Biosensors and Bioelectronics 65 (2015) 341–345

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Quartz crystal microbalance detection of protein amplified by nicked circling, rolling circle amplification and biocatalytic precipitation

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ARTICLE INFO

Article history:
Received 23 July 2014
Received in revised form
16 October 2014
Accepted 17 October 2014
Available online 31 October 2014

Keywords:
Quartz crystal microbalance
Rolling circle amplification
Biocatalytic precipitation
Lysozyme

ABSTRACT

A novel quartz crystal microbalance (QCM) assay was described for sensitive detection of protein. Lysozyme was used as a model of protein. To enhance the sensitivity of this QCM biosensor, biocatalytic precipitation (BCP) reaction combined with strand-scission cycle and rolling circle amplification (RCA) were applied together for the first time. As a result of the multi-signal amplification in this aptasensor, the detection limit for lysozyme was down to 0.3 fM. What is more, this amplified QCM biosensor also showed good selectivity and practical usage in human serum.

1. Introduction

QCM sensor is a piezoelectric device that uses the frequency shifts on quartz crystals to measure the changes of mass on the surface (Janshoff et al., 2000). Measurements can be performed in real time without any supplementary labeling and has been widely applied in biochemical analysis (Pei et al., 2007). However QCM measurement is often insufficient to produce a large signal change for trace detection due to the relatively small number of targets specifically bound to the QCM surface (Abad et al., 1998). To further increase the mass load on the surface of quartz crystals and amplify the frequency shift aimed at the trace analysis, several signal amplification strategies have been developed (Tang et al., 2012), for example the usage of gold nanoparticle (Song et al., 2014), enzyme (Fei et al., 2011), liposome (Patolsky et al., 2000) or self-assembled DNA nanostructure (Tang et al., 2012) as an efficient mass amplifier. Among them, enzymatic biocatalytic precipitation (BCP), involving the formation of insoluble products on electronic transducers (Yang et al., 2014), has been utilized as an important amplification route for sensing and biorecognition events. Now, BCP reaction has been used in the field of faradaic impedance spectroscopy (Hou et al., 2014), photoelectrochemical measurement (Zhao et al., 2012; Zhang et al., 2014) as well as QCM analysis (Fei et al., 2011; Feng et al., 2007).

Rolling circle amplification (RCA) is a simple and efficient isothermal enzymatic process that utilizes unique DNA and RNA polymerases to generate a long single DNA/RNA strand within a short period of time (Ali et al., 2014, Ji et al., 2012). This amplification method is achievable at a constant temperature, in solution, on a solid support or in a complex biological environment without specialized instrumentation (Zhao et al., 2008). Herein, we develop a sensitive QCM biosensing platform by combining three effective amplification strategies—nicked circling, RCA and BCP reactions, to develop a new method for straightforward and cost-effective detection of protein *via* aptamer-based bioassay. To the best of our knowledge, such amplified QCM aptasensor has not yet been reported.

2. Experiment

2.1. Materials and apparatus

2.1.1. Materials

Deoxyribonucleoside 5′-triphosphates (dNTPs) mixture and all oligonucleotides as depicted in Table S1 of Supporting information were purchased from SBS Genetech. Co. Ltd. (China). The Phi29 DNA polymerase (10,000 U/mL) and T4 DNA ligase (5000 U/mL) were obtained from Thermo Scientific. The restriction endonuclease Nb.BbvCI (10,000 U/mL) was purchased from NEW ENGLAND Biolabs (NEB). 4-Chloro-1-Naphthol (4-CN), lysozyme,

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http://dx.doi.org/10.1016/j.bios.2014.10.055
0956-5663/

thrombin, bovine serum albumin (BSA), and the IgG were purchased from Sigma-Aldrich. HRP conjugated Streptavidin was purchased from Sangon Biotech Co., Ltd. (China). The carboxyl-modified magnetic beads (MBs, 1.0–2.0 μm) were purchased from Tianjin Baseline ChromTech Research Centre (China). Milli-Q-deionized water was used throughout the experiments.

The stock solution of 4-CN was prepared by dissolving 4-CN in ethanol to give ethanolic solution of 0.05 M. Then, 1 mL ethanolic solution prepared above was diluted with 0.1 M phosphate buffered saline (PBS, pH 7.4) to 50 mL.

2.1.2. Apparatus

The resonance frequency was monitored by Q-Sense E1 QCM-D instrument (Q-Sense AB, Västra Frölunda, Sweden). QCM experiments were performed at a 5 MHz AT-cut quartz crystal (Q-Sense E1). The tapping mode AFM images were obtained using a Being Nano-Instruments CSPM-4000 (Benyuan, China) operating under ambient conditions. The imaging measurements were performed using AN-NSC10 Probe (NTI Co. Ltd., force constant 40 N/m, driving frequencies in the range of 220.4–394.6 kHz). The ratio of the setpoint and free cantilever vibration amplitudes Asp/A0 was maintained at 0.7–0.8. AFM was operated at a scan speed of 2 Hz.

Impedance measurements were carried out on CIMPS-1 electrochemical workstation (Germany). The supporting electrolyte is 10 mM PBS containing 0.1 M KCl and 2.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ as a redox couple.

