A G-quadruplex-based Label-free Fluorometric Aptasensor for Adenosine Triphosphate Detection

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A G-quadruplex-based, label-free fluorescence assay was demonstrated for the detection of adenosine triphosphate (ATP). A double-stranded DNA (dsDNA), hybridized by ATP-aptamer and its complementary sequence, was employed as a substrate for ATP binding. SYBR Green I (SG I) was a fluorescent probe and exonuclease III (Exo III) was a nuclease to digest the dsDNA. Consequently, in the absence of ATP, the dsDNA was inset with SG I and was digested by Exo III, resulting in a low background signal. In the presence of ATP, the aptamer in dsDNA folded into a G-quadruplex structure that resisted the digestion of Exo III. SG I was inserted into the structure, showing high fluorescence. Owing to a decrease of the background noise, a high signal-to-noise ratio could be obtained. This sensor can detect ATP with a concentration ranging from 50 μM to 5 mM, and possesses a capacity for the sensitive determination of other targets.

Keywords Label-free, G-quadruplex, fluorescence, ATP

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Introduction

Adenosine-5′-triphosphate (ATP) is a major energy-storage molecule in cell physiology. It plays an important role in various cellular metabolic functions and biochemical pathways of living organisms.1,2 Abnormal concentrations of ATP are tightly associated with a series of diseases, such as angiocardiopathy,3 Alzheimer’s4 and Parkinson’s.5 Therefore, the detection of ATP is highly demanded for biochemical studies and clinical diagnosis.

Many traditional methods for ATP assays have been developed, such as luciferase mediated bioluminescence,6 high-performance liquid chromatography7 and mass spectrometry.8 These methods may suffer from expensive cost or tedious sample separation, and thus limiting their wide applications. As a result, it has stimulated the development of aptamer-based biosensors for the sensitive and selective detection of ATP.

Aptamers are single-stranded DNA or RNA oligonucleotides selected through a systematic evolution of ligands by exponential enrichment (SELEX), which possess high-recognition ability to specific targets.9,10 Aptamer-based biosensors have previously been employed to detect ATP using fluorimetric,11,12 colorimetric,13,14 and electrochemical15,16 signal-transduction methods. To some extent, these approaches have exhibited some advancements concerning the sensitivity and selectivity of ATP detection. However, fluorescent aptasensors usually require fluorophore and quencher moieties labeling at strategic sites within the aptamers. These characteristics may make the sensors subject to the influences of high cost and complex purification steps.11 The electrochemical methods usually attach the probes on electrode surfaces, the process of which is time-cost and complex.17 Thus, the design of new strategies for the sensitive and selective detection of ATP is in need.

Recently, label-free biosensors have been developed that use photoactive substances or intercalating dyes to monitor the conformational changes of aptamers upon the target’s binding.18–20 This method has been applied to detect ATP. Lin et al.21 reported a label-free strategy for ATP detection employing a loop DNA probe with low-background noise. Exonuclease I (Exo I) and exonuclease III (Exo III) completely digested the loop DNA probe in the absence of ATP. However, in the presence of ATP, the T4 DNA ligase sealed the probe, which resisted digestion by Exo I and Exo III. When adding SYBR Green I (SG I), a strong fluorescence signal could be obtained. This method was sensitive and effective, but it needed several enzymes to participate in the assay, which was laborious, expensive and complicated. An aptamer-based, label-free fluorescence strategy for the detection of thrombin and ATP was designed by Kong and Xu.22 SG I was employed as a fluorescent probe; it bound to duplexes formed by the aptamer and its complementary DNA, and exhibited strong fluorescence in the absence of the target. However, upon the addition of the target, the aptamer became bound to the target, released the probes on electrode surfaces, the process of which is time-cost and complex.17 Thus, the design of new strategies for the sensitive and selective detection of ATP is in need.

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selective methods for ATP detection.

In this strategy, an ATP-aptamer hybridized in a DNA duplex was employed for the formation of an ATP-dependent G-quadruplex structure. SG I was employed as the readout signal, recognizing the dsDNA and G-quadruplex structure. Exo III catalyzes the stepwise removal of mononucleotides from a blunt or recessed 3’-end of DNA duplexes, but its activity on G-quadruplex is limited. Upon the addition of Exo III, the dsDNA was digested, showing a low background fluorescence, and thus the S/N and sensitivity were improved. In comparison with many previous methods, our approach has the advantages of simplicity, low cost and high specificity.

