Original article

A fluorescent aptasensing strategy for adenosine triphosphate detection using tris(bipyridine)ruthenium(II) complex containing six cyclodextrin units

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A S T R A C T

A sensitive label-free fluorescent aptasensing strategy for the detection of adenosine triphosphate (ATP) has been developed with a metallocyclodextrin, tris(bipyridine)ruthenium(II) complex containing six cyclodextrin units (6CD-Ru), which exhibited much stronger emission signal compared to the parent compound Ru(bpy)\textsubscript{3}Cl\textsubscript{2}. Furthermore, the emission spectrum showed that the ATP-aptamer (ssDNA) could increase the fluorescence intensity of 6CD-Ru dramatically, attributed to the interaction between aptamer and cyclodextrin, which could provide protection to the ruthenium core from the quenching of emission by oxygen in the solution. With the addition of ATP, the interaction between aptamer and cyclodextrins on 6CD-Ru was diminished, since the ATP/aptamer complex had the priority to be formed, leading to the corresponding reduction of fluorescence intensity, which could be utilized to detect ATP quantitatively. A linear relationship was displayed between the fluorescence and the logarithm of ATP concentrations in the range from 1 nmol/L to 1 \mu mol/L with the detection limit of 0.5 nmol/L (S/N = 3). The proposed fluorescent aptasensing strategy exhibited high sensitivity and specificity, without any labeling or amplification procedures, and it could also be applied for the detection of many other aptamer-specific targets.

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1. Introduction

Adenosine triphosphate (ATP), generally acknowledged as “energy currency” in most animate beings, plays an essential role in most enzymatic activities \cite{1} and it is a vital component of many biological cofactors \cite{2}. In addition, ATP has also been used as an indicator for cell viability and cell injury \cite{3}. Thus, developing simple, sensitive and specific methods for ATP detection is much important in clinic diagnosis. The key factors, including a highly selective ATP recognition element and a novel mechanism of signal enhancement, should be integrated for a successful detection method \cite{4}. Aaptamers, as recognition element, are synthetic oligonucleotides, which are generated by an \textit{in vitro} selection technique known as SELEX (systematic evolution of ligands by exponential enrichment) from a nucleic acid library \cite{5}. They have gained increasing attention due to the unique character of specifically targeting for a wide variety of substances, such as proteins \cite{6,7}, small molecules \cite{8,9}, metal ions \cite{10} and cells \cite{11,12}. The ATP sensing based on the aptamer-binding methods usually offer advantages in desirable specificity and affinity. At the present, a number of detection systems have been developed, including fluorescence \cite{13}, electrochemistry \cite{14}, colorimetry \cite{15}, piezoelectric mechanisms \cite{16} and electro-chemiluminescence \cite{17}. Among these methods, fluorescence-based detection method exhibits excellent benefits in sensitivity, simplicity, convenience and diversity \cite{18,19}, thus it has been widely used and continues to act as an important role in future research.

Cyclodextrin, cyclic oligosaccharides, composed of glucose units at different numbers, presents bucket structure with a hydrophobic inner cavity and a hydrophilic outer side \cite{20}, which can accommodate a wide range of molecules to form stable inclusion complexes \cite{21,22} and this effect is named as host–guest recognition. This unique property promises a wide application in various fields, especially in biomolecules aptasensing. By introducing luminescent transition metal, such as rhenium \cite{23},