2.2. Assembly on magnetic beads

Preparation of MB–aptamer–S1 conjugates was described in Supplementary material. During test, different concentrations of lysozyme (100 μL) were added to the conjugated MBs system. After shaking at 37 °C for 60 min and magnetic separation, the supernatant containing the released DNA S1 (100 μL) was obtained and kept at 4 °C before use.

2.3. Assembly on quartz crystal

Before use, the crystal chips were cleaned thoroughly by immersion in piranha solution (30% H_2O_2/70% concentrated H_2SO_4), followed by rinsing with deionized water and drying under a nitrogen stream. The pre-treated gold electrode was then incubated with 30 μL of capture probe DNA (S2, 100 nM) in pH 7.4 PBS at 37 °C for 16 h in a humidity chamber. After DNA S2 immobilization, 1% BSA was added and incubated for 2 h to passivate the electrode. After each step of fabrication process, the gold electrode was washed three times with pH 7.4 PBS and deionized water respectively, then dried under N_2 atmosphere.

A series of 25 μL volumes of reaction mixture containing the 21.5 μL DNA S1 supernatant obtained above, 2.5 μL 10 × NEB buffer and 1 μL Nb.BbvCI (10 U/μL) was dropped onto the electrode surface and incubated at 37 °C for 2 h.

2.4. RCA and BCP reaction

21.5 μL of padlock DNA (S3, 10 nM), 2.5 μL T4 DNA ligase buffer and 1 μL T4 DNA ligase (5 U/μL) were mixed in a centrifugal tube, then this mixture was transferred to the electrode surface and incubated at 22 °C for 1 h. Subsequently, the electrode was washed with PBS buffer and doubly distilled water, followed by drying under nitrogen gas. The RCA reaction was carried out by adding a mixture of 19 μL H_2O, 2.5 μL 10 mM dNTPs, 2.5 μL Phi29 DNA polymerase buffer and 1 μL Phi29 DNA polymerase (10 U/μL) onto the electrode. After incubation at 37 °C for 1.5 h, the electrode with the long single DNA strands produced by RCA was washed as before.

An aliquot of 25 μL biotin modified detection probe (S4, 10 nM) in PBS was dropped onto the electrode to form lots of DNA duplexes. After incubation at 37 °C for 30 min and washing, 25 μL HRP conjugated streptavidin (50 nM) was transferred to the electrode surface and incubated again at 37 °C for 30 min. Finally, the electrode was thoroughly rinsed as described above.

2.5. QCM measurement

During QCM measurement, 300 μL of the BCP solution containing 1 mM 4-CN and 1 mM H_2O_2 was pumped into reaction pool. The resonance frequency of the QCM was recorded until equilibrium was reached. The temperature was controlled at 25 °C throughout the measurement.

3. Results and discussion

3.1. Strategy for amplified QCM detection and its feasibility

This strategy can be used for the detection of nucleic acid as well as protein when aptamer was used. By combination of DNA nicked circling, RCA and BCP, a sensitive QCM detection of lysozyme was developed and described in Fig. 1A. Aptamer immobilized on MBs and its complementary oligonucleotides S1 were first hybridized to form a rigid duplex. When target lysozyme was added, due to the recgnition between aptamer and lysozyme, the single-stranded DNAs S1 were released and acted as triggers in following reaction. The released DNA S1 was then introduced to the detection system and hybridized with hairpin DNA S2 immobilized on gold electrode. The formed duplex DNA (S1/S2) contained a restriction endonuclease Nb.BbvCI recognition site, which led to the scission of DNA S2. Then the thermally unstable structure DNA S1 spontaneously dissociated and amplification was accomplished by the hybridization of free DNA S1 with another hairpin DNA S2, which led to the strand-scission cycle. In this way, one trigger DNA S1 results in the cleavage of many DNA S2 and large quantities of hairpin fragments are formed.

After incubating hairpin fragment immobilized on the gold electrode with padlock probe S3 and T4 ligase, the circular template was formed. The following RCA reaction could be initiated by adding Phi29 DNA polymerase and dNTPs to form a long single-stranded DNA, which stimulated the binding of abundant biotin-labeled DNA S4. Finally, the streptavidin-HRP could be captured onto the assembled gold electrode through the recognization between biotin and streptavidin. In the presence of H_2O_2, the HRP-biocatalyzed oxidation of 4-chloro-1-naphthol occurred and the formed precipitation deposited on the gold electrode surface was determined by QCM. Through the nicked circling, rolling cycle amplification reaction and biocatalytic precipitation, the QCM signals were significantly enhanced. Thus, a sensitive QCM biosensor for the detection of lysozyme was obtained.

As a proof-of-concept experiment, a series of control experiments was monitored (shown in Fig. 1B). In the absence of lysozyme, no DNA S1 was produced. While, in the presence of target lysozyme but no nicking enzyme Nb.BbvCI, T4 DNA ligase and Phi29 DNA polymerase, the formed duplex DNA (S1/S2) could not be cut. In both of these cases, RCA primer probe cannot be liberated and the RCA reaction cannot be preceded, let alone the incorporation of HRP probes. Therefore, the frequency shifts were negligible (curves *a* and *b*). In contrast, by the combination of DNA nicked circling, RCA and BCP amplification system, even when the concentration of lysozyme was as small as 1.0×10^{-13} M, frequency shift increased greatly (curve *c*), which indicating the proposed QCM strategy could be amplified by cycle system and enzymatic biocatalytic reaction.