Sensors and Actuators B 238 (2017) 175–181

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Label-free chemiluminescent strategy for highly selective and sensitive detection of adenosine triphosphate by cofactor-dependent enzymatic ligation-triggered polymerase chain reaction

Lu-Yan Yao [a,b], Xiao-Qian Yu [a,b], Rui-Jin Yu [c], Yan-Jun Zhao [a,b], Ai-Ping Fan [a,b,*]

[a] School of Pharmaceutical Science and Technology, Tianjin University, Tianjin 300072, PR China
[b] Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Tianjin 300072, PR China
[c] College of Science, Northwest A&F University, Yangling, Shaanxi 712100, PR China

ARTICLE INFO

Article history:
Received 19 February 2016
Received in revised form 27 June 2016
Accepted 13 July 2016
Available online 15 July 2016

Keywords:
ATP-dependent enzymatic reaction
Rolling circle amplification
Adenosine triphosphate
Graphene oxide
Chemiluminescence
Label free

ABSTRACT

In this work, a novel label-free chemiluminescent (CL) assay system was developed for highly sensitive and selective detection of adenosine triphosphate (ATP). The strategy relies on the powerful signal amplification capability of rolling circle amplification (RCA), the preferential binding ability of graphene oxide (GO) to single-stranded DNA (ssDNA), and instantaneous derivatization reaction between phenylglyoxal (PGO) and guanine nucleobases (G). In the presence of ATP, the T4 DNA ligase catalyzes the ligation reaction between the two ends of the padlock probe producing a closed circular DNA template that initiates the RCA reaction with phi29 DNA polymerase and dNTP. Therein, many complementary copies of the circular template can be generated. The formed long single-stranded DNA which contained an amount of guanine bases could be adsorbed on the surface of GO forming DNA-GO complexes. And the CL signal of DNA-GO complexes can be obtained *via* the instantaneous derivatization reaction between PGO and guanine bases. The CL signal increased linearly with the concentration of ATP from 0.1 to 2.5 nM with a detection limit of 0.03 nM. The system also showed high specificity to ATP against its analogues such as CTP, GTP, UTP, AMP, ADP, BSA and CaM. In addition, ATP has been determined in diluted serum indicating the applicability of this assay.

© 2016 Published by Elsevier B.V.

1. Introduction

ATP, as an universal energy storage molecule in all living organisms, plays an important role in regulation of cellular metabolism [1,2]. The intracellular ATP level has been used as an indicator for cell viability and cell injury [3]. The detection of ATP therefore is of great importance in clinical diagnosis, food quality control and environmental analyses. In recent years, various sensing system for ATP detection have been developed using CL [4–6], electrochemiluminescence (ECL) [7], electrochemistry [8,9], fluorescence [10,11], colorimetric [12,13], and surface plasmon resonance (SPR) [14] methods. Compared with other methods [15,16], CL analysis attracted a lot of attention owing to the superiority of CL analysis such as high sensitivity, broad linear range, simple devices, and low cost. For example, Zhao group [4] developed an aptasensor for ATP based on chemiluminescence resonance energy transfer (CRET) with a detection limit of 185 nM. Bi group [5] developed a sensing probe for ATP based on CRET with a detection limit of 110 nM. Our group [6] developed a label-free CL aptasensor for the detection of ATP using GO nano-platform with a detection limit of 70 μM. However, most of these methods suffered from moderate sensitivity and selectivity because most of these methods developed in recent years depended on anti-ATP DNA aptamer. The low sensitivity may be derived from low affinity of anti-ATP DNA aptamer to ATP because of the relatively low association constant between anti-ATP DNA aptamer and ATP. And the low selectivity may derive from the disability of anti-ATP DNA to discriminate ATP from its analogues, such as AMP and ADP.

