires.[40] However, most of these reports are based on fluorescent phenomena related to the recognition between the cyclodextrin cavity and various guests.[41] Apart from the previous work, the ECL properties of polynuclear metallocyclodextrins as a single complex have seldom been studied. We synthesized a series of multi-tris(bipyridine)ruthenium(II)-*β*-cyclodextrin (multi-Ru-*β*-CD) complexes including tris-tris(bipyridine)ruthenium(II)-*β*-cyclodextrin (tris-(bpyRu)-*β*-CD) (Figure S1).[42-44] Exploiting the luminescent properties and supramolecular structure of a film of tris(bpyRu)-*β*-CD, we developed a sensitive ECL sensor for DNA using a 4-dimethylaminoazoben-4'-caroxylic acid-labeled hairpin DNA.[45] Furthermore, a label-free ECL aptasensor was designed to detect thrombin by use of tris(bpyRu)-*β*-CD with the emergence of single-stranded DNA (ss-DNA) as guests of cyclodextrins.[46]

The label-free ECL aptasensor exhibited excellent operability, sensitivity and stability. However, the above sensor was dependent on the decrease of ECL intensity involved in the combination between thrombin and its aptamer in the ECL determination process. This is similar with most of the "signal-off" ECL sensors, which are limited in their signal gain as the target protein can suppress only 100% of the original ECL intensity. On the contrary, a new type of "signal-on" ECL aptasensors has been reported, in which ECL intensity is increased by the formation of aptamer-protein complex.[47,48] Herein, a signal-on ECL aptasensor is developed to determine target protein with improved sensitivity and specificity. Both the photoactivities of the multi-tris(bipyridine)ruthenium(II)-*β*-cyclodextrin complexes and their specific molecular recognition with ss-DNA were investigated. Because of the outstanding ECL performance of tris(bpyRu)-*β*-CD, it was then used as a typical signal probe to construct an aptasensor. As illustrated in Scheme 1a, ss-DNA was attached to a glassy carbon electrode (GCE) via coupling interaction and showed a strong ECL intensity after recognition with tris(bpyRu)-*β*-CD in aqueous solution containing 2-(dibutylamino) ethanol (DBAE), which was employed as a co-reactant in the ECL system of tris(bpyRu)-*β*-CD. Comparing to the ss-DNA, ds-DNA led to a low ECL intensity in the same experimental conditions (Scheme 1b). Therefore, on the basis of above facts, a signal-on ECL aptasensor was designed for thrombin or lysozyme detection. Upon addition of thrombin or lysozyme (Scheme 1c), the ECL intensity increased when ss-DNA was released by virtue of the higher stability of aptamer-thrombin or aptamer-lysozyme.[49-51] Rather than the traditional chemical labeling of the probe DNA, the signal-on aptasensor was carefully constructed by use of the specific recognition between ss-DNA and the host complex. And the mechanism is established on the direct proportion between the enhancement of ECL intensity and the concentration of target proteins. As a consequence, this signal-on ECL aptasensor exhibits higher sensitivity and wider detection range, along with good specificity and simplicity, showing promise for protein monitoring and disease diagnosis.

Scheme 1 Schematic of an aptasensor for the detection of thrombin or lysozyme



Experimental

Apparatus

Electrochemical measurements were performed on an electrochemical workstation (AUTOLAB PGSTAT302N, Metrohm, The Netherlands), composed of GCE working electrode, platinum wire counter electrode and Ag/AgCl reference electrode. ECL measurements were obtained on an ECL analyzer (LK5100, Tianjin Lanlike High-Tech Company, Tianjin, China).

