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Electrochemiluminescence aptasensor for adenosine triphosphate detection using host–guest recognition between metallocyclodextrin complex and aptamer

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

Department of Chemistry, State Key Laboratory of Precision Spectroscopy, East China Normal University, Shanghai 200241, PR China

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ABSTRACT

A sensitive and label-free electrochemiluminescence (ECL) aptasensor for the detection of adenosine triphosphate (ATP) was successfully designed using host–guest recognition between a metallocyclodextrin complex, i.e., tris(bipyridine)ruthenium(II)-β-cyclodextrin [tris(bpyRu)-β-CD], and an ATP-binding aptamer. In the protocol, the NH_2-terminated aptamer was immobilized on a glassy carbon electrode (GCE) by a coupling interaction. After host–guest recognition between tris(bpyRu)-β-CD and aptamer, the tris(bpyRu)-β-CD/aptamer/GCE produced a strong ECL signal as a result of the photoactive properties of tris(bpyRu)-β-CD. However, in the presence of ATP, the ATP/aptamer complex was formed preferentially, which restricted host–guest recognition, and therefore less tris(bpyRu)-β-CD was attached to the GCE surface, resulting in an obvious decrease in the ECL intensity. Under optimal determination conditions, an excellent logarithmic linear relationship between the ECL decrease and ATP concentration was obtained in the range 10.0–0.05 nM, with a detection limit of 0.01 nM at the *S*/*N* ratio of 3. The proposed ECL-based ATP aptasensor exhibited high sensitivity and selectivity, without time-consuming signal-labeling procedures, and is considered to be a promising model for detection of aptamer-specific targets.

1. Introduction

Nucleic acid aptamers are engineered through repeated rounds of selection in vitro, a process called systematic evolution of ligands by exponential enrichment (SELEX) [1]. Various molecular targets such as small molecules, proteins, nucleic acids, and even cells and organisms can be specifically recognized by the corresponding aptamers [2–5]. Because of their low cost, high affinity, and simplicity, aptamer-based approaches have attracted much attention from researchers and have been widely used in the determination of various biomolecules [6–8]. Aptasensors for different purposes have been developed by combining aptamers with different detection techniques such as electrochemistry [9,10], fluorescence [11–13], and electrochemiluminescence (ECL) [14–16].

ECL is the emission of light resulting from the electron-transfer reaction between electrochemically generated ion radicals on the surface of an electrode [17]. In the past few years, as a result of advantages such as high sensitivity, good selectivity, and easy controllability [18], ECL-based aptasensors have been used extensively for target determination with low detection limits [19–21]. In most cases, nanoparticles (e.g., gold or silica nanoparticles) have been used as carriers to amplify the ECL signals, improving the sensitivity of the aptasensor [19,22]. As the use of quantum dots (QDs) has increased, increasing numbers of studies have been successfully performed using QDs, because of their effective and valuable ECL behaviors [23]. In our work, tris(bipyridine)ruthenium(II)-β-cyclodextrin [tris(bpyRu)-β-CD; Scheme 1), a typical polynuclear metallocyclodextrin, was used to promote the ECL performance of an aptasensor, based on multiple ruthenium centers. Although polynuclear metallocyclodextrins have aroused great interest as a result of their applications in the construction of light-activated miniature devices [24], few studies have focused on their potential in ECL-based aptasensors. Polynuclear metallocyclodextrins are excellent receptors for some guest molecules because of the cyclodextrin cavity [25], so aptasensors could be constructed based on host–guest recognition rather than luminescent-labeling procedures. Furthermore, in-depth research on the recognition properties of cyclodextrins has shown that single-stranded (ss) DNAs or bases are good guests for cyclodextrins [26–28]. Polynuclear metallocyclodextrins such as the tris(bpyRu)-β-CD, which was first synthesized by our group [29], consisting of a cyclodextrin unit could therefore directly recognize ss-DNA (as an aptamer) based on host–guest recognition, opening up new possibilities for the design of novel label-free aptasensors.