Experimental

Reagents and chemicals

Exo III was obtained from New England Biolabs Ltd. (Beijing, China), and used without further purification. SG I (10000×) was purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). ATp was brought from Sangon Biotechnology Co., Ltd. (Shanghai, China). Uridine triphosphate (UTP), guanosine triphosphate (GTP) and cytidine triphosphate (CTP) were all purchased from Shanghai yuanye Bio-Technology Co., Ltd. (Shanghai, China). DNA oligonucleotides used in this work were synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China). The sequences of oligonucleotides are given as follows:

ATP-aptamer: 5’-AAC CTG GGG GAG TAT TGC GGA GGA AGGT-3’. Complementary-DNA: 5’-ACC TTC CTC CGC AAT ACT CCC CCA GGT-3’.

An ultrapure water from Millipore Milli-Q water purification system (Billerica, MA) with a resistivity higher than 18.2 MΩ cm⁻¹ was used for all experiments. All samples were prepared by dissolving in 20 mM Tris-HCl buffer (pH 8.3) containing 2 mM MgCl₂. All other reagents were of analytical grade, and were used freshly without further purification.

Apparatus

A Hitachi F-7000 fluorescence spectrometer (Hitachi Ltd., Japan) was used to record the fluorescence spectra. The optical path length of a quartz fluorescence cell was 1.0 cm. The excitation wavelength was set at 497 nm, and the emission spectra were recorded in the range from 508 to 600 nm with both excitation and emission slits of 5 nm; the PMT detector voltage was 950 V. All fluorescence detections were carried out under room temperature, unless otherwise indicated.

Procedure

ATP-aptamer and its complementary strand were used as received, and diluted in sterilized water to give stock solutions of 10 μM. To form dsDNA, the prepared ATP-aptamer was hybridized with its complementary strand to the same concentration (10 μM) and volume (10 μL) in Tris-HCl buffer (20 mM, pH 8.3, 2 mM MgCl₂) in order to obtain a total volume of 100 μL. The mixture was heated at 90°C for 10 min, followed by slowly cooling down to room temperature. The obtained dsDNA (1 μM) was stored at –20°C for further use. It was diluted into a suitable concentration during the experiment. The diluted dsDNA (10 nM) was mixed with SG I (1×) in Tris-HCl buffer (20 mM, pH 8.3, 2 mM MgCl₂) with different amounts of ATP, which was kept at 25°C for 40 min. 10 U Exo III was added to the above-mentioned solution for a final volume of 100 μL, and then incubated at 37°C for 30 min. After that, the fluorescence was measured and recorded on a Hitachi F-7000 fluorescence spectrometer.

Results and Discussion

Principle of the ATP sensing system

The sensing mechanism of the label-free fluorescent aptasensor is shown in Scheme 1. The ATP-aptamer (pink) was initially hybridized to its complementary sequence (blue). In the absence of ATP, the dsDNA bound to SG I and showed enhanced fluorescence. The complex was digested by Exo III from the 3’-end of the dsDNA, resulting in the release of SG I into solution. As a result, a reduced background signal was obtained. However, in the presence of ATP, the ATP-aptamer in the dsDNA structure bound specifically to its target while generating a G-quadruplex structure, and the complementary DNA was released. When stained with SG I, a strong fluorescence signal could be observed. Upon the addition of Exo III, the fluorescence intensity was supposed to remain unchanged because that the G-quadruplex structure was resistant to Exo III digestion. Based on the big difference of the fluorescence signal, the detection of ATP was expected to be realized.

In order to verify the feasibility of the present assay, an assay for proof of principle was performed. As shown in Fig. 1A, the fluorescence response of SG I (1×) in Tris-HCl buffer (20 mM, pH 8.3, 2 mM MgCl₂) was extremely low (Fig. 1A, red line). After the addition of 10 nM dsDNA, the fluorescence intensity of the system showed a great increase (Fig. 1A, green line) due to the specific recognition of SG I to dsDNA. The fluorescence further increased in the presence of 5 mM ATP (Fig. 1A, blue
line) for the structure switching of ATP-aptamer and the generation of G-quadruplex structure. As can be seen from Fig. 1B, after the addition of Exo III to the above systems, the fluorescence intensity decreased for both the complex with and without ATP (Fig. 1B, blue and green line); the fluorescence response of SG I (Fig. 1B, red line) was almost the same as it was in the absence of Exo III. Note that the fluorescence of the system with Exo III (Fig. 1B, blue line) decreased compared to system without Exo III (Fig. 1A, blue line), because that a portion of the G-quadruplex structure was digested by Exo III. However, the $S/N$ was highly different between the complex with and without Exo III. In the absence of Exo III, the $S/N$ was 1.41. However, in the presence of Exo III, the $S/N$ reached 6.92. Based on the big difference of $S/N$, our proposed assay can be applied to detect ATP. Further, we tested the response of a system containing only ATP-aptamer in a single-stranded conformation and without the

Fig. 1 Fluorescence spectra of the proposed sensing ATP platform: A. without or B. with Exo III (10 U) in Tris–HCl buffer (20 mM, pH 8.3, 2 mM). C. Fluorescence spectra of the single-stranded ATP aptamer (10 nM) system. Condition: SG I (1×), dsDNA (10 nM), ATP (5 mM).

