Sensors and Actuators B 246 (2017) 402–407

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Research paper

Electrochemical proximity assay-coupled highly nonenzymatic amplifying strategy for total protein of *Nosema bombycis* detection

Yali Yuan*, Guangpeng Liu, Yaqin Chai, Ruo Yuan*

Key Laboratory of Luminescent and Real-Time Analytical Chemistry (Southwest University), Ministry of Education, Hanhong College, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China

ARTICLE INFO

Article history:
Received 12 October 2016
Received in revised form 9 January 2017
Accepted 17 February 2017
Available online 20 February 2017

Keywords:
Electrochemical proximity assay
Nonenzymatic amplification
Catalytic efficiency
Host-guest interaction
DNA concatamers

ABSTRACT

In this study, an electrochemical proximity assay (ECPA)-coupled highly nonenzymatic amplifying strategy with electron mediator collected by a host-guest interaction was proposed for total protein of *N. bombycis* (TP N.b) detection. Antibody-dsDNA-gold nanoparticles functional Fe_3O_4 probe (termed as Ab-dsDNA-Au@Fe_3O_4NPs), in which the Ab-dsDNA composed by adenine rich ssDNA (A_1) and Ab labeled capture ssDNA 1 (Ab-S_1), was first incubated with target TP N.b and another Ab labeled capture ssDNA 2 (Ab-S_2) to perform the proximity immunoreaction. Satisfyingly, the immunoreaction could not only produce sandwich immunocomplex with the proximity hybridization of S_1 and S_2, but also exposed the trigger ssDNA A_1 to in situ form substantial methylene blue (MB) intercalated DNA concatamers via hybrid chain reaction (HCR). To further realize the nonenzymatic amplification, the MB intercalated in DNA concatemers was released into solution by duplex specific nuclease (DSN) and subsequently collected by prepared cucurbit(7)-uril (CB[7])/Au@Fe_3O_4NPs/GCE via host-guest interaction, which thus produced a significantly amplified signal for quantitative detection of target. Since the catalyst Fe_3O_4NPs on electrode surface could directly electrocatalyze the reduction of coimmobilized MB, the proposed system possessed superior advantages compared with that of traditionally enzymatic systems, which not only simplified the operation process, decreased measurement error but also improved catalytic efficiency and stability.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Highly sensitive detection of protein is particularly important for the diagnosis, treatment and prognosis of diseases [1–3]. *Nosema bombycis* (*N. bombycis*), a parasite of *bombyx mori* that can be transmitted to the progeny through eggs, is considered as a pathogen cause to silkworm pebrine disease with destructive disaster to sericulture [4–7]. To meet the urgent demands for *N. bombycis* analysis, several analytical approaches, from the earlier microscopic examination [8] to serological and molecular biology assays [9–11], have been developed up to now. Unfortunately, there are some shortcomings with respect to labor-intensive work, large sample demand, and low accuracy or sensitivity. Thereby, powerful and straightforward electrochemical protein detection is an attractive method because of its rapid response, small volumes of samples, good specificity and so on [12–14]. Our group originally proposed DNAzyme amplified electrochemical methods to realize the spore wall protein of *N. bombycis* detection [15,16]. However, some performances were still needed to be improved, especially on the catalytic efficiency and simplified operation of signal amplification.

As is well-known, traditional protein enzyme catalytic activities are susceptible to external conditions such as temperature and pH, while minor effects for mimic enzymes including DNAzyme [17–19], metal nanoparticles [20,21] and Fe_3O_4 nanoparticles (Fe_3O_4NPs) [22]. Nevertheless, the currently reported electrocatalytic amplifying systems are almost involved in the extra addition of catalytic substrates into supporting electrolyte [23,24], which result in complex operation, tedious assay time and increased measurement deviation. More importantly, since the substrates exist in supporting electrolyte, the catalytic efficiency is relatively low due to the long operating distance between catalyst and substrate. Based on the above observation, in this work, we proposed a catalyst and small dye molecules coimmobilized electrocatalytic amplifying system, in which the Fe_3O_4NPs on electrode surface served as superior catalyst [25] to directly electrocatalyze the reduction of coimmobilized small dye molecules. The

