Sensors and Actuators B 261 (2018) 441–450

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

A sandwiched electroanalysis method for probing Anthrax DNAs based on glucose-induced gold growth and catalytic coupling of tyramine using gold-mineralized glucose oxidase

Hua Wang*,[1], Liyan Zhang, Yao Jiang, Lijun Chen, Zhiqiang Duan, Xiaoxia Lv, Shuyun Zhu

Institute of Medicine and Materials Applied Technologies, College of Chemistry and Chemical Engineering, Qufu Normal University, Qufu City, Shandong Province 273165, PR China

ARTICLE INFO

Article history:
Received 5 August 2017
Received in revised form
21 December 2017
Accepted 21 January 2018

Keywords:
Gold nanoparticles
Glucose oxidase
Double catalysis activities
Tyramine-labelled
Glucose-triggered gold growth
DNA detection

ABSTRACT

Gold nanoparticles was doped into the protein matrix of glucose oxidase (GOx) by the in-site biomineralization route, yielding the GOx with mineralized gold (GOx-Gold) showing the double catalysis activities of GOx and peroxidase-like gold catalysis. A magnetic separation-based detection method was thus tailored for the sandwiched electroanalysis of Anthrax DNAs using GOx-Gold and tyramine linker as the probe labels. After the DNA hybridization reactions, the tyramine-mediated linking of DNA capture and detection probes was conducted through gold-catalytic oxidization of tyramines labelled at the probe terminuses, followed by the glucose-triggered gold growth catalyzed by GOx. Highly amplified electrochemical output of gold signals was thus achieved toward the ultrasensitive detection of DNAs in blood, with the detection limit down to ~0.10 fM. Also, the discrimination of DNAs with single-base mutation could be expected. Importantly, such a biomimic gold mineralization route can be tailored for remolding various enzymes with improved intrinsic catalysis and electrocatalysis, thus promising the wide applications in the catalysis, biosensing, and biomedical fields.

1. Introduction

Recent decades have witnessed the increasing applications of various enzymes as the labels for the catalytic signal amplification for the highly sensitive detections. It is widely recognized that the catalysis-active centers of enzymes are commonly inaccessible, since they are either located close to the electrically insulated protein shells of enzymes like horseradish peroxidase (HRP) [1,2], or embedded deep in their glycoprotein sheath like glucose oxidase (GOx) [3–6]. Establishment of an efficient electron transfer between enzymes and their supports like the electrodes is vital for the development of various enzymatic catalysis-based biosensors (or bioelectronics) [7–9], catalysts [10,11], and biofuel cells [12,13]. Aiming to build up the electrical contacts toward the enzymatic redox centers, historically, the use of electron mediators [14,15], the modification of enzymes with redox relays [16,17], and the incorporation of enzymes in redox-active polymer matrices [18,19] were commonly practiced, but received the limited success in improving the electron transportation of enzymes. In recent years, a variety of efforts have been devoted to the development of direct electron transfer (DET) between the redox enzymes and electrode surfaces by using enzymatic labels of nano-sized materials, typically as gold nanoparticles (NPs) [20,21] and carbon nanotubes (CNTs) [6,22–27]. Nevertheless, the so obtained DETs of most enzymes are still compromised, since it is hard for the labelled or coated nanomaterials to electrically plug into the catalysis-active sites of enzymes that are embedded deeply aforementioned. Better enzymatic DETs have been alternatively proposed by the reconstitution of apo-enzymes with cofactors that are functionalized with some conductive nanomaterials (i.e., gold NPs and CNTs) or redox tethers [6,26,28,29]. For example, Willner et al. [6] extracted the catalysis-active center from GOx to be functionalized with gold NPs and further reconstituted with the apo-enzyme yielding the enzyme mimics with the improved electrocatalysis. Takashi and coworkers reconstituted myoglobin with the prosthetic groups that were bound with cationic cytochrome c to produce the highly catalytic complex [30]. Our group also developed a kind of peroxidase mimics with the enhanced catalysis and electrocatalysis by reconstituting hemoglobin with the gold-remolded active centers [31]. While these pioneering methods can greatly improve the DETs of some enzymes, their practical applicability may be substantially

