Optimization of fluorescent aptamer sensor for ATP detection

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Abstract. Abnormal concentrations of ATP are associated with many diseases and cancers, and quantitative detection of ATP is thus of great importance for disease diagnosis and prognosis. In order to obtain a simple and quickly detection method of ATP by fluorescence aptasensor, the principle of ATP detection based on resonance energy transfer between FAM and GO was studied. And then the optimization of process conditions was carefully researched. The results showed that when the concentration and reaction time of GO was 10μg/mL and 5min, respectively, the quenching light intensity was the easiest to recover. With the optimized parameters, the concentration of ATP in the range of 1-250μmol/L had a good linear relationship with the fluorescence intensity, and the detection limit was 1μmol/L. Besides, the selectivity experiments proved the selectivity of the constructed aptasensor toward ATP. This method creates the opportunity to detect a broad range of targets by using appropriate aptamers, holding great potential in the field of food safety and biomedical diagnostics.

1. Introduction
Abnormal concentrations of ATP are associated with many diseases and cancers, such as Hypoxia, hypoglycemia, Parkinson's disease and some malignant tumors [1-4]. In addition, the utilization efficiency of ATP is also used as an important indicator of cell survival energy and cell injury [5]. So quantitative detection of ATP is thus of great importance for disease diagnosis and prognosis [6]. At present, conventional methods, such as chemiluminescence [7-9], high performance liquid chromatography (HPLC) [10-12], and mass spectrometry [13,14] have been widely used in the detection of ATP. However, these methods are not suitable for real-time analysis because of their costly apparatus and complicated pretreatment procedures. So there is a demand for biosensors capable of rapid, accurate and reliable determination of low concentrations of ATP.

Aptamers have prominent advantages in detecting small molecular biological targets due to their high stability, low cost, simply synthesis, easy marking, no immunity source and toxicity [15-17]. But, aptamers cannot produce any light or electrical signals. Fluorescence aptasensor has been widely researched for biomolecular detection in in clinical diagnostics and food safety fields, because of its high sensitivity from fluorescence detection and its high selectivity according to nucleic acid aptamer [18]. Recently, fluorescent aptasensors have been widely researched for detections of many biomolecules, such as AFB1 [19,20], DA [21-23], AD [24] and so on. These aptasensors recognize and capture targets with aptamers which exhibit their high affinity and specificity, and then measuring the concentrations of target molecules by fluorescent signal variation.

In the present work, we report a simple and quickly detection method of ATP by fluorescence aptasensor based on resonance energy transfer. And by optimizing the detection parameters, a wide detection range with high detection limit of ATP were obtained. The principle of ATP detection was
shown in Figure 1. When the graphite oxide is added to nucleic acid aptamer solution modified by FAM, fluorescence intensity of FAM will decreases significantly due to resonance energy transfer. After the target is added, the aptamer will be first combined with the target, leading to FAM dissociation from graphene oxide and fluorescence recovery. The amount of restoring fluorescence intensity is related to the concentration of the target.

Figure 1. Schematic diagram of ATP detection principle.

2. Materials and Methods

2.1. Reagents and materials
Tris (2-carboxyethyl) phosphine (TCEP) and adenosine triphosphate (ATP) aptamer were purchased from Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. (Shanghai, China). ATP aptamer: 5’-FAM-ACCTGGGGAGTATTGCGGAGGAAGGT-3’. ATP were purchased from Beijing Solarbio Science & Technology Co., Ltd (Beijing, China). Phosphate buffer solution (PBS, 10mM, pH 7.4), Tris-acetate buffer (10mM, pH 5.2). All chemical reagents were of analytical grade and the ultrapure water used throughout the study was prepared by PURELAB Option-R (ELGA LabWater, UK).

2.2. Instruments
Fluorescence measurements were performed on a F-7000 fluorescence spectrophotometer (Hitachi, Japan). Eppendorf centrifuge 5418 (Hamburg, Germany) was used for the centrifugation. All pH values were acquired on a FE-20K pH-meter (METTLER TOLEDO, Switzerland). GL-16II centrifuge (Shanghai Anting Scientific Instrument Factory) and 07HWS-2 digital thermostat magnetic stirrer (Hangzhou Instrument Motor Co., Ltd.) were also used in the experiment.