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http://dx.doi.org/10.1016/j.ccl.2016.11.013
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ruthenium [24], and metal binding sites into cyclodextrins framework, metalloccyclodextrin have been synthesized and they are appealing in building blocks for the development of sophisticated supramolecular devices [25]. Since they combine the attractive encapsulated features of cyclodextrin with the inherent properties of metal center in a single molecule, it creates new opportunities for the development of biosensing methods [26,27], especially based on fluorescence change. A luminescent system for steroids detection has been designed using tris-(bipyridine)ruthenium(II) complexes containing multiple cyclodextrin binding sites [28]. It was noteworthy that this synthesized metalloccyclodextrin displayed bright luminescence, twice as intense as the reference metal core, which could be attributed to that the shielding effects of cyclodextrins to the metal core strongly decreased the quenching effects by dissolved oxygen in aqueous solution [29–31]. Our group has also successfully synthesized a series of tris(bipyridine)ruthenium(II) complexes containing multi-ruthenium(II) and multi-cyclodextrins, respectively [32,33]. The former type exhibited excellent electrochemi-luminescence (ECL) properties as well as host–guest recognition ability, so it has been applied to the determination of DNA [34], proteins [35,36] and small molecules [37]. The latter type, especially with six cyclodextrin units (6CD–Ru) (Scheme 1), exhibited great fluorescence performance, thus it has been exploited to develop an aptasensing method for the determination of lysozyme [38].

Herein, a sensitive fluorescent aptasensing strategy without any signal labeling or amplification procedures for ATP detection was proposed based on the host–guest recognition of cyclodextrin on 6CD–Ru to the aptamer (Scheme 1). The fluorescence intensity of 6CD–Ru was firstly enhanced by the addition of aptamer, and then, the presence of ATP reduced the intensity. The signal change could be utilized for the quantitative determination of target molecule—ATP. This proposed fluorescent aptasensing strategy performs the satisfactory sensitivity, simplicity and operability, and it can be further applied to the detection of other aptamer-specific targets.

2. Results and discussion

2.1. Photophysical properties of 6CD–Ru and fluorescence enhancement induced by ssDNA/aptamer

In order to investigate the photophysical properties of the metalloccyclodextrin–6CD–Ru, its fluorescence spectrum (curve c) was measured in aqueous solution as illustrated in Fig. 1, with the fluorescence spectrum of the parent compound Ru(bpy)₃Cl₂ (curve a) as a comparison. 6CD–Ru fluoresces with a maximum peak at approximately 620 nm, and the intensity is nearly 4 times as that of Ru(bpy)₃Cl₂, due to the protecting effect of CDs.

It has been reported [39,40] that single bases, as the key components of ssDNA/aptamer, can be encapsulated into the cyclodextrin cavity freely, thus ssDNA/aptamer can bind cyclodextrin through the host–guest recognition, and then it is supposed to form the noncovalent complex with 6CD–Ru. As shown in Fig. 1, the fluorescence intensity of 6CD–Ru has been further improved drastically after the addition of ssDNA (ATP-aptamer here) (curve d), with 60.4% increase. While, as a comparison, the parent compound Ru(bpy)₃Cl₂ (curve b) has only obtained 19.8% increase under the same conditions due to the electrostatic effect existed between Ru(bpy)₃²⁺ and ssDNA [41]. This result verifies Scheme 1a and suggests that the cyclodextrin rings play an important role in the interaction between ssDNA and 6CD–Ru.

This interaction, enhancing the fluorescence intensity, was further investigated by switching to nucleotides—the monomer of ssDNA. As shown in Fig. 2A, the interaction between 6CD–Ru and single nucleotides just caused 6.8% increase in fluorescence intensity (curve b). While, the addition of ssDNA could improve the fluorescence intensity of 6CD–Ru drastically with 117.8% increase (curve c). It is known that fluorescence generated by Ru(bpy)₃²⁺ could be quenched by dissolved oxygen. 6CD–Ru presents stronger fluorescence property since cyclodextrins could shield the core of Ru(bpy)₃²⁺ from dissolved oxygen. On this basis, the fluorescence intensity would be enhanced further by the interaction between 6CD–Ru and ssDNA, which causes ssDNA chains to wrap around 6CD–Ru, thus leading to the increased shielding effect. While, this wrapping conformation could not be formed with single nucleotides, so they do not have the ability to enhance the fluorescence intensity. To further verify this deduction, amino acids and the polymerized peptide were employed to interact with 6CD–Ru by indole group (Fig. 2B), obtaining 4.3% increase (curve b) and 59.3% increase (curve c), respectively.

