Biosensors and Bioelectronics 63 (2015) 562–565

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Short communication

A label-free fluorescence strategy for sensitive detection of ATP based on the ligation-triggered super-sandwich

Chunshui Lin [a], Yiying Chen [a], Zhixiong Cai [a], Zhi Zhu [a], Yaqi Jiang [a], Chaoyong James Yang [a,c,*], Xi Chen [a,b,*]

[a] Department of Chemistry and the MOE Key Laboratory of Spectrochemical Analysis & Instrumentation, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China
[b] State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361005, China
[c] State Key Laboratory of Physical Chemistry of Solid Surfaces, The Key Laboratory for Chemical Biology of Fujian Province, Xiamen University, Xiamen 361005, China

ARTICLE INFO

Article history:
Received 2 June 2014
Received in revised form
24 July 2014
Accepted 6 August 2014
Available online 15 August 2014

Keywords:
ATP
T4 DNA ligase
Super-sandwich
SYBR Green I
Fluorescence
Label-free

ABSTRACT

In this study, a label-free fluorescence strategy for sensitive detection of ATP based on the ligation-triggered super-sandwich is reported. We designed a double-stranded DNA (ds-DNA) probe as the substrate of ATP-dependent ligation. SYBR Green I (SG I), a double-duplex DNA specific dye, was employed as the readout signal. In the absence of ATP, the ligation would not occur and the ds-DNA remained intact. Further, a weak fluorescence could be observed due to the intercalation of SG I into the grooves of the ds-DNA probe. In the presence of ATP, T4 DNA ligase would catalyse the ligation between 3′-OH and 5′-PO_4 ends between ds-DNA probes. As a result, more binding sites of the SG I were generated and a fluorescence enhancement was obtained. This method showed a good sensitivity with a detection limit of 200 pM and could perfectly discriminate ATP from its analogs.

1. Introduction

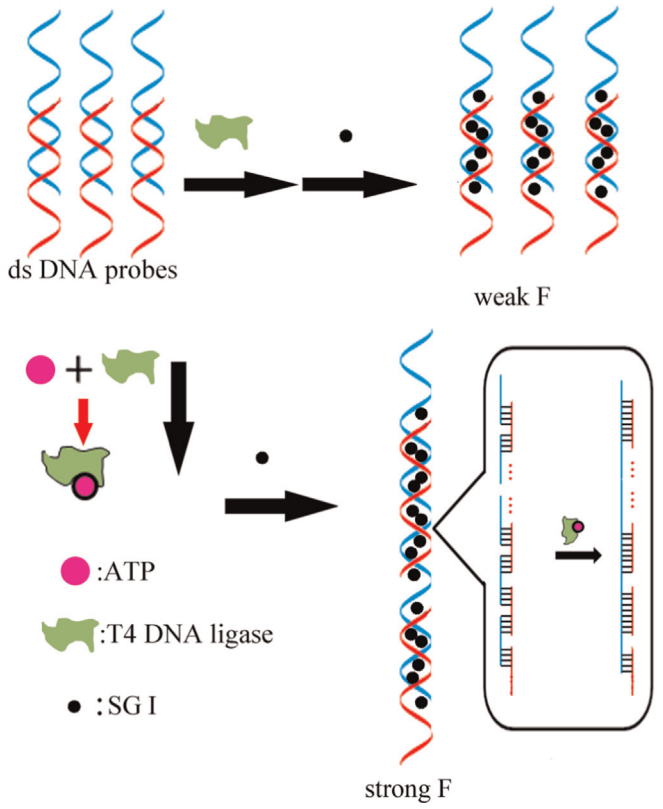
Adenosine triphosphate (ATP) is a universal energy source that plays an important role in many biological processes such as the regulation of cellular metabolism and biochemical pathways in cell physiology (Abraham et al., 1997; Compagnone and Guilbault 1997; Perez-Ruiz et al., 2003). Many human diseases such as cardiovascular diseases and Parkinson's and Alzheimer's disease are associated with changes in the ATP level (Sriskanda and Shuman, 2001; Yokoshiki et al., 1998) In addition, ATP has been widely used as an indicator of cell viability and injury. Therefore, it is of particular importance to develop a sensitive and specific method (Ma et al., 2008) for its determination.

Several strategies to assess the ATP level have been developed. Traditional methods are mainly based on high-performance liquid chromatography (Khlyntseva, 2009), mass spectrometry (Kennedy et al., 1999) and bioluminescence (Zyryanov et al., 2007). However, most of these ATP-detection approaches are complicated and expensive and cannot distinguish ATP from its analogs, such as adenosine (A), adenosine 5′-monophosphate (AMP), and adenosine diphosphate (ADP). Recently, an aptamer-based ATP-sensing strategy has shown tremendous potential and versatility due to the binding between the aptamer and ATP (Wang et al., 2005, 2008). The aptamer-based ATP methods generally use the conformational change of the aptamers upon ATP binding which needs a long incubation time. Among these methods, nanoparticles (Wang et al., 2008), graphene oxide (Wang et al., 2010) and enzymes (Ma et al., 2013) have been widely applied to improve their performance. These methods have shown high sensitivity and selectivity but it usually takes several hours to finish the detection. Several novel strategies for ATP detection based on the ATP-dependent ligation reaction have been reported (Ma et al., 2008, 2012, 2013). Owing to the high sensitivity of the molecular beacon and the high substrate dependence of T4 DNA ligase, these methods have shown very good analytical performances. However, these reported fluorescence-based ATP detection strategies rely on a probe that is modified with a fluorophore and a quencher and thus suffer from increased complexity and high cost.