Fig. 2 A. Dependency of $(F - F_0)/F_0$ on the concentration of SG I for ATP detection. B. Effect of the incubation time of SG I (1×) with dsDNA (10 nM) and ATP (2.5 mM) on the fluorescence intensity of the mixture. C. Dependency of $(F - F_0)/F_0$ on the amount of Exo III for ATP detection. The error bars represent the standard deviations based on three independent measurements.
Addition of Exo III. As can be seen from Fig. 1C, the fluorescence intensity became enhanced in the presence of ATP due to the formation of the G-quadruplex structure. However, the S/N of the single aptamer system was 5.50 at a concentration of 5 mM ATP, which was lower than the system of the duplex DNA structure was chosen for the detection of ATP.

Optimization of the experimental conditions

In order to achieve the best assay performance, several experimental parameters, including the concentration of SG I, the intercalating time of SG I to dsDNA and ATP and the amount of Exo III were optimized. The optimization of the experimental conditions for the present system is illustrated in Fig. 2. Figure 2A reveals optimization of the concentration of SG I for ATP detection. It shows that when the concentration reached 1×, the value of \((F - F_0)/F_0\) was the highest; here, \(F\) and \(F_0\) represent the fluorescence intensity in both the presence and absence of ATP. As a consequence, SG I (1×) was selected for the proposed assay. Figure 2B displays the effect of the intercalation time of SG I (1×) to dsDNA (10 nM) and ATP (2.5 mM) on the fluorescence intensity of the mixture at 25°C. As shown in the figure, when the time reached 30 min, the fluorescence intensity almost became a horizontal line. In order to obtain the best performance, we chose a time of 40 min for the incubation. The amount of Exo III was also optimized, as shown in Fig. 2C. As can be seen from the figure, when the units of Exo III became 10 U, \((F - F_0)/F_0\) reached the maximum value. Accordingly, 10 U Exo III was used for the following assay.

Sensitivity and selectivity

The detection of varying concentrations of ATP was conducted under the optimal experimental conditions; definitely, 1× SG I intercalated with 10 nM dsDNA at 25°C for 40 min, followed with 10 U Exo III incubation at 37°C for 30 min. As such, we obtained the fluorescence emission spectra of different amounts of ATP. The results are displayed in Fig. 3. It can be seen that the fluorescence intensity of the system increased with the increasing concentration of ATP in the range of 50 μM to 5 mM (Fig. 3A). There was a good linear relationship between the peak intensity of the spectrum and the concentration of ATP in the range from 50 μM to 5 mM (Fig. 3B). The increased fluorescence emission can be ascribed to the formation of the G-quadruplex structure induced by ATP binding, the structure of which was resistant to the digestion of Exo III. The detection limit of ATP was estimated to be 5 μM based on the criterion of three-times the standard deviation of the blank, which was comparable to those of previous label-free fluorescence assays reported.23,24

To demonstrate the selectivity of the analytical strategy toward ATP, the fluorescence responses of three ATP analogs were evaluated under the same experimental conditions. As shown in Fig. 4, when the system contained 2.5 mM GTP, CTP, or UTP, no obvious increase in the fluorescence intensity could be observed. However, when 2.5 mM ATP was added to the system, a strong fluorescence intensity could be observed. Owing to the high specificity of the ATP-aptamer for its target ATP and the resistance of the formed G-quadruplex structure to Exo III digestion, our proposed method shows excellent selectivity.

Conclusions

In conclusion, we designed a label-free, simple aptamer sensor...
to selectively detect ATP. The presence of the target ATP led to a structure transformation of the ATP-aptamer in dsDNA, and subsequent formation of the G-quadruplex structure. The structure was highly fluorescent when stained with SG I, and was resistant to the cleavage of Exo III. Thereby, the addition of Exo III reduced the background signal. Our approach exhibited great selectivity for ATP against its analogues. Moreover, this label-free method avoided laborious and time-consuming labeling and separation steps. In view of the mentioned merits, the proposed method may offer a direction in the design of simple and sensitive strategies for the detection of other small molecules.

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References