Chemicals and materials

N-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), 2-(dibutylamino) ethanol (DBAE), anthranilic acid (ABA), Tween-20 and thrombin (from human plasma) were obtained from Sigma-Aldrich (Shanghai, China). Tris-(hydroxymethyl)aminomethane (Tris) was obtained from Sangon Biotech Co., Ltd (Shanghai, China). Lysozyme, bovine serum albumin (BSA) and human IgG were purchased from Dingguo Biotechnology Inc (Beijing, China). Mono(bpyRu)-*β*-CD and tris(bpyRu)-*β*-CD (Figure S1) were synthesized as we reported previously.[42,43] The following oligonucleotides were prepared by Sangon Biotech Co., Ltd (Shanghai, China): thrombin-binding aptamer (TBA): 5'-GGTTGGTGTGGTTGG-3'; complementary ss-DNA (ss-DNA_1): 5'-$(CH_2)_6$-NH_2-CCAACCACACCAACC-3'; lysozyme-binding aptamer (LBA): 5'-ATCAGGGCTAAAGAGTGCAGAGTTACTTAG-3'; complementary ss-DNA (ss-DNA_2): 5'-$(CH_2)_6$-NH_2-CTAATAACTCTGCACTCTTTAGCCCTGAT-3'. Thrombin and lysozyme were prepared in binding buffer (0.56 mmol/L Tris-HCl, 4.7 mmol/L NaCl, 0.14 mmol/L KCl, pH 6.5). All oligonucleotides were prepared in TE buffer (0.01 mol/L Tris-HCl, 0.1 mol/L NaCl, pH 7.4). Human serum samples provided by Shanghai Electric Power Hospital were stored at 4 ℃. All of the solution was prepared with ultrapure water from a Millipore Milli-Q water purification system.

The immobilization of DNA probes on the electrodes

Prior to DNA probes immobilization, the GCE was polished sequentially with 1.0-, 0.3- and 0.05-μm alumina slurries, cleaned ultrasonically in ethanol and ultrapure water and then dried under nitrogen gas. ABA was electropolymerized on the polished GCE by immersing the electrode in a solution of 0.05 mol/L ABA/1 mol/L H_2SO_4 as reported (0–1 V, 8 cycles).[52] After rinsing thoroughly with ultrapure water, the poly(ABA)-coated electrode was covered with 4 mmol/L EDC/1 mmol/L NHS solution (1.5 μL) for 15 min. Then, ss-DNA solution at various concentrations (1.5 μL) was added to the activated solution for 3 h. Finally, the ss-DNA modified with amino-group was immobilized on the electrode via a coupling reaction, and the electrode was rinsed thoroughly with washing buffer before protein detection.

For the immobilization of double-stranded DNA (ds-DNA) on the electrode, ss-DNA_1 or ss-DNA_2 (1.0 nmol/L) was hybridized with TBA or LBA (1.0 nmol/L) in TE buffer and incubated for 1 h at 37 ℃ to form the corresponding ds-DNA_1 (ss-DNA_1/TBA) or ds-DNA_2 (ss-DNA_2/LBA), respectively. The ds-DNA mixture was heated at 65 ℃ for 5 min and then slowly cooled to room temperature. Finally, ds-DNA (1.5 μL, 0.5 nmol/L) formed by hybridization of the aptamer and its target DNA was attached to the electrodes in the same way of the ss-DNA probes.

ECL measurements for proteins

To detect thrombin or lysozyme, the electrodes immobilized with ds-DNAs were incubated in thrombin or lysozyme solution at various concentrations for 1 h. After rinsing in washing buffer (25 mmol/L Tris-HCl, 100 mmol/L NaCl, 5 mmol/L $MgCl_2$, 1.0% (*V*/*V*) Tween-20, pH 7.4) to remove nonspecifically bound species, the electrode was immersed in 10 μmol/L tris(bpyRu)-*β*-CD solution for 1.5 h at room temperature. Following washing thoroughly, the electrode with tris(bpyRu)-*β*-CD captured by ss-DNA was placed in 0.1 mol/L PBS buffer (pH 7.5) containing 20 mmol/L DBAE. The ECL behavior was monitored over a scan range of 0.4—1.25 V versus Ag/AgCl at a scan rate of 100 mV/s.