* Corresponding author.
E-mail address: huawangqfnu@126.com (H. Wang).
[1] http://wang.qfnu.edu.cn.

https://doi.org/10.1016/j.snb.2018.01.171
0925-4005/

limited by the complicated experimental procedure. Furthermore, the biomineralization route has emerged as a facile and highly efficient way for the synthesis of various inorganic materials like gold NPs by using some biological materials such as proteins and enzymes [21,32–37]. For example, Zhang's group employed HRP to synthesize gold nanoclusters (NCs) with strong fluorescence through the biomineralization process for H_2O_2 sensing [38]. Gold NCs were also in site incorporated into alkaline phosphatase by the protein-mediated biomineralization for a catalytic amplification technique for the gene analysis [21]. In addition, some noble metal nanomaterials with small sizes can intrinsically present the peroxidase-like catalysis activities [39–41], thus promising the extensive catalysis applications.

Moreover, many modern biosensors have been established to quantify DNAs to date [42,43], especially those by way of the sandwiched DNA hybridization and enzyme catalysis-based signal amplification. However, the detection sensitivities of these sandwiched analysis methods can still be trapped generally by the limited signal amplification, due to that the hybridized double chains, which can be formed either between targets and the capture probes or between targets and the detection probes, may not be robust enough to withstand the enzyme-catalytic signal amplification at a large scale, during which the hybridized targeting DNAs might risk the unwound or detached issues.

In the present work, we have developed a magnetic separation-based detection method for the sandwiched electroanalysis and discrimination of DNAs using GOx with mineralized gold NPs (GOx-Gold) as the probe labels. As schematically illustrated in Scheme 1, herein, gold were doped into the protein matrix of GOx by the biomineralization route showing the greatly improved DETs and catalysis of GOx. Importantly, the resulted GOx-Gold composites were confirmed with the double catalysis activities of enhanced GOx catalysis and the peroxidase-like catalysis of mineralized gold. Furthermore, after the hybridization of DNA targets, the capture and detection DNA probes would be linked through the oxidative coupling reaction of their tyramine labels catalyzed by gold NPs of GOx-Gold. Meantime, GOx of GOx-Gold would catalyze the oxidization of glucose to produce H_2O_2 to facilitate the reductive gold growth. The maximized and stable gold signals would be thereby achieved because of the tyramine-mediated linking of DNA probes. A magnetic separation-based sandwiched detection method was demonstrated by using GOx-Gold catalyst and tyramine linker as the labels of DNA probes towards the sensitive electroanalysis and discrimination of targeting Anthrax DNAs with the single-base mutation.

2. Experimental section

2.1. Reagents and apparatus

Glucose oxidase (GOx, EC 1.1.3.4, 200 U mg^{-1}), hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4 \cdot 3H_2O$), Nafion (5.0 wt.%) solution, horseradish peroxidase (HRP) (E.C. 1.11.1.7, 200 U mg^{-1}), tyramine, *N*-hydroxysuccinimide (NHS), *N*-ethyl-*N*'-(3-dimethylaminopropyl) carbodiimide (EDC), and sulfosuccinimimidyl 4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate (Sulfo-SMCC) were purchased from Sigma-Aldrich. Magnetic Dynabeads M-270 carboxylic acid (~30 mg mL^{-1}) was obtained from Invitrogen Dynal AS. The blood samples were kindly provided from the local hospital. The targeting DNAs were spiked in blood to yield the DNA samples of different concentrations. All other chemicals were of analytical grade. Deionized water was used throughout in the preparation of aqueous solutions like phosphate buffer solution (pH 7.2), and 2-(*N*-morpholine) ethanesulfonate (MES) buffer (pH 5.0). Anthrax fetal factor DNA oligonucleotides of terminus-functionalized capture and detection DNA probes, and matched and one-base mismatched DNAs were synthesized by Shanghai Sangon (Shanghai), including:

(1) Capture probe: 5′-Thiol-$(CH_2)_6$-TAA CAA TAA TCC-$(CH_2)_6$-amine-3′;
(2) Detection probe: 5′-Amine-$(CH_2)_6$-ATC CTT ATC AAT ATT-$(CH_2)_3$-Thiol-3′;
(3) A-T match: 5′-GGA TTA TTG TTA AAT ATT GAT AAG GAT-3′;
(4) A-G mismatch: 5′-GGA TGA TTG TTA AAT ATT GAT ATG GAT-3′;
(5) A-C mismatch: 5′-GGA TCA TTG TTA AAT ATT GAT ATG GAT-3′;
(6) A-A mismatched: 5′-GGA TAA TTG TTA AAT ATT GAT ATG GAT-3′.

2.2. Synthesis and characterization of GOx-Gold

All glassware were first washed with nitro-hydrochloric acid (HNO_3: HCl volume ratio = 1:3) (*Caution: the mixture is a very corrosive oxidizing agent, which should be handled with great care.*), and then rinsed with ethanol and ultrapure water. The synthesis of GOx-Gold was conducted by a modified procedure previously reported [32]. In a typical experiment, 2.0 mL aqueous $HAuCl_4$ solution (15 mM, 37 °C) was mixed with a 2.0-mL aliquot of GOx with different protein concentrations (5.0, 10.0, 20.0, 30.0, 40.0, and 50.0 mg mL^{-1}) under the vigorous stirring at 37 °C. An aliquot of NaOH (1.0 M) was then introduced into the mixture, and the reaction proceeded at 37 °C overnight. The resulting GOx-Gold composites were dialyzed in water overnight using the membranes (pore size of 1.8 nm or molecular weight of 20 KD). Finally, they were stored at 4 °C for short-term usage, or dried by freezing in the solid form for long-term usage.

Transmission electron microscopy (TEM) was operated on a FEI Tecnai TF-20 field-emission high-resolution transmission electron microscope at 200 Kv for imaging GOx-Gold products, which were pre-purified by the centrifuge. X-ray photoelectron spectroscopy (XPS) was applied to explore the oxidation states of Au in GOx-Gold. Moreover, a microplate reader (Tecan, Austria) and 96-well plates were utilized in the tests for the peroxidase-like catalysis of GOx-Gold. The absorbance intensities of the reactions of 3,3′,5,5′-tetramethylbenzidine and H_2O_2 (TMB-H_2O_2) were terminated with 2.0 M H_2SO_4 to be measured at 450 nm.

2.3. Electrochemical measurements using the GOx-Gold electrodes

Glassy carbon electrodes (GCE, 3.0 mm in diameter) were cleaned following a common polishing procedure. An aliquot of 5.0 μL GOx-Gold (5.0 mg mL^{-1} in GOx) that was pre-mixed with Nafion (final concentration of 0.50 wt.%) was dropped onto the surface of a cleaned GCE to be dried overnight at 4 °C. Then, 5.0 μL Nafion (0.25 wt.%) was casted and dried as a net to hold firmly the GOx-Gold onto the electrodes, resulting in the GOx-Gold modified electrodes. The similar procedures were employed to fabricate the GOx-modified electrodes as the controls using native GOx with corresponding enzyme concentrations. All of the resulting electrodes were finally stored at 4 °C.

Electrochemical voltammetric measurements were carried out with a CHI 760B electrochemical workstation (CH Instruments Co., USA) using a conventional three-electrode cell. The GOx-Gold or GOx modified electrodes were used as the working electrodes. The Pt wire and Ag/AgCl (3.0 M KCl) electrodes were used as the counter and reference electrodes, respectively. All measurement experiments were performed in the PBS that was piped with oxygen gas for 10 min to facilitate the GOx-Gold-catalytic reactions.