2.3. Preparation of detection probes
Briefly, the aptamer stored at -20 °C was centrifuged for 30 s (12,000 rpm), then added Tris-HCl to bring the final concentration of FAM-aptamer to 1 μmol/L. The solution was incubated at room temperature for 2 hours under dark. The FAM-aptamer were mixed with the oxidized octaene solution, stand at room temperature for a period of time to immobilize the FAM-aptamer on the graphene oxide and the fluorescence of the FAM was quenched (Figure 1).

2.4. Fluorescent detection of ATP
Various concentrations of ATP (1 to 250 μmol/L) were prepared in a stock solution (50mmol Tris, 30mmol NaCl, 50mmol KCl) and added to the prepared probe solution. After incubation for a period of time, 400μL was taken into a fluorescence spectrometer to detect fluorescence. Then, the ATP working solution was used as a blank control group to calculate the fluorescence intensity of the different concentrations of the test solution and draw a standard curve.

3. Results and Discussion

3.1. Feasibility of the fluorescent aptasensor
The fluorescence emission spectra of FAM-DNA, FAM-DNA+GO, FAM-DNA+GO+ATP were shown in Fig.2. The fluorescence intensity of FAM sharp dropped with the addition of GO (10μg/mL, 900μL), which due to the fluorescence resonance energy transfer from FAM to GO. When ATP (1μM, 100μL) was added, the ATP was specific binding with its aptamer. Thus, the FAM-DNA would detach from the surface of GO, and the fluorescence intensity would recovered. The amount of restoring fluorescence intensity would be related to the concentration of the ATP.

3.2. Optimization of experimental conditions

In this method, the degree of fluorescence quenching will directly influence the intensity of light recovery, which will affect the measurement results. The degree of fluorescence quenching is affected by the concentration and reaction time of GO. To optimize these parameters, fluorescence spectra of ATP aptamer modified by FAM solution added by different concentrations of GO solution (2, 5, 10, 12, 15, 20μg/mL, Figure 3) and different reaction times (1-15min, Figure 4) were measured. The results showed that when the concentration and reaction time of GO was 10μg/mL and 5min, respectively, the quenching light intensity was the easiest to recover.
3.3. Quantitative detection of ATP

With the optimized parameters, different concentrations of ATP were measured. Fig. 5(a) showed the fluorescence spectra of restoring fluorescence intensity of QDs/AuNPs-aptamer/DA system with different concentrations (0.05, 25, 100, 150, 200, 250 μM) of DA. The calibration curve of ATP concentration and fluorescence intensity was shown in Figure 5(b). The results showed that the concentration of ATP in the range of 1-250 μmol/L had a good linear relationship with the fluorescence intensity. The linear regression equation was $Y=1.5266X+96.16572$ (Y was the fluorescence intensity and X was the ATP concentration). The regression coefficient is 0.94905, and the detection limit (The minimum that the sample can be accurately detected) was 1 μmol/L.

![Figure 5(a). Fluorescence signal curves of ATP.](image1)

![Figure 5(b). Calibration curve of ATP concentration and fluorescence intensity.](image2)

3.4. Selectivity of DA based on the assay

In order to analyze the specificity of this method to ATP, Na+, K+, DA, Biotin, adrenaline (AA), and ATP were all tested with the same parameters (100 μL, 1 μM) and the same experimental conditions. Compared with other subjects, ATP has significant specificity which were shown in Figure 6.

![Figure 6. The specific detection of ATP (1 μmol/L).](image3)
4. Conclusions

In conclusion, we report a simple and quickly detection method of ATP by fluorescence aptasensor based on resonance energy transfer. The principle of ATP detection was studied, the detection parameters were optimized, and then different concentrations of ATP were detected. Finally, The specificity of this method for ATP detection was tested. The results showed that with the optimized parameters, the concentration of ATP in the range of 1-250μmol/L had a good linear relationship with the fluorescence intensity, and the detection limit was 1μmol/L. Besides, compared with other subjects, ATP has significant specificity of this method, which may be applied for detection of pathogenic bacterium in food safety and biomedical diagnostics.

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