Results and Discussion

ECL properties of the multi-Ru-*β*-CD complexes

As previously reported,[53] $Ru(bpy)_3^{2+}$ shows intense ECL signal with DBAE in an eco-friendly manner. In this work, DBAE was employed as a co-reactant, which was applied recently in many $Ru(bpy)_3^{2+}$ ECL detecting systems.[6] Because the same photoactive component was present in our multi-Ru-*β*-CD complexes, they also exhibited high ECL intensities. As shown in Figure S2, the measured intensity of tris(bpyRu)-*β*-CD (curve a) is approximately three times that of $Ru(bpy)_3Cl_2$ (curve c). As anticipated, the multiple luminophores in tris(bpyRu)-*β*-CD enhanced its ECL performance. Compared with mono(bpyRu)-*β*-CD (curve b), tris(bpyRu)-*β*-CD gave more intense ECL at the same concentration, which could improve the sensitivity of the corresponding ECL biosensor. The ECL intensity of mono(bpyRu)-*β*-CD was not as high as that of $[Ru(bpy)_3]^{2+}$ in DBAE, which was attributed to the influence of the ligand cyclodextrin.

The recognition of DNA probes to the multi-Ru-*β*-CD complexes

Recently, the host-guest recognition between cyclodextrin and ss-DNA has been reported, since the bases, the key components of ss-DNA, can be encapsulated into the cyclodextrin cavity freely.[54-56] Based on the unique property, we hoped that the multi-Ru-*β*-CD complexes would be specifically captured by ss-DNA on the electrode, which was characterized by ECL

measurements. In our approach, ss-DNA_1 (0.5 nmol/L) or corresponding ds-DNA_1 (0.5 nmol/L) was immobilized on a poly(ABA)-modified GCE via a coupling reaction in the presence of EDC and NHS. Following immersion in the multi-Ru-*β*-CD complexes and $Ru(bpy)_3Cl_2$, multi-Ru-*β*-CD/ss-DNA_1/GCE and $Ru(bpy)_3Cl_2$/ss-DNA_1/GCE or multi-Ru-*β*-CD/ds-DNA_1/GCE and $Ru(bpy)_3Cl_2$/ds-DNA_1/GCE were procured after sufficient washing.

As indicated in Figure 1, the multi-Ru-*β*-CD complexes exhibited more excellent ECL performances than $Ru(bpy)_3Cl_2$. Compared with $Ru(bpy)_3Cl_2$, the multi-Ru-*β*-CD complexes showed much greater ECL disparity toward ss- and ds-DNA (Figure 1D), which indicated that the cyclodextrin component played an important role in discriminating between ss- and ds-DNA. Furthermore, the disparity in ECL intensity increased with the increasing number of ruthenium cores in the multi-Ru-*β*-CD complexes, so tris(bpyRu)-*β*-CD exhibited the maximum discrimination between ss- and ds-DNA among these complexes. As a result, tris(bpyRu)-*β*-CD was used to construct aptasensors because of its strong ability to recognize ss-DNA and excellent sensitivity.

In addition, compared with bare electrodes (curves a in Figure 1A, 1B and 1C) and multi-Ru-*β*-CD/ds-DNA_1/GCE (curves c in Figure 1A, 1B and 1C), a low intensity increment can be seen, which might be due to the electrostatic interaction between the phosphate backbone of ds-DNA and the $Ru(bpy)_3^{2+}$ cores of tris-(bpyRu)-*β*-CD.

Effects of the concentrations of DNA probes on the ECL responses of tris(bpyRu)-*β*-CD

We found that the ECL signal of tris(bpyRu)-*β*-CD corresponded to the concentrations of ss- or ds-DNA on the electrodes. As seen in Figure 2A, the intensity of ECL from tris(bpyRu)-*β*-CD/ss-DNA_1/GCE (curve a) was obviously higher than that from tris(bpyRu)-*β*-CD/ds-DNA_1/GCE (curve b). The stronger recognition between tris(bpyRu)-*β*-CD and ss-DNA than that with ds-DNA was reflected by the considerable enhancement (about 400%) of ECL intensity under the same experimental conditions. Moreover, the DNA concentration of 0.5 nmol/L led to the largest absolute difference in ECL intensity between tris(bpyRu)-*β*-CD/ss-DNA_1 and tris(bpyRu)-*β*-CD/ds-DNA_1 in the concentration range of 0.5 μmol/L to 0.5 pmol/L. Therefore, 0.5 nmol/L DNA probes were selected to fabricate a novel aptasensor for protein (thrombin or lysozyme) detection (Scheme 1).