Adenosine triphosphate (ATP) is considered to be an "energy currency" in all living organisms, and is an indicator of cell

Abbreviations: tris(bpyRu)-β-CD, tris(bipyridine)ruthenium(II)-β-cyclodextrin

* Corresponding author. Tel./fax: +86 21 54340057.
E-mail address: pghe@chem.ecnu.edu.cn (P. He).

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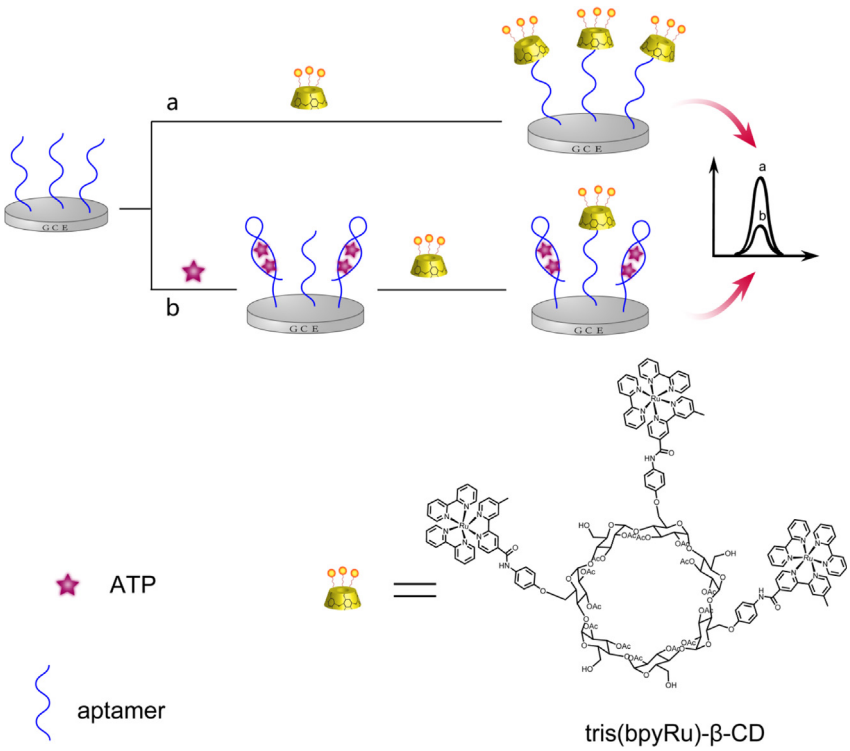

Scheme 1. Schematic diagram of proposed ECL-based aptasensor for ATP detection.

viability or cell injury [30]. The dissipative rate and concentration of ATP are closely related to common diseases such as hypoglycemia, Parkinson′s disease, and ischemia. Sensitive and selective detection of ATP therefore has practical implications in modern scientific research. Until now, various aptasensors for ATP detection have been designed by transducing aptamer/ATP interactions into fluorescent [31,32], electrochemiluminescent [15,33], electrochemical [34,35], and colorimetric [36] signals. However, ATP-binding aptamers seem to have weak target-binding affinities compared with those for other biomolecules [37], which makes the determination of ATP with low detection limits difficult.

In this study, an ultrasensitive label-free aptasensor for ATP detection was developed using the excellent photoactive and macromolecular characteristics of tris(bpyRu)-β-CD (Scheme 1). Tris(bpyRu)-β-CD, consisting of a cyclodextrin unit and photoactive multimetal centers, is therefore expected to recognize an aptamer specifically and produce good ECL signals, making it useful for the construction of label-free ECL-based aptasensors. First, the NH_2-terminated aptamer was immobilized on an activated glassy carbon electrode (GCE) by a coupling interaction. As a result of specific recognition between tris(bpyRu)-β-CD and the aptamer, tris(bpyRu)-β-CD was successfully attached to the aptamer/GCE. Because of the excellent photoactive properties of tris(bpyRu)-β-CD, the tris(bpyRu)-β-CD/aptamer/GCE produces a good ECL signal (Scheme 1a). However, in the presence of ATP, an aptamer/ATP complex was formed preferentially, hindering the host–guest recognition between tris(bpyRu)-β-CD and the aptamer. Consequently, less tris(bpyRu)-β-CD was attached to the electrode surface, leading to an obvious decrease in the ECL intensity (Scheme 1b). It is also worth mentioning that the concentration of ATP could be quantitatively monitored based on the reduction in the ECL intensity. The proposed ECL-based aptasensor exhibited good sensitivity, simplicity, and operability. Since the ATP aptasensor is just one model, this approach could be further applied to the detection of other aptamer-specific targets.

