Biosensors and Bioelectronics 105 (2018) 103–108

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

An electrochemical biosensor for microRNA-196a detection based on cyclic enzymatic signal amplification and template-free DNA extension reaction with the adsorption of methylene blue

Jing Guo, Changjing Yuan, Qi Yan, Qiuyue Duan, Xiaolu Li, Gang Yi*

Key Laboratory of Medical Diagnostics of Ministry of Education, Department of Laboratory Medicine, Chongqing Medical University, Chongqing 400016, PR China

ARTICLE INFO

Keywords:
Electrochemical biosensor
MicroRNA
Cyclic enzymatic signal amplification
Template-free DNA extension reaction
Methylene blue

ABSTRACT

A simple and sensitive electrochemical biosensor was developed for microRNA-196a detection, which is of important diagnostic significance for pancreatic cancer. It was based on cyclic enzymatic signal amplification (CESA) and template-free DNA extension reaction. In the presence of microRNA-196a, duplex-specific nuclease (DSN) catalyzed the digestion of the 3′-PO_4 terminated capture probe (CP), resulting in the target recycling amplification. Meanwhile, the 3′-OH terminal of CP was exposed. Then, template-free DNA extension reaction was triggered by terminal deoxynucleotidyl transferase (TdT), producing amounts of single-stranded DNA (ssDNA). After ssDNA absorbed numerous methylene blue (MB), an ultrasensitive electrochemical readout was obtained. Based on this dual amplification mechanism, the proposed biosensor exhibited a high sensitivity for detection of microRNA-196a down to 15 aM with a linear range from 0.05 fM to 50 pM. This biosensor displayed high specificity, which could discriminate target microRNAs from one base mismatched microRNAs. It also showed good reproducibility and stability. Furthermore, it was successfully applied to the determination of microRNA-196a in plasma samples. In conclusion, with the excellent analytical performance, this biosensor might have the potential for application in clinical diagnostics of pancreatic cancer.

1. Introduction

MicroRNAs (miRNAs) are single-stranded non-protein-coding RNAs with approximately a length of 22 nucleotides. They play important roles in regulating gene expression (Bartel, 2004; Eulalio et al., 2008). Research evidence showed that aberrant expression of miRNAs has the high correlation with the occurrence of various cancers (Kent and Mendell, 2006; Ventura and Jacks, 2009). Recently, pancreatic cancer has become one of the most common causes of cancer-related death. Three independent groups have reported that the levels of several specific miRNAs (miR-21, miR-196a, miR-155 and so on) in humans peripheral blood are closely related to pancreatic cancer (Huang et al., 2016; Liu et al., 2012; Wang et al., 2009). These strongly suggest that miRNAs have great potentials to work as promising biomarkers for the diagnosis of pancreatic cancer. However, miRNAs are high sequence homology, small size, and low expression levels in plasma (Li et al., 2016). Thus, it is quite challenging to sensitively and specifically analyze miRNAs. Therefore, it is urgent to develop a sensitive approach for miRNAs detection.

Electrochemical sensors have the advantages of high sensitivity, low cost and few sample consumption (Liu et al., 2016). They have gained wide attention in miRNAs detection. To achieve high sensitivity, amplification strategies are usually used in biosensors, such as hybridization chain reaction (HCR) (Jie et al., 2017), rolling circle amplification (RCA) (Zhang et al., 2015b) and catalytic hairpin assembly (CHA) (Zhang et al., 2016). Nevertheless, these strategies often involve complex probe design process or complicated DNA sequence analysis. Thus, a kind of cyclic enzymatic signal amplification (CESA) catalyzed by duplex-specific nuclease (DSN) has been developed owing to its simplicity and high efficiency (Yin et al., 2012). In CESA, the specific cleavage preference of DSN for DNA in DNA-RNA hybrid duplexes is mainly used (Lu et al., 2016). During the digestion process, RNA can be reused through repeated cycles of hybridization, digestion and dissociation. As a result, the responding signal can be amplified significantly (Zhang et al., 2015a). More importantly, the nuclease has a wonderful capability to discriminate perfectly matched from slightly mismatched (even one base mismatch) short duplexes (Shagin et al., 2002). Despite many advantages, CESA suffers a relatively low sensitivity (Ren et al., 2013), which may counteract the analytical performance of biosensors.