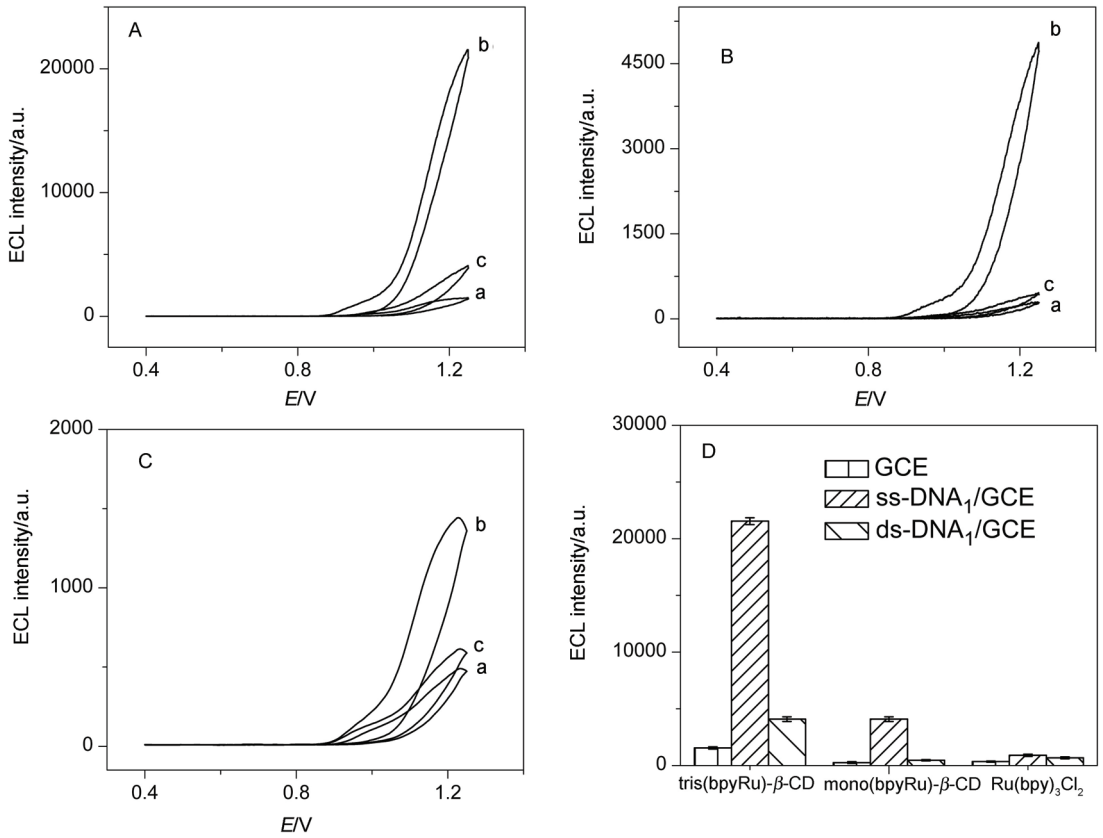


Figure 1 ECL spectra of (A) tris(bpyRu)-*β*-CD, (B) mono(bpyRu)-*β*-CD and (C) $Ru(bpy)_3Cl_2$ on different electrodes: (a) bare GCE, (b) ss-DNA_1/GCE, (c) ds-DNA_1/GCE; (D) bar chart of the peak intensities of tris(bpyRu)-*β*-CD, mono(bpyRu)-*β*-CD and $Ru(bpy)_3Cl_2$ on different electrodes. ECL behaviors were performed in 0.1 mol/L PBS containing 20 mmol/L DBAE at a scan rate of 100 mV/s ranging from 0.4 V to 1.25 V (vs. Ag/AgCl). The error bars shown were derived from the standard deviation of four replicate experiments.