Instead of aptamer-based ATP sensors, some enzyme reactions, namely ATP-dependent enzymatic reaction (ATP-DER), show specific dependence on ATP providing an efficient platform for constructing highly selective and sensitive sensing systems for ATP detection [17]. For example, ATP is a cofactor for the activation of T4 DNA ligase. In other words, in the presence of ATP, T4 DNA ligase can ligate the 3′-OH and 5′-PO_4 ends of the pad-

* Corresponding author at: School of Pharmaceutical Science and Technology, Tianjin University, Tianjin 300072, PR China.
E-mail address: aipingfan1980@163.com (A.-P. Fan).

http://dx.doi.org/10.1016/j.snb.2016.07.061
0925-4005/© 2016 Published by Elsevier B.V.

lock probe resulting in the formation of a closed circular DNA template. This kind of enzymatic ligation reaction shows specific dependence on its cofactor ATP, which, in turn, provides an efficient platform for constructing highly selective biosensing systems for the target molecule ATP. Using ATP as a cofactor of T4 DNA ligase, several fluorescent [18,19], electrochemical [20] and CL [21] sensing systems have been reported for the detection of ATP. These ATP-DER-based ATP sensing system exhibited high sensitivity and selectivity for ATP detection. Recently, our group [21] has developed an ATP-DER-based CL detection system for ATP by taking advantage of the powerful signal amplification capability of RCA and hydroxylamine-amplified gold nanoparticles. RCA, an isothermal amplification method, could amplify DNA by generating thousands of long, tandemly repetitive ssDNA strands that are complementary to the circular DNA template. As low as 100 pM of ATP could be sensitively detected using ATP-triggered RCA. However, the proposed method is time consuming because several tedious processes were involved such as preparation of gold probe, gold staining, and gold dissolution. In order to overcome these problems, the present study aimed to develop a rapid and simple CL method for label-free detection of ATP by using GO.

GO, a mono-layer carbon arranged in a honeycomb structure has recently attracted intense interest due to its unique electrochemical properties, high thermal conductivity, excellent mechanical flexibility, large accessible surface area, as well as good biocompatibility [22,23]. GO exhibits strong adsorption ability to ssDNA *via* π-π stacking interaction between the hexagonal cells of GO and the ring structure in the nucleobases and nucleosides [24–26]. In recently, our group [6] found that GO could be used as nanoplatform for the construction of label-free CL biosensor because instead of CL quenching GO-ssDNA complexes could emit light *via* a specific instantaneous derivatization reaction between PGO and guanine nucleobases contained in ssDNA. Based on the unique characteristic of GO, the present study developed a simple and label-free CL method for highly sensitive and selective detection of ATP by taking advantage of the powerful signal amplification capability of RCA, strong adsorption ability of GO to ssDNA, and instantaneous derivatization reaction between PGO and guanine nucleobases (Scheme 1). The 3′- and 5′- terminal bases of the padlock probe were designed to be complementary to primer probe. In the presence of its cofactor ATP, T4 DNA ligase is activated, and can ligate the 3′-OH and 5′-PO_4 ends of the padlock probe resulting in the formation of a closed circular DNA template in an amount that is positively related to the concentration of the cofactor ATP. With addition of phi29 DNA polymerase and dNTP to the ligation products the RCA reaction is initiated leading to the production of long single-stranded DNA by replicating the circular template hundreds to thousands of times. The formed long single-stranded DNA which contains an amount of guanine bases can adsorb on the surface of GO forming DNA-GO complexes. And the CL signal of DNA-GO complexes can be obtained *via* the instantaneous derivatization reaction between PGO and guanine nucleobases.