2. Materials and methods

2.1. Chemicals

ATP, cytosine triphosphate (CTP), guanosine triphosphate (GTP), and uridine triphosphate (UTP) were obtained from Sinopharm (Shanghai, China). *N*-hydroxysuccinimide (NHS), 2-(dibutylamino)ethanol (DBAE), Tween-20, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), and anthranilic acid (ABA) were obtained from Sigma-Aldrich (Shanghai, China). Tris (hydroxymethyl)aminomethane (tris) was purchased from the Sangon Biotech Co., Ltd. (Shanghai, China). The aptamer, 5′-$NH_2(CH2)_6$-ACCTGGGGGAGTATTGCGGAGGAAGGT-3′, was prepared by the Sangon Biotech Co., Ltd. (Shanghai, China). All solutions were prepared using ultrapure water from a Millipore Milli-Q water purification system. Tris(bpyRu)-β-CD (Scheme 1) was synthesized and characterized as previously reported by our group [29].

2.2. Instrumentation

All electrochemical measurements were carried out using a CHI 660C electrochemical analyzer (CH Instruments Co., Shanghai, China). The electrochemical system was a conventional three-electrode system: a GCE as the working electrode, a platinum wire as the counter electrode, and an Ag/AgCl electrode as the reference electrode. All ECL measurements were performed using an LK5100 ECL analyzer (Lanlike Electronic Science Tech. Co., Ltd., China).

2.3. Preparation of ATP aptasensor

First, a GCE was polished sequentially with 1.0 μm, 0.3 μm, and 0.05 μm alumina slurries, ultrasonically cleaned with ethanol and ultrapure water for 10 min each, and dried under nitrogen gas. Next, ABA was electropolymerized on the GCE in a solution of

0.05 M ABA/1 M H_2SO_4 (0–1 V, 8 cycles), as previously reported [38]. After cleaning with ultrapure water, the poly(ABA)-coated GCE was activated with 4 mM EDC/1 mM NHS solution for 30 min. Then 5 nM NH_2-aptamer was immediately dropped on the modified GCE for 3 h and then immobilized on the surface of GCE via a coupling interaction. Through washing gave the aptasensor (aptamer/GCE) for ATP detection.

As shown in Scheme 1, the aptamer/GCE was incubated in various concentrations of ATP (10.0–0.05 nM) in the binding buffer (20 mM Tris–HCl, 0.1 M NaCl, 5 mM $MgCl_2$, pH 7.4) for 1 h at 37 °C. The reagents of the binding buffer are benefit for aptamer to combine with ATP. After rinsing with washing buffer (20 mM Tris–HCl, 0.1 M NaCl, 5 mM $MgCl_2$, 0.05% (v/v) Tween-20, pH 7.4) to remove nonspecifically bound ATP, the ATP/aptamer/GCE was immersed in 10 μM tris(bpyRu)-β-CD for 1.5 h. Followed with extensively rinsed with ultrapure water, the fabricated electrode was carried out by the ECL measurement.

2.4. ECL measurements of ATP

The ECL behavior was monitored over the scanning range 0.4–1.25 V vs. Ag/AgCl in 0.1 M phosphate buffer solution (PBS) containing 20 mM DBAE (pH 7.4), at a photomultiplier tube voltage of 900 V and a scanning rate of 100 mV/s. The potential range of ECL measurement was 0.4–1.25 V vs. Ag/AgCl as the electrochemical oxidation of $Ru(bpy)_3^{2+}$ was carried out at the potential range as reported by most of the literatures [39–41]. Furthermore, the selectivity of the ATP aptasensor was investigated using UTP, GTP, and CTP to perform comparative experiments under the same conditions.