Characterization of the ECL aptasensor

Through the specific recognition of ss-DNA_1 (Figure 2B, curve b) and ss-DNA_2 (Figure 2C, curve e) by tris(bpyRu)-β-CD on GCE, the highest ECL intensities have been obtained. In contrast, ds-DNA_1 (Figure 2B, curve a) and ds-DNA_2 (Figure 2C, curve d) were only weakly recognized by tris(bpyRu)-β-CD, and thus little tris(bpyRu)-β-CD attached to the surface of the GCE, which led to low ECL intensity. In the presence of the target protein, the ds-DNA (aptamer/ss-DNA) was converted into ss-DNA because the stability of the aptamer-protein complex was higher than that of the ds-DNA. Therefore, addition of tris(bpyRu)-β-CD induced a high intensity of ECL through the specific recognition between tris(bpyRu)-β-CD and ss-DNA. Correspondingly, tris(bpyRu)-β-CD/thrombin/ds-DNA_1/GCE (Figure 2B, curve c) and tris(bpyRu)-β-CD/lysozyme/ds-DNA_2/GCE (Figure 2C, curve f) exhibited much stronger ECL signals than tris(bpyRu)-β-CD/ds-DNA_1/GCE (Figure 2B, curve a) and tris(bpyRu)-β-CD/ds-DNA_2/GCE (Figure 2C, curve d), respectively. Consequently, the developed ECL-based aptasensor can be used for protein detection.

ECL detection of thrombin or lysozyme

In our aptasensor, each protein molecule can switch an aptamer from an aptamer/complementary DNA duplex to an aptamer/target protein complex with the release of ss-DNA, which served as an excellent guest. In addition, our aptasensor is expected to offer high sensitivity because tris(bpyRu)-β-CD contains multiluminophore with the spontaneous recognition property between ss-DNA and the host cavity of tris(bpyRu)-β-CD. The sensitivity of the aptasensor was investigated by ECL measurements, which were performed in 0.1 mol/L PBS buffer containing 20 mmol/L DBAE over the potential range from 0.4 V to 1.25 V.

Figure 3 illustrates the ECL responses of the detection system to thrombin or lysozyme at different concentrations, respectively. As shown in Figure 3A and 3C, the addition of thrombin or lysozyme at different concentrations, respectively, to the aptasensor induced different increase of ECL intensity. In addition, a relatively small background ECL response is obtained in the absence of the target protein. This phenomenon was attributed to the electrostatic interaction between the phosphate backbone of ds-DNA and the $Ru(bpy)_3^{2+}$