![Scheme 1. Schematic representation of fluorescence detection of ATP based on the change of fluorescence intensity.](image-url)

![Fig. 1. Fluorescence spectra of (a) Ru(bpy)₃Cl₂ (1 μmol/L), (b) Ru(bpy)₃Cl₂ (1 μmol/L) with ATP-aptamer (1 μmol/L), (c) 6CD–Ru (1 μmol/L), and (d) 6CD–Ru (1 μmol/L) with ATP-aptamer (1 μmol/L) excitation at 450 nm in aqueous solution at 25 °C.](image-url)
Clearly, the interaction with the polymerized peptide generated more significant enhancement of fluorescence intensity. Thus, the development of aptasensing strategy can be realized based on this unique property of 6CD-Ru.

2.2. Development of the aptasensing strategy

Scheme 1 displays a sensitive and label-free aptasensing strategy to detect ATP. Firstly, the fluorescence intensity of 6CD-Ru is improved sharply by the addition of ATP-aptamer via the interaction between cyclodextrin and aptamer (Scheme 1a). When ATP was presented in the mixture solution, ATP/aptamer complex prefers to be formed and the conformation changes of ATP-aptamer weakens the interaction with cyclodextrins on 6CD-Ru, which causes the fluorescence reduction (Scheme 1b).

In order to verify the proposed strategy, the ATP detection was then performed, as shown in Fig. 3. Compared to the original fluorescence signal of 6CD-Ru (curve a), the addition of ATP-aptamer increases the emission signal (curve c), corresponding to Scheme 1a. Then, ATP solution was added into the mixed system for incubation and the portion formation of ATP/aptamer complex was promoted, leading to the obvious fluorescence signal decrease in the spectrum (curve b), corresponding to Scheme 1b. This fluorescence aptasensing strategy is proved feasible for ATP determination.

2.3. Optimization of experimental variables

The fluorescence aptasensing is affected by various experimental variables. Thus, in order to achieve the most excellent performance of 6CD-Ru, pH value and ATP-aptamer concentration have been optimized. As illustrated in Fig. 4A, the fluorescence enhancement (ΔI, ΔI=I – I0, where I0 and I are the fluorescence intensities of 6CD-Ru in the absence and presence of ATP-aptamer, respectively) increases with pH value increasing until pH value comes to 8.5. Then, ΔI reduces as pH value continues to increase. Thus, pH 8.5 is selected as the optimum condition. The effect of ATP-aptamer concentration on fluorescence enhancement of 6CD-Ru was examined by adding ATP-aptamer at different concentrations (from 0.25 μmol/L to 3.5 μmol/L) into 0.5 μmol/L 6CD-Ru solution (Fig. 4B). Clearly, the fluorescence intensity of 6CD-Ru is sharply enhanced by ATP-aptamer at the increasing concentration until up to 2.0 μmol/L, and then continues with almost no change in ΔI for the higher concentrations, indicating that the largest absolute fluorescence enhancement could be obtained with 2.0 μmol/L ATP-aptamer for 0.5 μmol/L 6CD-Ru. Therefore, 2.0 μmol/L ATP-aptamer was selected as the optimum concentration.

2.4. Quantitative determination of ATP

Based on the developed aptasensing strategy, the determination of ATP is performed quantitatively. From Fig. 5A, it can be seen that the fluorescence intensity increases with the decreased concentration of ATP, where the curves from a to h corresponded to the ATP concentrations ranging from 0 to 1 μmol/L, respectively. Fig. 5B displays a linear relationship between the difference in the fluorescence signal (ΔI, ΔI=I0 – I, where I0 and I are the fluorescence intensities in the absence and presence of ATP, respectively) and the logarithm of ATP concentrations in the range of 1 nmol/L–1 μmol/L. The calibration equation obtained from this curve is ΔI=278.5 × lgC_ATP + 0.6382 with a correlation coefficient of 0.991. The detection limit is found to be 0.5 nmol/L (S/N=3), which is close to or lower than the previously reported detection methods for ATP [6,42]. It is indicative of an acceptable quantitative behavior, and this approach possesses excellent...
sensitivity without any further signal labeling or amplification procedures.

