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Signal-on electrochemiluminescent biosensor for ATP based on the recombination of aptamer chip†

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A novel signal-on electrochemiluminescent (ECL) biosensor based on adenosine triphosphate (ATP)-induced recombination of split aptamer chips is presented.

Aptamers are nucleic acid macromolecules of single-stranded DNA/RNA oligonucleotides which can bind with a wide array of targets with high affinity and specificity. They are originated from large random-sequence nucleic acid libraries *via* an *in vitro* evolution process called SELEX (Systematic Evolution of Ligands by Exponential Enrichment).[1,2] Due to their easy labelling, high stability and specificity,[3,4] particular attention has been paid to the development of aptamer-based sensors.[5–8] Electrochemiluminescence (ECL) is a kind of chemiluminescent reaction triggered by electrochemical methods; it has a remarkable feature of high sensitivity. In comparison to other sensors based on fluorescence or other spectroscopic methods, most sensors based on ECL detection own the remarkable features of high sensitivity and low detection limits.[9–12] The combination of high sensitivity of the ECL technique and high selectivity of aptamer has led the emergence of aptamer-based electrochemiluminescent (ECL-AB) biosensors.[13,14] In particular, $Ru(bpy)_3^{2+}$ and its derivatives are the commonly used ECL reagents.[15–19] As a novel ECL signal amplifier, tris(2,2′-bipyridyl)-ruthenium(II)-doped silica nanoparticle (Ru-SiNP) has been applied to modify the DNA. Since each particle contains thousands of $Ru(bpy)_3^{2+}$ molecules, the detected ECL signal is at least 1000-fold higher than that on the single $Ru(bpy)_3^{2+}$ modified DNA.[20] Hence, a much lower detection limit can be achieved.

Adenosine triphosphate (ATP) is vital to living organisms; it provides the support for the metabolism and maintains life in biological tissues. The detection of ATP is not only of research interest but of clinical importance. Various methods such as fluorescence and colorimetric approaches have been developed to detect ATP.[21,22] Recently, with the increasing applications of aptamer as a new recognition element, some novel aptamer-based approaches for ATP determination have been developed. For example, Du *et al.*[23] designed a multifunctional reusable label-free aptamer electrochemical biosensor for ATP detection using electrochemical impedance spectroscopy and cyclic voltammetry (CV). The reported sensor could be reused many times. Unfortunately, its detection limit was not good enough. Zuo and coworkers[24] adopted the electrochemical sandwich assay for the direct detection of ATP based on a single aptamer sequence. The sensor developed by this method was reusable and also showed a low detection limit. However, its sensitivity was not satisfactory. An ECL-AB biosensor was developed by Yao and coworkers,[25] in which a sharp variation of ECL response had to be accompanied by enough target molecules to induce aptamer conformation change. Therefore, there were substantial background signals in their sensor. Moreover, in their approach, each DNA chain was connected only to one ECL reagent, so the ECL signal was weak. It is supposed that if one DNA can be modified with multiple ECL reagents, the detected ECL signal can be enhanced and so the detection limit can be further lowered.

Herein, a novel signal-on (the presence of target causes the increase of the signal strength) ECL biosensor based on ATP-induced recombination of split aptamer chips is developed using Ru-SiNPs as ECL signal producer. The principle of the biosensor is shown in Fig. 1. A single aptamer for ATP is split into two fragments (ssDNA1 and ssDNA2): a thiolated aptamer-chip (ssDNA1) which is immobilized onto the surface of gold electrode through Au–thiol interaction working as the capture probe, while the other one (ssDNA2) functionalized with ECL reagent (Ru-SiNPs) is regarded as the detection probe. It has been proved that the cutting site doesn't hinder target combination by circular permutation.[24] In the absence of ATP, there is inferior interaction between the two fragments, which results in a weak ECL signal from the electrode. In the presence of ATP, owing to the formation of a stable complex between the ssDNA1 and Ru-SiNPs modified ssDNA2, Ru-SiNPs are caused to be immobilized onto the electrode surface, resulting in a much larger ECL signal. The ECL signals detected are correlated to the concentration of ATP. It is demonstrated that the sensor developed in this way owns