As a proof of concept, we developed a fast and label-free strategy for highly sensitive and selective detection of ATP. Some enzymatic reactions show specific dependence on certain

* Corresponding authors at: Department of Chemistry and the MOE Key Laboratory of Spectrochemical Analysis & Instrumentation, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China.
Tel./fax: +86 592 2184530.
E-mail address: xichen@xmu.edu.cn (X. Chen).

http://dx.doi.org/10.1016/j.bios.2014.08.011
0956-5663/



Scheme 1. The detection principle for ATP based on an ATP-triggered super-sandwich.

cofactors (Lu et al., 2011), which provides an efficient platform for constructing a highly selective sensing system for the cofactors. In order to develop the sensing system for ATP with high selectivity, T4 DNA ligase was applied as it specifically employs ATP as a cofactor and its catalytic activity is cofactor dependent. In this work, we selected a double-stranded DNA (ds-DNA) probe which can form a sticky end called Pst I (Scheme 1). In the presence of the T4 DNA ligase and its cofactor ATP, the ds-DNA probes can form a super-sandwich which can be detected using ds-DNA-specific fluorescent SYBR Green I (SG I). The strategy could be confirmed by gel-electrophoresis results and it provided a detection limit as low as 200 pM of ATP. The detection process only took about 40 min, revealing it as a fast fluorescence strategy in ATP determination.

2. Materials and methods

2.1. Materials

The T4 DNA ligase was purchased from Takara Biotechnology Co., Ltd. (Dalian, China). SG I, ATP, adenosine, adenosine diphosphate, adenosine 5′-monophosphate, uridine triphosphate, cytidine triphosphate and guanosine triphosphate were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). The original solution of SG I was 10,000-fold concentrated and about 19.8 mM, based on the Lambert–Beer measurement. SG I was first diluted to 19.8 μM with ultrapure water before use. All other reagents were of analytical grade and were used without additional purification. All solutions were prepared with ultrapure water (≥ 18 MΩ, Milli-Q, Millipore).

The oligonucleotides were synthesized and purified by Sangon Biotechnology Co., Ltd. (Shanghai, China) and their base sequences in detail were as follows:

SP: 5′-PO_4-ACGTCCAGCACTCCGAGCG-3′
AP: 5′-TGGACGTCGCTCGGAGTGC-3′

2.2. Ligation-triggered super-sandwich assay protocol

A buffer containing 50 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$ and 1 mM dithiothreitol (DTT) was used for the ligation reaction and fluorescence assay. In a typical procedure, first, 20 μL of 100 nM ds-DNA probe solution was achieved by mixing SP and AP solutions (50 mM Tris–HCl pH 7.4, 5 mM $MgCl_2$, 1 mM DTT) and it was denatured at 90 °C for 5 min and cooled down to room temperature for further experiments. Then, T4 DNA ligase (final concentration 0.05 U/μL) with varying concentrations of ATP was introduced into the 20 μL 100 nM ds-DNA solution for 30 min to induce ligation at room temperature (25 °C). After that, 20 μL of 19.8 μM SG I was added for 5 min of incubation. Finally, the mixture was diluted to 200 μL and its fluorescence intensity was measured.

2.3. Fluorescence measurement

All fluorescence measurements of samples were performed on an F-4500 spectrophotometer (Hitachi, Japan) at room temperature. The excitation wavelength was set at 497 nm and the emission spectra were collected from 505 nm to 600 nm with both excitation and emission slits of 5 nm.

2.4. The verification of the ATP-triggered super-sandwich reaction using nondenaturing (PAGE)

A 12% native polyacrylamide gel was prepared using 1 × TBE (89 mM Tris–HCl, pH 8.0, 89 mM Boric acid, 2.0 mM Na_2EDTA). 10 μL mixtures before and after reaction with ATP were used for the PAGE. The gel was run at 100 V for 60 min in 1 × TBE buffer. Then, it was stained in the Stains-all solution (0.1 g Stains-all, 450 mL formamide, 550 mL H_2O) for 30 min. After that, the gel was illuminated under sunlight for 5–10 min to obtain the stained bands. Finally, the gel was photographed with a digital camera.

3. Results and discussion

3.1. Design principle of the strategy

The method design is shown in Scheme 1. The ds-DNA probe was prepared using two short DNA probes: SP and AP. SP was 5′-end PO_4 modified. In the absence of the cofactor ATP, the ligation reaction could not occur. SG I would intercalate into the short ds-DNA probes, giving a weak background noise. Whereas, when ATP was added to the system, T4 DNA ligase would catalyse the ligation between 3′-OH and 5′-PO4 ends between ds-DNA probes, leading to the formation of super-sandwich structured DNA. Thus, more SG I would be stained into the grooves of the probes, resulting in a fluorescence enhancement.

3.2. Feasibility of the strategy

To demonstrate the feasibility of this principle, the fluorescence emission spectra and gel-electrophoresis results are shown in Fig. 1. As shown in Fig. 1A, in the presence of the ds-DNA probe (100 nM), the fluorescence intensity (curve b) of SG I was slightly