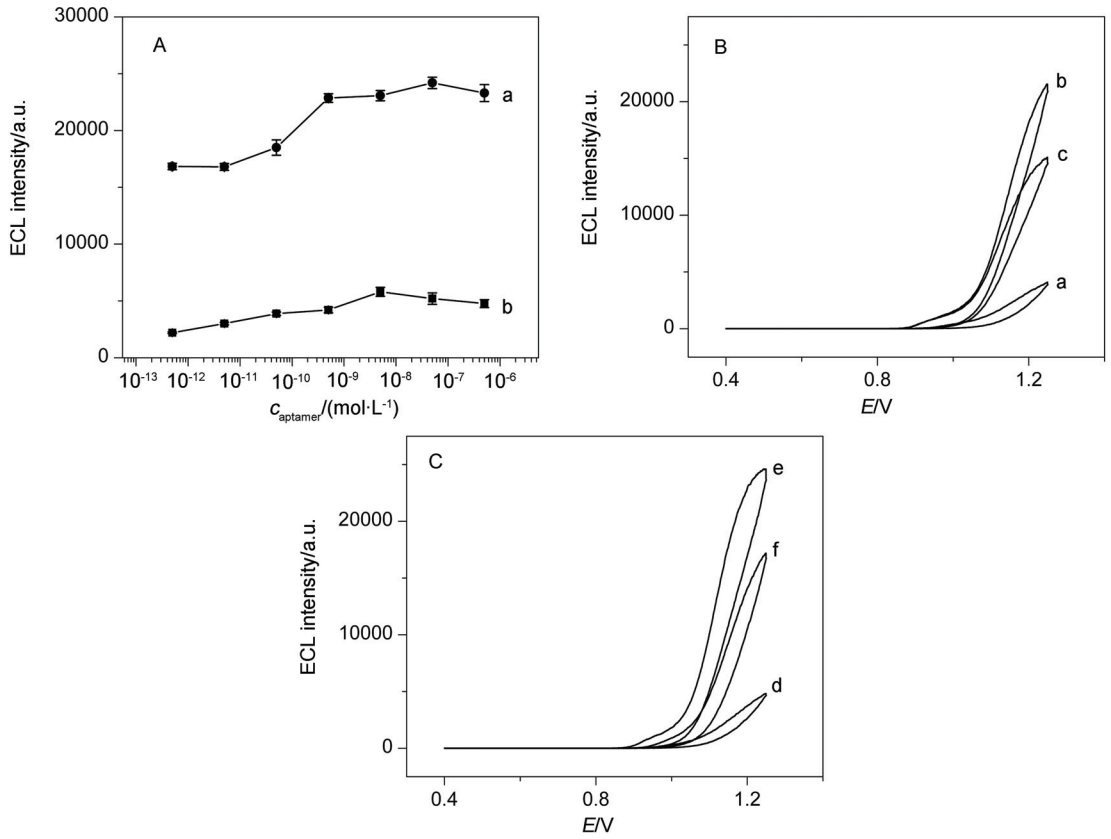


Figure 2 (A) ECL signal responses of (a) tris(bpyRu)-β-CD/ss-DNA_1/GCE, and (b) tris(bpyRu)-β-CD/ds-DNA_1/GCE to ss-DNA_1 or ds-DNA_1 at different concentrations; (B) ECL behaviors of (a) tris(bpyRu)-β-CD/ds-DNA_1/GCE, (b) tris(bpyRu)-β-CD/ss-DNA_1/GCE, and (c) tris(bpyRu)-β-CD/thrombin (10 pmol/L)/ds-DNA_1/GCE in 0.1 mol/L PBS containing 20 mmol/L DBAE; (C) ECL behaviors of (d) tris(bpyRu)-β-CD/ds-DNA_2/GCE, (e) tris(bpyRu)-β-CD/ss-DNA_2/GCE, and (f) tris(bpyRu)-β-CD/lysozyme (50 pmol/L)/ds-DNA_2/GCE in 0.1 mol/L PBS containing 20 mmol/L DBAE at a scan rate of 100 mV/s (vs. Ag/AgCl). The error bars shown were derived from the standard deviation of four replicate experiments.