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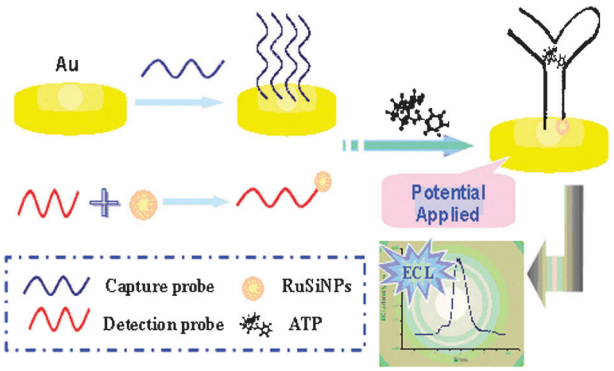

Fig. 1 Schematic of the aptamer-chip-based biosensor for the determination of ATP.

the merits of high sensitivity, lower detection limit, and lower background signal.

The immobilization process of oligonucleotide on the electrode surface was characterized by faradic electrochemical impedance spectroscopy (see Fig. S2 in Supporting Information for detail). As shown in Fig. 2A, the change of the ECL intensity depends on the concentrations of ATP. The measurement is performed in 2.0 mL PBS (Phosphate Buffered Solution, pH 7.4) containing 5.0×10^{-3} mol L^{-1} 2-(dibutylamino)ethanol (abbreviated as DBAE, working as the coreactant of $Ru(bpy)_3^{2+}$ [26]). Without addition of ATP into the solution, very low ECL signals can be detected. The background ECL signal is about 120 a.u. If a single aptamer like that reported in ref. 25 has been applied instead of the split aptamers, the background ECL signal is about 370 a.u.; this indicates that the proposed method has lower background ECL signal. The ECL intensities increase with the concentrations of ATP, and the enhanced ECL intensity (ΔI) has a good linear relationship with the logarithm of ATP concentrations (C(ATP)) in the range of 1.0×10^{-9}–1.0×10^{-12} mol L^{-1} (shown in Fig. 2B). The regression equation can be expressed as

$$\Delta I/\text{a.u.} = 311.26 \log C(\text{ATP}) + 3873.10$$

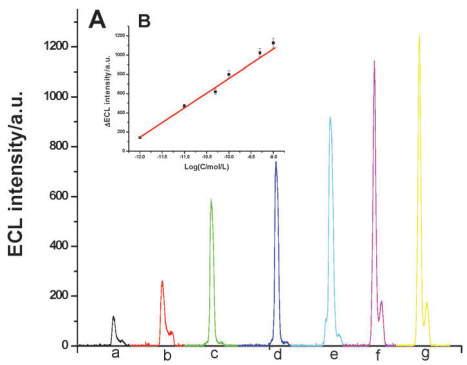

Fig. 2 (A) ECL intensity curves at different ATP concentrations. (a) 0; (b) 1.0×10^{-12} mol L^{-1}; (c) 1.0×10^{-11} mol L^{-1}; (d) 5.0×10^{-11} mol L^{-1}; (e) 1.0×10^{-10} mol L^{-1}; (f) 5.0×10^{-10} mol L^{-1}; (g) 1.0×10^{-9} mol L^{-1}. The electrodes were immersed in 1.0×10^{-2} mol L^{-1} PBS (pH 7.4) containing 5.0×10^{-3} mol L^{-1} DBAE. Scan mode: cyclic voltammetry; Scan rate: 50 mV s^{-1}; potential range: 0.8–1.6 V. (B) The relationship between the enhanced ECL intensity (average of three times detection) and the logarithm of ATP concentration.

The pregression coefficient R is 0.9968, and the detection limit is 2.0×10^{-13} mol L^{-1} (defined as S/N = 3), which is lower than that of the electrochemical[24] and ECL[25] approach. We believe that the Ru-SiNPs, assembled by a large number of $Ru(bpy)_3^{2+}$ molecules, have served as the signal amplifier and led to the lower detection limit.