2.5. Specificity of the proposed aptasensing strategy

The specificity of our proposed aptasensing strategy toward ATP detection was further investigated in the existence of other typical interfering analogous molecules, including CTP, UTP, GTP, AMP and ADP under the same experimental conditions. Fig. 6 shows that none of the above interfering molecules induces obvious fluorescence changes (ΔI = I₀ – I, where I₀ and I are the fluorescence intensities in the absence and presence of detected molecules) even their concentrations are 10 times larger than that of ATP, while the addition of ATP brings about a significant intensity decrease. The results suggest that the designed fluorescent aptasensing strategy performs excellent specificity for ATP detection.

3. Conclusions

In summary, the present work demonstrates a novel label-free fluorescent aptasensing strategy for sensitive detection of ATP with the synthesized compound—6CD-Ru, based on its remarkable photophysical properties. The fluorescent signal was further enhanced by ATP-aptamer due to the host–guest recognition between cyclodextrins on 6CD-Ru and ATP-aptamer. The addition of ATP switches the conformation of ATP-aptamer and diminishes the interaction existed between ATP-aptamer and 6CD-Ru, due to the priority formation of ATP/aptamer complex. Under the optimized condition, the detection limit of 0.5 nmol/L was successfully obtained. The proposed fluorescent aptasensing strategy exhibits high sensitivity and specificity, without any signal labeling or amplification procedures. Moreover, it can be expanded into detecting other biomolecules with the corresponding aptamers.

4. Experimental

4.1. Chemicals and materials

The metallo-cyclodextrin compound—6CD-Ru was synthesized by our group [32,33]. ATP, guanosine triphosphate (GTP), uridine triphosphate (UTP), and cytosine triphosphate (CTP) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai,
Adenosine monophosphate (AMP) and adenosine diphosphate (ADP) were purchased from Sigma-Aldrich Trading Co., Ltd. (Shanghai, China). The oligonucleotides used here were synthesized and purified by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). The sequence of the ATP aptamer is 5'-ACCTGGGGAGATCGGAGAAAG-3'. All solutions were prepared with double-distilled water, which was purified with a Millipore Milli-Q purification system to a specific resistance of >18.3 MΩ cm. The binding buffer for the incubation of ATP and its aptamer was made up of 0.56 mM tris-HCl, 4.7 mM KCl and 0.14 mM KCl. All reagents used in this work are of analytical reagent grade.

### 4.2. Instrumentation

Fluorescence spectra were collected on a F-7000 fluorescence spectrophotometer (Hitachi High-Technologies Co., Ltd. Japan). HQQ-C swing bed machine (Donglian Electronic Technology Co., Ltd., China) was used for facilitating the incubation of ATP and its aptamer. The centrifugation of DNA was performed with a TDL–16B centrifugal machine (Shanghai Anting Scientific Instruments Factory, China).

### 4.3. Optimization of experimental variables

The effect of pH value and ATP-aptamer on the performance of GDC–Ru was investigated by mixing 6GCD–Ru (0.5 μmol/L) with ATP-aptamer (0.5 μmol/L) at various pH values (pH= 6.0, 7.0, 8.0, 8.5, 9.0), and ATP-aptamer at various concentrations (0.25 μmol/L–3.5 μmol/L) at 25 °C for 2 h, respectively, followed by the fluorescence measurement. The fluorescence spectra were collected at the excitation wavelength of 450 nm with the slit width of 10 nm and the photomultiplier voltage of 700 V at 25 °C.

### 4.4. ATP determination

The ATP solution at various concentrations was added into the binding buffer containing 6GCD–Ru and the aptamer under the optimized condition. The mixture was incubated at 37 °C for 2 h, and then the fluorescence intensity was measured with the same parameters. The detection was performed by independent experiments with repetition for at least three times.

Furthermore, the specificity of this aptasensing strategy was investigated using some typical interference targets (100 nmol/L), including GTP, UTP, CTP, AMP and ADP to perform comparative experiments under the same conditions.

### Acknowledgment

This work was financially supported by the National Natural Science Foundation of China (Nos. 21275054, 21405049, 31300819).

### References