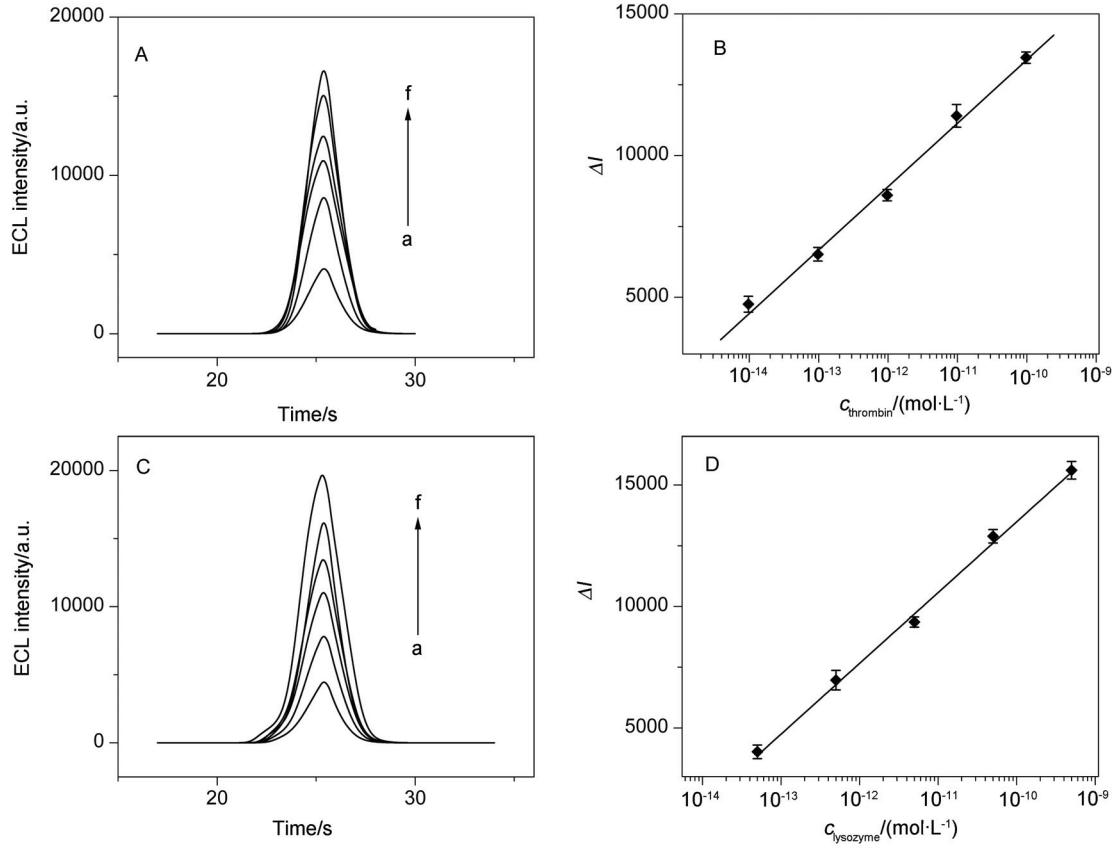


Figure 3 (A) ECL responses of the aptasensor to thrombin at different concentrations: (a) 0 mol/L, (b) 0.01 pmol/L, (c) 0.1 pmol/L, (d) 1 pmol/L, (e) 10 pmol/L, and (f) 100 pmol/L. (B) The resulting calibration curve showing the absolute difference of ECL intensity as a function of the logarithm of thrombin concentration. (C) ECL responses of the aptasensor to lysozyme at different concentrations: (a) 0 mol/L, (b) 0.05 pmol/L, (c) 0.5 pmol/L, (d) 5 pmol/L, (e) 50 pmol/L, and (f) 500 pmol/L. (D) The resulting calibration curve depicting the absolute difference of ECL intensity as a function of the logarithm of lysozyme concentration ($\Delta I_{ECL}=I-I_0$, where I_0 and I are the ECL intensity of the aptasensor in the absence and presence of target protein, respectively). The error bars shown were derived from the standard deviation of four replicate experiments.

cores of tris(bpyRu)-β-CD as discussed above. Figure 3B shows that the absolute difference of the ECL intensity of the aptasensor exhibited a linear correlation with the logarithm of thrombin concentrations ranging from 0.01 pmol/L to 100 pmol/L, with a linear coefficient of 0.9973 and a detection limit of 8.5 fmol/L (S/N=3). Meanwhile, as shown in Figure 3D, the linear range of the lysozyme concentrations was from 0.05 pmol/L to 500 pmol/L (R=0.9935), and the detection limit was 33 fmol/L (S/N=3).

Furthermore, the stability of the ECL aptasensor was also investigated. As illustrated in Figure S3, after the storage in 0.1 mol/L PBS (pH 7.5) at 4 ℃ for one week, the ECL aptasensor showed a satisfying stability, which retained about 91.7% and 87.7% of its initial response for thrombin and lysozyme determination, respectively. The results demonstrated that the developed aptasensor possesses good potential stability at least during one week.

Specificity of the ECL aptasensor

We evaluated the specificity of the aptasensor for thrombin or lysozyme against other common typical interfering protein molecules, as displayed in Figure S4. Clearly, lysozyme, BSA and IgG (1 nmol/L) did not induce large changes in the ECL intensity of the aptasensor compared with that of 10 pmol/L thrombin (Figure S4A). Meanwhile, thrombin, BSA and IgG (5 nmol/L) did not cause any apparent increase in ECL intensity, whereas 50 pmol/L lysozyme led to a much stronger response (Figure S4B). These results demonstrate that the developed ECL aptasensor exhibits sufficient specificity for thrombin or lysozyme detection in the presence of the interfering proteins.