* Corresponding author.
E-mail address: yigang666@126.com (G. Yi).

https://doi.org/10.1016/j.bios.2018.01.036
Received 1 November 2017; Received in revised form 6 January 2018; Accepted 17 January 2018
Available online 31 January 2018
0956-5663/

Template-free DNA extension reaction is another amplification method popular used in different sensing strategy. This amplification method is assisted by terminal deoxynucleotidyl transferase (TdT). TdT is a template-independent DNA polymerase (Liu et al., 2014). It can catalyze the sequential addition of deoxyribonucleoside 5′-triphosphates (dNTP) at 3′-OH group of DNA to produce a long single-stranded DNA (ssDNA) structure directly (Shen et al., 2015). Since the template is not required in the extension reaction, the reaction system can be easily designed and operated. As a simple, direct and efficient isothermal amplification, it has become an essential step in the process of miRNA sensors design for nucleic acid detection and quantification. However, to the best of our knowledge, no electrochemical biosensor has been developed with the use of DSN assisted CESA and template-free DNA extension reaction.

Various metal nanoparticles are widely used, such as gold nanoparticles (AuNPs) (Saleh et al., 2017a), silver nanoparticles (AgNPs) (Alshalalfeh et al., 2016; Al-Shalalfeh et al., 2017; Saleh et al., 2016, 2017b) and copper nanoparticles (CuNPs) (Chi et al., 2017). Although these metal nanoparticles could generate excellent signals, they usually need a complicated process of fabrication. This may limit their application to some extent. Methylene blue (MB) was a redox complex. Compared with circular nanoparticles, Mb can bind specifically to guanine bases on ssDNA easily due to its unique planar structure (Nordin et al., 2016). And that it can be used without the complicated preparation. However, so far as we know, reports of MB used as a redox indicator for template-free DNA extension reaction are very rare.

To develop a simple and sensitive electrochemical biosensor for miRNA-196a detection, we combined CESA with template-free DNA extension reaction. In this cascade signal amplification strategy, only one nucleotide sequence was used. In addition, MB was properly used as an electroactive redox indicator in our strategy. What's more, because of the simple principle and probe design, the detection process is easy to implement, and the cost is reduced to a certain degree. Compared with other methods, our strategy exhibits superior specificity and lower detection limit. Due to the excellent analytical performance of this method, it may become a potential alternative tool for detection of miRNA in biomedical research and early clinical diagnosis.

2. Experimental section

2.1. Chemicals and materials

All HPLC-purified DNA oligonucleotides and miRNAs were synthesized by Sangon Biotech Co. (Shanghai, China), and their base sequences were listed in Table S1. 6-mercapto-1-hexanol (MCH) was purchased from Sigma (St. Louis, MO, USA). DSN and 10 × DSN master buffer (500 mM Tris-HCl, 50 mM $MgCl_2$, 10 mM DTT, pH 8.0) were obtained from Evrogen Joint Stock Company (Moscow, Russia). TdT, 10× terminal transferase reaction buffer (500 mM Potassium Acetate, 200 mM Tris-acetate, 100 mM Magnesium Acetate, pH 7.9) and 2.5 mM $CoCl_2$ were purchased from New England Biolabs. Ltd. (USA). Deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (dCTP), deoxythymidine triphosphate (dTTP), deoxyadenosine triphosphate (dATP) and methylene blue (MB) were obtained from Sangon Biotech Co. (Shanghai, China). Ultrapure water obtained from a Millipore water purification system (18.2 MΩ, MilliQ, Millipore) was used in all experiments. The ultrapure water used in miRNA-related experiments was treated with DEPC.