3. Results and discussion

3.1. ECL properties and host–guest recognition performance of tris(bpyRu)-β-CD

The tris(bpyRu)-β-CD, first synthesized by our group, consists of three ruthenium centers and a cyclodextrin unit, and is a bifunctional complex with excellent photoactive properties and host–guest recognition capabilities. Fig. 1A shows the ECL performances of 1 μM tris(bpyRu)-β-CD (curve *a*) and $Ru(bpy)_3Cl_2$ (curve *b*) in 0.1 M PBS buffer with 20 mM DBAE, using a bare GCE as the working electrode. It is clear that the ECL signal of tris(bpyRu)-β-CD was much stronger than that of $Ru(bpy)_3Cl_2$ at the same concentration, showing the satisfactory photoactive properties of tris(bpyRu)-β-CD and its potential for further promotion of sensitivity in ECL determination.

Host–guest recognition between cyclodextrin and ss-DNA or bases has been reported previously [26–28], so tris(bpyRu)-β-CD was expected to possess aptamer-specific recognition capabilities because of its cyclodextrin unit. In this work, the aptamer was first immobilized on the surface of GCE, this gave no ECL signal (curve *a* in Fig. 1B) in 0.1 M PBS containing 20 mM DBAE. After addition of 10 μM tris(bpyRu)-β-CD for 1.5 h, the formed tris(bpyRu)-β-CD/aptamer/GCE gave a high ECL signal (curve *c* in Fig. 1B) under the same conditions. However, when 10 μM $Ru(bpy)_3Cl_2$ was added to the aptamer/GCE surface instead of tris(bpyRu)-β-CD, the $Ru(bpy)_3Cl_2$/aptamer/GCE formed gave a weak ECL signal (curve *b* in Fig. 1B), this resulted from electrostatic interactions between $Ru(bpy)_3Cl_2$ and the phosphate backbone of the aptamer. These results showed that recognition between the cyclodextrin component and the aptamer was the key factor in producing a strong ECL signal, leading to a good ECL performance, as a result of the excellent photoactive properties of tris(bpyRu)-β-CD. Tris(bpyRu)-β-CD could therefore be a good choice for the construction of a sensitive label-free aptasensor, based on its good photoactive properties and recognition abilities.

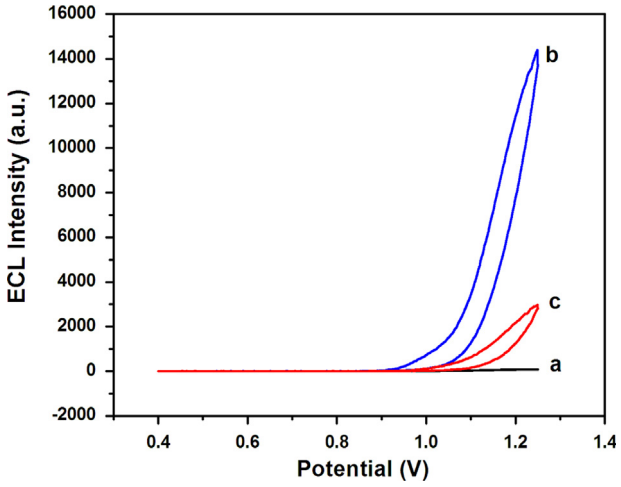

Fig. 2. ECL signals of (a) aptamer/GCE, (b) tris(bpyRu)-β-CD/aptamer/GCE, and (c) tris(bpyRu)-β-CD/ATP (10 nM)/aptamer/GCE in 0.1 M PBS containing 20 mM DBAE (pH 7.4), at a scanning rate of 100 mV/s, ranging from 0.4 V to 1.25 V (vs. Ag/AgCl).

