Biosensors and Bioelectronics 59 (2014) 269–275

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Amplified and selective detection of Ag^+ ions based on electrically contacted enzymes on duplex-like DNA scaffolds

Gang Xu [a,b], Guangfeng Wang [a,b,*], Yanhong Zhu [a,b], Ling Chen [a,b], Xiuping He [a,b], Lun Wang [a,b], Xiaojun Zhang [a,b,*]

[a] Key Laboratory of Chem–Biosensing, Anhui province; Key Laboratory of Functional Molecular Solids, Anhui province; College of Chemistry and Materials Science, Center for Nano Science and Technology, Anhui Normal University, Wuhu 241000, PR China
[b] State Key Laboratory of Chemo/Biosensing and Chemometrics, Hunan University, Changsha 410082, PR China

ARTICLE INFO

Article history:
Received 4 February 2014
Accepted 8 March 2014
Available online 15 March 2014

Keywords:
Electrical contacted enzyme
Carbon nanodots
Cytosine
Ag^+

ABSTRACT

In the present study, ultrasensitive detection of Ag^+ is demonstrated by a biocatalytic signal amplification system which is realized by only one DNA sequence based electrical contacted enzyme structure and the Au nanoparticles/Carbon nanodots (AuNPs/C-dots) composite immobilization platform. In the presence of Ag^+, with the interaction of cytosine–Ag^+–cytosine (C–Ag^+–C), cytosine-rich DNA sequence labeled with methylene blue (MB) molecules near 5′ end and Glucose Oxidase (GOx) at 3′ end, has a self-hybridization and then forms a duplex-like structure which makes MB and GOx approach the AuNPs/C-dots modified electrode. MB units can then act as a relay that electrically contacts GOx with the AuNPs/C-dots modified electrode and activate the bioelectrocatalyzed oxidation of glucose to glucose acid. In consequence, based on the bioelectrocatalyzed signal amplification on the AuNPs/C-dots platform, Ag^+ could be quantitatively detected in the range of 10^{-11}–10^{-5} M with a low detection limit of 3 pM. Also, there is an excellent selectivity against other interferential metal ions. The detection of Ag^+ ions was realized by Ag^+ self-induced conformational change of DNA scaffold which involved only one oligonucleotide showing its convenience and availability.

1. Introduction

Silver ions (Ag^+), surpassed only by mercury are recognized as one of the most hazardous metal pollutants due to their high potential toxicity to human health (Li et al., 2010; Sun et al., 2011; Lin et al., 2010) which may accumulate in the human body through the food chain or directly drinking, causing various serious problems such as cytotoxicity, organ failure, and reduction in mitochondrial function (Ryu et al., 2009). Thus, it is an important goal to develop methods for sensitive detection of Ag^+ in aqueous solution. Currently, numerous conventional methods have been used for the determination of Ag^+ ions including atomic absorption spectrometry (AAS) (Wu et al., 2008), inductively coupled plasma mass spectrometry (ICP-MS) (Li et al., 2007) and fluorescent or electrochemical chemosensing (Zhang et al., 2008; Taki et al., 2008). Among these methods, electrochemical methods offer good sensitivity and excellent selectivity (He et al., 2013; Xu et al., 2013). In addition, sophisticated instruments and complicated organic synthesis for special reagents is not needed. Therefore, it is very much required to develop an electrochemical method for Ag^+ ions detection.

It is widely reported that the electrical contact of redox enzymes with electrodes is one of the most fundamental processes required for the development of bioelectronic devices, such as amperometric biosensors (Heller, 1996; Zayats et al., 2008), biofuel cells (Barton et al., 2004; Heller, 2004; Willner et al., 2009), etc. However, redox enzymes in bioelectronic devices usually lack direct electrical contact with electrodes because of the spatial separation of their redox centers from the conductive surfaces by the protein shells (Degani and Heller, 1988). So different methods to assemble enzyme on electrodes exhibiting electrical contact between the redox protein and electrode are discussed, including the assembly of enzyme–nanoparticle (Yehezkeli et al., 2011; Xiao et al., 2003), enzyme–carbon nanotube hybrids (Patolsky et al., 2004) and enzyme–DNA composite (Piperberg et al., 2009; Mor-Piperberg et al., 2010) on electrodes. Among these assembling methods, DNA monolayers are good scaffold for the reorganization of relay (cofactor) and enzyme units because it could lead to effective and controllable electrical contact of the enzymes with the electrode (Piperberg et al., 2009; Fruk et al., 2007; Wang et al., 2012a, 2012b).