2. Experimental

2.1. Materials and chemicals

All chemicals were of analytical reagent grade and were used as received. Distilled water (18.2 MΩ cm^{-1}) was used throughout the current work. Graphite powder (99%) was purchased from Heowns Biochem. Technologies Co., Ltd. (Tianjin, China). PGO was purchased from Tokyo chemical industry Co., Ltd. (Tokyo, Japan). T4 DNA ligase, phi29 DNA polymerase and deoxynucleotides (dNTPs) were obtained from Thermo Fisher Scientific (China) Co., Ltd. Bovine serum albumin (BSA), dithiothreitol (DTT) and human serum were obtained from Puboxin Biotechnology Co. Ltd. (Beijing, China). Calmodulin was purchased from Sigma-Aldrich (St. Louis, MO). Tetrabutylammonium hydroxide, N, N-dimethylformamide (DMF), Tween 20 and other chemical reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). ATP, CTP, GTP, UTP, AMP, ADP, primer probe (5′-ATA TTA TTT ATT TTA TTT TTA ATA ATT AAT-3′), ATP primer-9G (5′-ATA TTA TTT ATT TTA TTT TTA ATA ATT AAT GGG TTA GGG TTA GGG-3′), ATP primer-15G (5′-ATA TTA TTT ATT TTA TTT TTA ATA ATT AAT GGG TTA GGG TTA GGG TTA GGG TTA GGG-3′) and padlock probe (5′-PO_4-TAA AAT AAA TAA TAT CCA CCT CCA CCT CCA CCT CCA CCT CCC TCC ACC TCC ACC TCC ACC TCC ACC ATT AAT TAT TAA AAA-3′) were acquired from Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China).

2.2. Apparatus

CL measurements were performed with BPCL chemiluminescence analyzer (Beijing, China).

2.3. Preparation of GO

Graphene oxide was synthesized from graphite powder by a modified Hummers method. In the pretreatment step, 25 mL of concentrated H_2SO_4 was heated to 90 °C in a 100 mL round bottomed flask containing 5 g $K_2S_2O_8$; 5 g P_2O_5 was added with stirring until all of the reactants were completely dissolved. The mixture was then cooled to 80 °C. 6 g of graphite powder was added into the mixture and then kept at 80 °C for 4.5 h. The mixture was finally diluted with 1 L of deionized water and purified using a 0.2 μm Nylon Millipore filter. The solid was transferred to a drying dish and then vacuum-dried.

In the oxidation step, 23 mL H_2SO_4 was placed into a 100 mL beaker and chilled to 0 °C using an ice bath. 0.5 g of pretreated graphite was then added to the acid and stirred. Three grams $KMnO_4$ was added slowly to the mixture that was then allowed to react for 2 h at 0 °C and another 2 h at 35 °C. The mixture was diluted with 46 mL deionized water and stirred for 2 h. After 140 mL of deionized water added in the solution, 10 mL of 30% H_2O_2 was added to the mixture resulting in a brilliant yellow color along with bubbling. The mixture was allowed to settle for at least a day, and then the clear supernatant was decanted. The soft sediment was centrifuged and washed with a total of 1 L of 10% HCl solution followed by 1 L of deionized water to remove the acid. The resulting solid was dried via lyophilization and diluted to make a 2% (w/w) dispersion that was put through dialysis for 2 weeks to remove any remaining metal.

2.4. Procedure of the CL detection of ATP

Before the ligation reaction, the padlock probe and primer probe was heated at 90 °C for 3 min, and then cooled down to room temperature. For the ligation reaction with T4 DNA ligase, 20 μL of reaction mixture which was consisted of 1 × ligation buffer (40 mM Tris–HCl, 10 mM $MgCl_2$, 10 mM DTT, pH 7.8), 0.5 U of T4 DNA ligase, 0.2 pmol of padlock probe, 1 pmol of primer probe, and different concentrations of ATP was incubated at 37 °C for 60 min. The reaction mixture was then heated at 65 °C for 10 min to denature the T4 DNA ligase and cooled down to room temperature to facilitate the hybridization reaction between primer probe and the circular DNA template which was formed during the ligation process. For RCA reaction, 10 μL of above ligation reaction mixture was mixed with 10 μL of reaction solution containing 33 mM Tris–acetate, 10 mM $Mg(Ac)_2$, 66 mM KAc, 1 mM DTT, 1 mM dATP, dTTP, and dCTP each, 0.1 mM dGTP, and 1 U of phi29 DNA polymerase. The resulting mixture was incubated 37 °C for 90 min, and then heated to 95 °C for 5 min to denature the phi29 DNA polymerase. The RCA product

Download English Version:

https://daneshyari.com/en/article/7142467

Download Persian Version:

https://daneshyari.com/article/7142467

Daneshyari.com