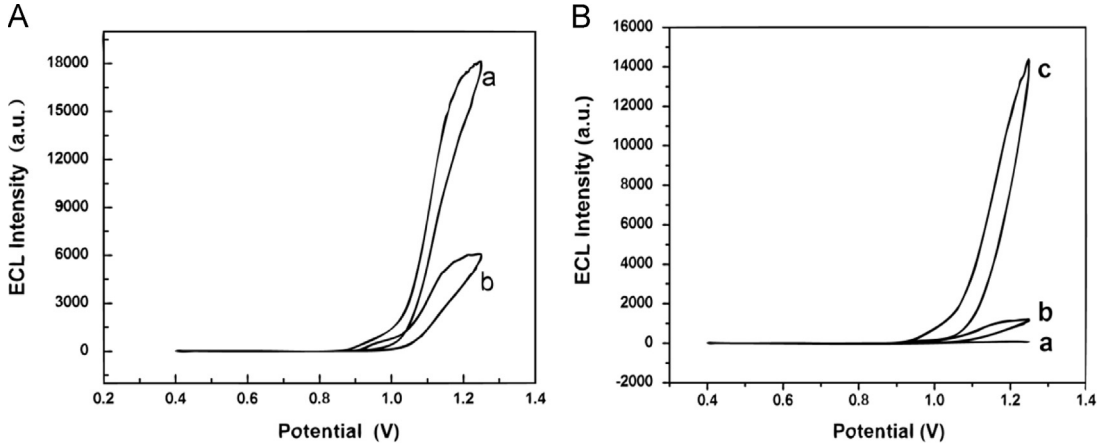

Fig. 1. (A) ECL performances of 1 μM (a) tris(bpyRu)-β-CD and (b) $Ru(bpy)_3Cl_2$ on a bare GCE; (B) ECL spectra of (a) aptamer/GCE, (b) $Ru(bpy)_3Cl_2$/aptamer/GCE, and (c) tris(bpyRu)-β-CD/aptamer/GCE. ECL experiments were carried out in 0.1 M PBS containing 20 mM DBAE (pH 7.4), and at a scanning rate of 100 mV/s and ranging from 0.4 V to 1.25 V (vs. Ag/AgCl).

3.2. ECL performance of proposed aptasensor

In our approach, the proposed aptasensor was successfully constructed as shown in Scheme 1. First, the NH_2-terminated aptamer was immobilized on the GCE. After application of tris (bpyRu)-β-CD for 1.5 h, the tris(bpyRu)-β-CD/aptamer/GCE gave a strong ECL signal (curve *b* in Fig. 2) because of the outstanding ECL properties of tris(bpyRu)-β-CD. However, in the presence of ATP, an ATP/aptamer complex was easily formed, restricting the host–guest recognition between tris(bpyRu)-β-CD and the aptamer, because of its higher stability. As a result, less tris(bpyRu)-β-CD was attached to the surface of the GCE, which gave rise to a sharp decrease in the ECL signal (curve *c* in Fig. 2). It can therefore be seen that the difference in the ECL intensity could be monitored to determine whether or not ATP was present in a sample solution. In other words, the proposed aptasensor could be used in ATP determination without any other signal-labeling or amplification procedures.

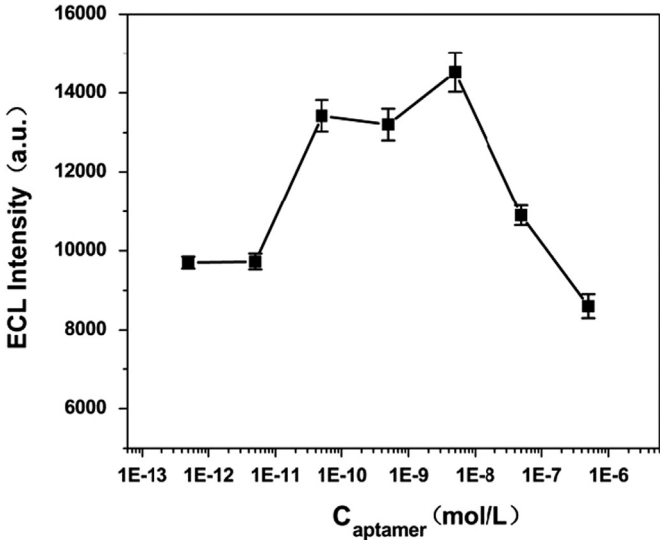

Fig. 3. ECL intensities of tris(bpyRu)-β-CD/aptamer/GCE with different concentrations of aptamer, ranging from 0.5 μM to 0.5 pM, in 0.1 M PBS containing 20 mM DBAE (pH 7.4), at a scanning rate of 100 mV/s, ranging from 0.4 V to 1.25 V (vs. Ag/AgCl). The error bars were derived from the standard deviation of three replicate experiments.