Application to the real sample of the ECL aptasensor

To further study the feasibility of the proposed ECL aptasensor for practical applications, the determination of thrombin[57] or lysozyme[58-60] in human real serum samples were carried out. As expected, the addition of thrombin[61] or lysozyme[62] did not affect the samples. Thus, the serum sample was first diluted with binding buffer and spiked with protein (thrombin or lysozyme) at different concentrations. Then, 5 μL of the serum sample spiked with thrombin or lysozyme was dropped on the electrode immobilized with ds-DNA$_1$ or ds-DNA$_2$,

Table 1 Analytical results of proteins detected in human serum sample and spiked samples

a Recovery results of thrombin at different concentrations spiked into human serum sample

Sample	Spiked/(mol•L^{-1})	Found/(mol•L^{-1})	Recovery/%	RSD/% n=3
Serum	1.0×10^{-12}	9.1×10^{-13}	91.3	6.6
	1.0×10^{-11}	9.1×10^{-12}	90.5	8.3
	5.0×10^{-11}	5.1×10^{-11}	102.0	4.8
	1.0×10^{-10}	9.6×10^{-11}	95.7	7.1

b Determination and recovery results of lysozyme in human serum sample and spiked samples

Sample	Spiked/(mol•L^{-1})	Found/(mol•L^{-1})	Recovery/%	RSD/% n=3
Serum 1	0	1.9×10^{-10}	N/A	6.9
Serum 1+2×10^{-10} mol/L	2.0×10^{-10}	3.5×10^{-10}	90.8	5.3
Serum 2	0	2.1×10^{-10}	N/A	8.7
Serum 2+2×10^{-10} mol/L	2.0×10^{-10}	4.0×10^{-10}	97.0	6.5

respectively. After incubated for 1 h at 37 ℃, the electrode was rinsed thoroughly to reduce the nonspecific binding. Following immersion in 10 μmol/L tris-(bpyRu)-β-CD solution for 1.5 h at room temperature and through washing, the ECL measurement was performed. The calibration method was used to determine proteins concentrations, and the results of these recovery tests were demonstrated in Table 1a and Table 1b, respectively.

As can be seen in Table 1a, the analytical results of thrombin determination in the serum sample showed that the spiked recoveries were changing from 90.5% to 102%, and the relative standard deviation (RSD) was no higher than 8.3%. Meanwhile, in the diluted serum sample, the lysozyme concentrations were found as 0.19 nmol/L and 0.21 nmol/L in different samples (Table 1b). According to the procedures based on spiked standards, the recovery results in Table 1b presented the satisfactory recoveries as 90.8% and 97.0% and the acceptable RSD with the highest value of 8.7%. In general, the results demonstrated that the proposed ECL aptasensor could be potentially used for the proteins detection in a complex biological environment. Furthermore, we have summarized the performances of several aptamer-based sensing platforms[34,57,63-68] in comparison to this developed method (Table S1 and Table S2) and the results indicate that our method has comparable or even lower detection limits, demonstrating its high sensitivity without any further labelling or amplification process.

Conclusions

In summary, an ultrasensitive and signal-on ECL aptasensor for protein detection without directly labelling a signal molecule has been developed based on the host-guest recognition between ss-DNA and tris(bpyRu)-β-CD. Endowed with high ECL efficiency induced by the multi-luminophores of tris(bpyRu)-β-CD, this aptasensor can detect as low as 8.5 fmol/L (S/N=3) thrombin or 33 fmol/L (S/N=3) lysozyme, and it also exhibits good specificity with the existence of interfering proteins. More importantly, the practical applicability of the proposed aptasensor was investigated by detecting target protein in human real serum samples, demonstrating a promising feature for the analytical application in complex biological samples. In view of these advantages, the ECL-based aptasensor presented in this study shows a great potential for protein determination and clinical applications.

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