* Corresponding authors.
E-mail addresses: y198688@swu.edu.cn (Y. Yuan), yuanruo@swu.edu.cn (R. Yuan).

http://dx.doi.org/10.1016/j.snb.2017.02.106
0925-4005/© 2017 Elsevier B.V. All rights reserved.

interesting amplifying system would effectively resolve the above mentioned problems, which not only simplified the operation process, decreased the measurement deviation but also improved catalytic efficiency owing to the shortened operating distance between Fe_3O_4NPs and coimmobilized small dye molecules. Additionally, the Fe_3O_4NPs used here can also served as nanocarrier to load substantial probes in biological sample separation [26–28], providing a possibility to construct nonenzymatic and simple signal amplified electrochemical immunoassay.

Proximity immunoassay, a novel sandwich-typed immune approach extended by DNA hybridization, is based on the target protein binding with two DNA strands-coupled antibodies. Significantly, these DNA strands come to a close proximity upon target binding and usually would be followed by the release or assembly a DNA segment which is expected to subsequently serve as trigger for DNA propagation or recycling [29,30], resulting in an enhanced signal output that indirectly reflect the amount of target protein present. In this regard, by combining electrochemical detection with the proximity immunoassay concept termed electrochemical proximity assay (ECPA), we proposed an ECPA-coupled nonenzymatic amplifying strategy with highly catalytic efficiency of Fe_3O_4NPs toward coimmobilized methylene blue (MB) for total protein of *N. bombycis* (TP N.b) detection (Scheme 1). Proximity immunoreaction was first used to expose the adenine rich ssDNA (A_1) on Au@Fe_3O_4NPs surface. The exposed ssDNA A_1 then served as trigger to induce hybrid chain reaction (HCR), aiming to *in situ* form extended DNA concatamers for loading substantial amount of the electron mediator methylene blue (MB). To obtain further amplified electrochemical signal through nonenzymatic amplification, the MB intercalated in DNA concatemers was released into solution with duplex specific nuclease (DSN)-catalyzed cleaving reaction of double-stranded DNA [31–33] and subsequently collected by prepared cucurbit(7)-uril (CB[7])/Au@Fe_3O_4NPs/GCE via the host-guest interaction between CB[7] and MB [34,35]. Because of the superior electrocatalytic performance and shortened operating distance of Fe_3O_4NPs toward coimmobilized MB, our approach had many advantages, such as high sensitivity, satisfactory stability and easy operation, which provided a promising prospect in real-time testing and commercial application.

2. Experimental section

2.1. Reagents

Normal silkworm blood, total protein of *Nosema bombycis* (TP N.b), infected silkworm blood and polyclonal antibody (Ab) were obtained from State Key Laboratory of Silkworm Genome Biology of China Southwest University. Methylene blue (MB) was obtained from Shanghai Aladdin Industrial Corporation (Shanghai, China). Duplex-specific nuclease (DSN) was purchased from Evrogen Joint Stock Company (Moscow, Russia). Cucurbit [7] uril (CB[7]), *N*-hydroxy succinimide (NHS) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimidehydrochloride (EDC) were bought from Sigma Chemical Company (St. Louis, MO, USA). All HPLC-purified DNA oligonucleotides were purchased from Sangon Biotech Company (Shanghai, China), and the corresponding sequences were listed as follows (Table 1):

2.2. Apparatus

All electrochemical measurements including cyclic voltammetry (CV) and alternating current voltammetry (ACV) were carried out on a CHI 660D electrochemical workstation (Shanghai Chenhua Instrument, China). A traditional three electrode system with a bare or modified glassy carbon electrode (GCE, Φ = 4 mm) served as working electrode, a platinum wire as auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode.

Table 1
Sequence of synthesized oligonucleotides used in this work.