3.3. Optimization of experimental conditions

To further optimize the sensitivity of the ATP aptasensor, various conditions were optimized by single factor experiments. In this work, DBAE was employed as a co-reactant in the electrochemiluminescent system of tris(bpyRu)-β-CD [39]. The concentration of DBAE is 20 mM as the optimization experiment condition according to the experiment results of dependences of ECL intensities on the concentrations of DBAE (Fig. S1 in Supporting information), which is also according with the previous report [39]. Additionally, the effect of scan rate on cyclic voltammograms of tris(bpyRu)-β-CD was shown in Fig. S2 of Supporting information. And the pH and ionic strength of the PBS were also investigated, (the details were shown as Figs. S3 and S4 in Supporting information).

Furthermore, the concentration of the aptamer on the electrode surface has great impact on the ECL performance. Different concentrations of aptamer (from 0.5 μM to 0.5 pM) were dropped onto the surfaces of activated electrodes for 3 h, and then the GCEs were immersed in 10 μM tris(bpyRu)-β-CD for 1.5 h. The fabricated GCEs were monitored using an ECL analyzer in 0.1 M PBS containing 20 mM DBAE. As shown in Fig. 3, the ECL intensity increased with increasing aptamer concentration from 0.5 pM to 5.0 nM. A larger amount of aptamer on the electrode surface led to recognition of a greater number of tris(bpyRu)-β-CD molecules, explaining the enhancement of the ECL intensity in this aptamer concentration range. However, when the concentration of the aptamer was further increased from 5.0 nM to 0.5 μM, the ECL intensity decreased. This could be caused by steric-hindrance effects at very high aptamer concentrations on the electrode surface, which would be unfavorable for recognition by tris (bpyRu)-β-CD. The detection principle of the proposed aptasensor was based on quenching of the ECL intensity after the addition of ATP, so it was preferable for the initial ECL intensity to reach a maximum, to promote sensitivity. An aptamer concentration of 5.0 nM was therefore selected for construction of aptasensors in the following experiments.

3.4. ECL detection of ATP

The aptasensor performance was evaluated by detection of different concentrations of ATP. Fig. 4A shows the ECL profiles obtained from the aptasensor after immersion in different concentrations of ATP; curves *a*–*g* correspond to ATP concentrations