After reacting with 1.0×10^{-11} mol L^{-1} ATP, the ECL intensity from the modified electrode has been recorded under continuous CV scanning in PBS (pH 7.4) with 5.0×10^{-3} mol L^{-1} DBAE (see Fig. S3 in Supporting Information for detail). The relative standard deviation (RSD) of ECL response is about 4.3% (n = 5). The sensor remains bioactive after four-week storage at 4 °C and the detected ECL signal is reduced less than 5%. This indicates that the biosensor proposed has good stability. Five different biosensors have been developed to detect the same concentration of ATP solution (1.0×10^{-11} mol L^{-1}). The RSD is 5.4%, which means the proposed method has good reproducibility.

Moreover, the biosensor can be regenerated by soaking in hot water followed by several times of rinsing with double-distilled water to wipe out the rudimental aptamer. After this regeneration treatment, the ECL intensity detected from the sensor is about 130 a.u., very close to the background ECL signal (about 120 a.u.). This means that the majority of Ru-SiNPs-modified aptamers have been removed from the electrode surface. The ECL intensity detected for 1.0×10^{-11} mol L^{-1} ATP by the regenerated one is almost the same as that on the freshly prepared one, indicating that the ssDNA1 modified on the electrode surface has not been destroyed during hot water treatment. After 25 times regeneration, the ECL signals (1.0×10^{-11} mol L^{-1} ATP) are reduced by only 5.8%, which means that the sensor can be reused at least 25 times.

Three ATP analogues of cytidine triphosphate (CTP), guanosine triphosphate (GTP) and uridine triphosphate (UTP) (all concentrations are 1.0×10^{-10} mol L^{-1}) are employed to examine the selectivity of the sensor to ATP (1.0×10^{-12} mol L^{-1}). As illustrated in Fig. 3, the enhanced ECL intensities from the analogues are very low, but it increases greatly from ATP. The comparative results show that the proposed biosensing system has an excellent affinity for ATP determination.

The proposed biosensor has been applied to detect the ATP in human serum samples. The recoveries of ATP by the

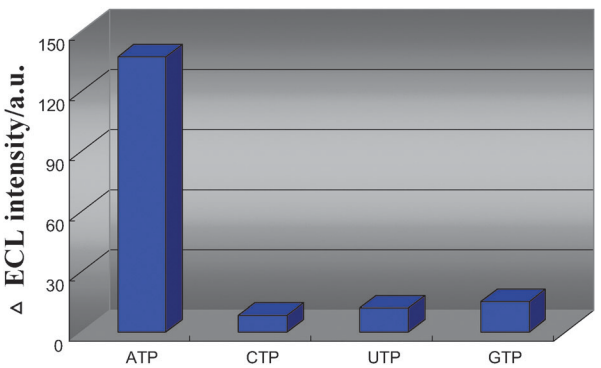

Fig. 3 Selectivity of biosensor to ATP, CTP, UTP and GTP. Concentration of ATP is 1.0×10^{-12} mol L^{-1}; the others are 1.0×10^{-10} mol L^{-1}.

Table 1 Detection of ATP in human serum samples

Sample No.	Detected (mol L^{-1})	Added (mol L^{-1})	Found (mol L^{-1})	Recovery (%)	RSD (%) (n = 5)
1	1.79×10^{-10}	1.00×10^{-10}	0.97×10^{-10}	97.0	5.03
2	1.81×10^{-10}	2.00×10^{-10}	2.16×10^{-10}	108.0	4.56
3	1.85×10^{-10}	5.00×10^{-10}	4.98×10^{-10}	99.6	4.19

standard addition have been detected and are used to evaluate the veracity of the proposed method. The results are shown in Table 1. The ATP concentration in human serum samples is about 1.81×10^{-10} mol L^{-1}. The recoveries are between 97.0% and 108.0%, the RSDs are between 4.19% and 5.03%. The results suggest that the proposed method can be successfully applied for detection of ATP in serum samples.

In summary, a signal-on ECL biosensor was developed based on ATP-induced recombination of split aptamer chips. The Ru-SiNPs containing thousands of $Ru(bpy)_3^{2+}$ molecules have been used as a signal amplifier to improve the sensitivity of the biosensor. The detection limit of the proposed sensor is 2.0×10^{-13} mol L^{-1}. The sensor has the characteristics of good stability, reproducibility and reusability. It has been demonstrated that the sensor can be stored for at least 4 weeks and reused at least 25 times with reasonable reduction rate of ECL intensity. The biosensor has also been successfully applied to analyze ATP in human serum samples with satisfactory results.

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