Several buffers were used in this study: Saline sodium citrate (SSC) buffer as the hybridization buffer (pH 7.4) contained 30 mM Sodium Citrate and 300 mM NaCl. 0.1 M phosphate buffered solution (PBS, pH 7.4) containing 0.1 M Na_2HPO_4, 0.1 M NaH_2PO_4, and 0.1 M $MgCl_2$ was served as the working buffer to perform electrochemical measurements. The washing buffer was PBS (0.01 M, pH 7.4) containing 0.05% (w/v) Tween-20.

2.2. Apparatus

All electrochemical assay measurements were performed on the CHI660D electrochemical workstation (Shanghai Chen Hua Instruments, Shanghai, China). A conventional three-electrode system was employed: Ag/AgCl electrode as reference, platinum wire as auxiliary and gold disk electrode (3 mm in diameter, 5 mm in length) as the working electrode (GaossUnion Technology Co., Ltd, Wuhan, China). Gel images were recorded on an imaging system (Bio-Rad Laboratories, USA). All measurements were carried out at room temperature.

2.3. Preparation of sensors

The bare gold disk electrode with a diameter of 3 mm was polished with 0.05 mm alumina slurry to a mirror-like finish and treated ultrasonically in ultrapure water for 5 min. The electrode was then soaked in freshly prepared piranha solution (the mixture of 98% H_2SO_4 and 30% H_2O_2 at a volume ratio of 3:1) for 10 min followed by rinsing thoroughly with ultrapure water to eliminate residual alumina powder. Afterwards, the pretreated electrode was dried at room temperature. Then, the obtained electrode was immersed into 10 μL of 10 μM MCH solution for 20 min. After washing with ultrapure water, 10 μL of 500 nM thiol-modified capture probe (CP) was dropped on the prepared electrode surface. Then, the electrode was incubated for 12 h at 4 °C. The electrochemical biosensor was rinsed with 0.01 M PBS (pH 7.4) containing 0.05% (w/v) Tween-20 and used for the following operation.

2.4. Amplified detection of miRNA

Droplets of 10 μL mixtures containing a series of different concentrations of the miRNA-196a and 0.06 U DSN in 1× DSN master buffer (50 mM Tris-HCl, 5 mM $MgCl_2$, 1 mM DTT, pH 8.0) were incubated with sensors for 100 min at 37 °C. Rinsed with the washing buffer and water, 10 μL of reaction mixture containing 5 U TdT, 4 mM dGTP, 0.8 mM dNTPs (dATP, dCTP and dTTP), 1× terminal transferase reaction buffer (50 mM Potassium Acetate, 20 mM Tris-acetate, 10 mM Magnesium Acetate, pH 7.9) and 0.25 mM $CoCl_2$ was dropped on the electrode surface and incubated at 37 °C for 60 min to form long ssDNA. After washing with PBS and ultrapure water, 10 μL of 0.1 M PBS buffer containing 0.5 mM MB was dropped on the electrode surface for 30 min in the dark. Finally, the electrode was washed with washing buffer. The differential pulse voltammetry (DPV) measurement was performed in the working solution (0.1 M PBS, pH 7.4) with a pulse period of 0.5 s, a pulse width of 0.2 s, a pulse amplitude of 50 mV and potential scan from − 0.4 V to 0 V.

2.5. Native polyacrylamide gel electrophoresis

The dual signal amplification strategy was verified by 8% native polyacrylamide gel electrophoresis (PAGE) in 1× TBE buffer (90 mM Tris-boric acid, 2 mM EDTA, pH 8.0). In this analysis, each sample contained 10 μL of the reaction solution and 2 μL of 6× loading buffer. The concentration of CP was 2 μM. CP was incubated with the same amount of miRNA-196a in the SSC hybridization buffer for 1 h at 37 °C. For CESA, 2 μM CP and 50 pM miRNA-196a was incubated with 0.06 U DSN at 37 °C for 2 h. Template-free DNA extension reaction was obtained by the incubation of the product of CESA, 5 U TdT, 4 mM dGTP and 0.8 mM dNTPs (dATP, dCTP, dTTP) for 1 h at 37 °C. After samples were added into the gel, they were run at 110 V constant voltage at room temperature for 30 min. Then, they were stained by GoldView I Nuclear Staining Dyes for 30 min and detected under UV light. Images were recorded by an imaging system.