Oligonucleotide	Sequence (from 5′ to 3′)
Adenine rich ssDNA (A_1)	TAT GTG ATA CAA TAG ATC AAA AAA AAA A
Capture ssDNA 1 (S_1)	GCG GAT CTA TTG TAT CAC ATA TTT TTT TTT TTT TTT TTT CAC CGT ATG CTA CTG TAG AT-NH_2
Capture ssDNA 2 (S_2)	NH_2-TAG GAA AAG GAG GAG GGT GGT TTT TTT TTT TTT TTT TTT TTT AGA TAC AAT AGA TC
Auxiliary DNA 1 (H_1)	GAT CTA TTG TAT CAC ATA GGT AAC TAT GTG ATA AAC
Auxiliary DNA 2 (H_2)	TAT GTG ATA CAA TAG ATC GTT TAT CAC ATA GTT ACC

2.3. Synthesis of Au@Fe_3O_4NPs magnetic microspheres

Gold nanoparticles (Au) with negative charges (about 0.045 M) were first synthesized according to the literature [36]. Then, 1 mL phthalic diglycol diacrylate solution (PDDA, 0.20 wt%) was subjected to 2 mg Fe_3O_4 nanoparticles (Fe_3O_4NPs, 2 mg mL^{-1}) and reaction for 30 min to obtain positive charges functional Fe_3O_4. Subsequently, 5 mL prepared Au solution was added into functional Fe_3O_4 and stirred for 1 h to obtain the Au functional Fe_3O_4 (abbreviated as Au@Fe_3O_4NPs). Finally, the resulting Au@Fe_3O_4NPs magnetic microspheres were isolated with a magnet and washed three times by ultrapure water, and then re-dispersed in 1 mL ultrapure water for future use.

2.4. Preparation of Ab-dsDNA-Au@Fe_3O_4NPs probes

To activate the carboxyl group of polyclonal antibody Ab, 75 mM EDC and 15 mM NHS were first added into 1 mL of 5 $\mu g \cdot mL^{-1}$ Ab and reacted for 1 h. Then, 100 μL amido terminal S_1 (100 μM) was added for reaction of 12 h under 4 °C [37], which finally obtained the Ab labeled S_1 (Ab-S_1). Moreover, we also prepared the Ab labeled S_2 (Ab-S_2) and the preparation process was the same with that of Ab-S_1. For the preparation of Ab-dsDNA-Au@Fe_3O_4NPs probes, 40 μL 1 μM adenine rich ssDNA A_1 and 40 μL synthesized Au@Fe_3O_4NPs magnetic microspheres were added into an eppendorf tube and incubated for 40 h [38], where the consecutive adenines of A_1 could play the role of −SH group to assemble ssDNA A_1 on Au@Fe_3O_4NPs surface (termed as A_1-Au@Fe_3O_4NPs). The supernatant was removed by magnetic separation and then 20 μL prepared Ab-S_1 was added into the precipitate (A_1-Au@Fe_3O_4NPs) for 2 h incubation. With the complementary base paring between S_1 and A_1, the Ab-S_1 could be assembled on A_1-Au@Fe_3O_4NPs and finally obtain the Ab-dsDNA-Au@Fe_3O_4NPs probes (Scheme 1).

2.5. Fabrication of biosensor

Prior to use, a GCE was polished with 0.3 and 0.05 μm alumina powder, respectively, and sequentially sonicated with ultrapure water, ethanol and ultrapure water for 2 min to obtain a mirror like surface. Then, 10 μL prepared Au@Fe_3O_4NPs magnetic microspheres was adopted on GCE for drying, which served as carrier to load abundant CB[7] [39]. Therefore, 10 μL 1 mM CB[7] was dropped on the electrode and incubated for 1 h at room temperature to obtain CB[7]/Au@Fe_3O_4NPs/GCE.

2.6. Principle of the biosensor for TP N.b detection

The prepared Ab-dsDNA-Au@Fe_3O_4NPs probe was first incubated with 10 μL target TP N.b of various concentrations and 10 μL

Download English Version:

https://daneshyari.com/en/article/5009802

Download Persian Version:

https://daneshyari.com/article/5009802

Daneshyari.com