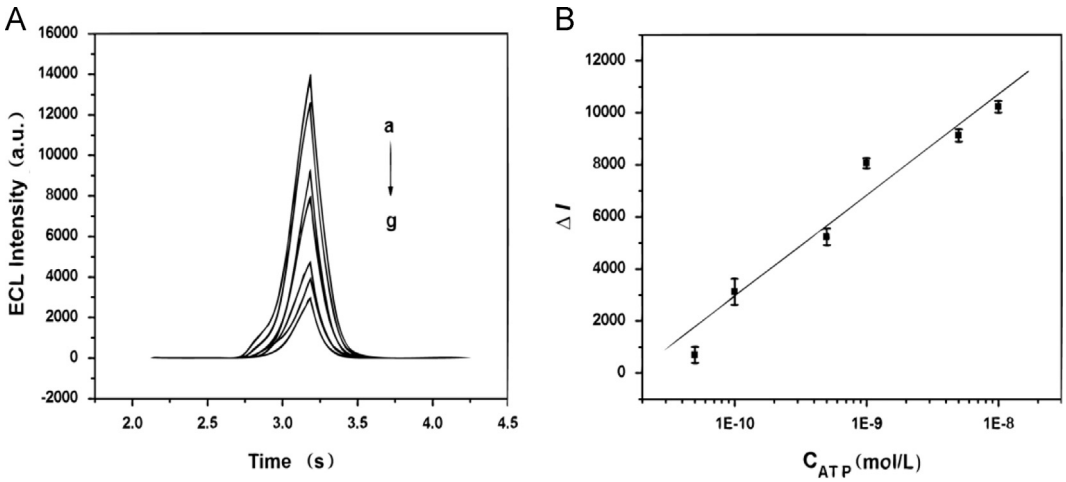

Fig. 4. (A) ECL responses of aptasensor incubated with different concentrations of ATP: (a) 0 M, (b) 0.05 nM, (c) 0.1 nM, (d) 0.5 nM, (e) 1.0 nM, (f) 5.0 nM, and (g) 10.0 nM. (B) The resulting calibration curve for the absolute difference in ECL intensity as a function of the logarithm of ATP concentration from 0.05 to 10.0 nM ($\Delta I = I_0 - I$, I_0 and I are the ECL intensities of the aptasensor in the absence and presence of ATP, respectively). The error bars were derived from the standard deviation of three replicate experiments.

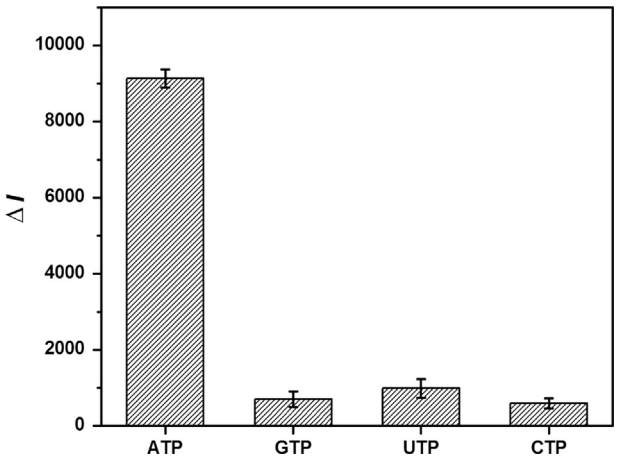

Fig. 5. Absolute differences of the ECL intensities of the ATP aptasensor responses to ATP (1 nM) against UTP (10 nM), UTP (10 nM), and CTP (10 nM). The error bars show the standard deviations of three replicate measurements.

from 0 nM to 10.0 nM. It can be seen that the ECL intensity decreased with increasing of ATP concentration, which demonstrated the success of the design strategy shown in Scheme 1. Fig. 4B showed the relationship between ΔI and ATP concentrations ($\Delta I = I_0 - I$, I_0 and I are the ECL intensities of the aptasensor in the absence and presence of ATP, respectively). The ΔI was found to be logarithmically related to the concentration of ATP in the range from 0.05 nM to 10.0 nM ($R = 0.9621$) with a detection limit of 0.01 nM at the S/N ratio of 3, demonstrating its acceptable quantitative behavior. Consequently, such a simply constructed label-free aptasensor with excellent sensitivity could be applied in the determination of biomolecules such as ATP.

3.5. *Selectivity of aptasensor*

To investigate the selectivity of the aptasensor, GTP, UTP, and CTP were chosen operate the proposed aptasensor instead of ATP under the same experimental conditions. Fig. 5 shows that, after the addition of a concentration as high as 10 nM GTP, UTP, or CTP, the ECL intensity of the aptasensor was decreased slightly. In contrast, 1 nM ATP led to a significant quenching of ECL signal. Additionally, three parallel experiments were conducted to demonstrate the specificity of the aptasensor. Therefore, the results proved that the proposed aptasensor performed excellent selectivity for ATP determination.

4. Conclusion

In this work, the host–guest recognition capabilities and excellent photoactive properties of a metallocyclodextrin complex, i.e., tris(bpyRu)-β-CD, were used to develop a sensitive and label-free aptasensor for ATP detection. The wide detection range, i.e., 10.0–0.05 nM, with a detection limit of 0.01 nM at the S/N ratio of 3, indicated that the proposed aptasensor had good sensitivity; this was considered to be one of the most important results of our approach. The label-free aptasensor was simple to construct and performed well in ATP detection without any other signal-labeling or amplification procedures. All the above features demonstrated the extensive potential of this sensing strategy in the determination or analysis of biomolecules, especially targets with specific binding aptamers.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.12.039.

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