Since Ag^+ ions were found to specifically interact with the cytosine–cytosine (C–C) mismatch in DNA duplexes

* Corresponding authors.
E-mail address: xjzhang@mail.ahnu.edu.cn (X. Zhang).

http://dx.doi.org/10.1016/j.bios.2014.03.017
0956-5663/

(Ono et al., 2008), it provides a novel rationale for Ag^+ detection (Li et al., 2009; Oh and Lee, 2010; Zhao et al., 2010). Various C–Ag^+–C based sensors have been developed with high sensitivity, convenience and good selectivity (Lin and Tseng, 2009; Li and Qi, 2011). Thus, inspired by the necessity of Ag^+ detection, the advantage of DNA scaffold for electrical contact of enzyme and the excellent property of C–Ag^+–C based sensor, can we study a C–Ag^+–C scaffold based DNA sensor for Ag^+ detection by the amplified signal generated from electrical contact of enzymes?

However, an effective immobilization platform for the DNA scaffold is also key in the detection system. With an explosion of interest in nanomaterial over the past decades (Rosi and Mirkin, 2005; Aragay et al., 2011), carbon nanodots (C-dots), first reported in 2004 (Xu et al., 2004), have subsequently attracted considerable attention (Cao et al., 2007; Yang et al., 2009). C-dots have a variety of applications, especially in the field of photocatalyst and electrocatalytic activities (Cao et al., 2007; Ray et al., 2009; Liu et al., 2009). The electronic properties of C-dots are suggestive of their potential ability as electrodes modifier to mediate electron transfer reactions with electroactive species in solution (Zhao et al., 2011; Cao et al., 2011; Long et al., 2012). In addition, besides C-dots exhibiting a high capability to promote some types of electron transfer reactions, it can also enhance the immobilization of molecules such as enzymes or antibodies on their surface (Li et al., 2010; Wang et al., 2011). Also, Au nanoparticles (AuNPs) have been extensively employed as immobilization platform because of their easy preparation, good biocompatibility, excellent catalytic effect and low toxicity (Lykakis et al., 2011; Fang et al., 2011; Cheng et al., 2003). What is more, it is very easy to immobilize thiolated DNA by the covalent bond of Au-S (Wang et al., 2012a, 2012b). In summary, the combination of C-dots with AuNPs (AuNPs/C-dots) as an immobilization platform will be fascinating and desirable.

Herein, a new DNA scaffold for the ultrasensitive detection of Ag^+ is demonstrated by a novel biocatalytic signal amplification system of the electrical contacted enzyme. With the help of methylene blue (MB) as electron mediator, the electrical communication between the glucose oxidase (GOx) and the electrode is realized by the conformational change of the DNA scaffold on the AuNPs/C-dots immobilization platform. Because C–Ag^+–C interaction is key for the formation of the DNA scaffold as designed, the biocatalytic response is relative with the amount of Ag^+ which becomes the base of detecting Ag^+ ions. In the present system, the bioelectrocatalytic signal of electrical contact of enzyme and nanocomposites platform is effective for the signal amplification.

2. Experiment sections

2.1. Materials and reagents

HPLC-purified oligonucleotides (sequences are listed, S1: 5′–SH-$(CH_2)_6$-ACTATGTTCCTCCTCCTTTTCCACCACCAA-Biotin-3′, MB–S1: 5′–SH-$(CH_2)_6$-ACT-(MB)-ATGTTCCTCCTCCTTTTCCACCACCAA-Biotin-3′ was obtained from Sangon Biotechnology Co. Ltd. (Shanghai, China). GOx modified with avidin was purchased from Sigma Aldrich. Gold (III) chloride trihydrate (99.9%), phosphate-buffered saline (PBS) and other reagents were obtained from the Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China) without further purification. Ultrapure water (PSDK2-10-C) was used throughout all experiments.

2.2. Preparation of the AuNPs/C-dots modified electrode

In order to fabricate an effective immobilization platform, the AuNPs/C-dots modified glassy carbon electrode (AuNPs/C-dots/GCE) was prepared. Firstly, C-dots/GCE should be prepared. C-dots with low toxicity and high conductivity (Ray et al., 2009) were prepared according to the literature (Mao et al., 2010). Briefly, 0.5 g of lampblack washed with acetone and dried at 110 °C was added into 150 mL of HNO_3 (5 mol/L), and the mixture was refluxed at 140 °C for 12 h. Then, the black solution was neutralized with sodium carbonate, loaded into dialysis bags (cutoff molecular weight 8000–14,000) for dialysis against ultrapure water for two days to purify the carbon dots. Acetone was added in the purified solution and then the bare C-dots were collected by centrifugation at 16,000 rpm for 15 min. The precipitation was dried under nitrogen flow and dispersed in ultrapure water. At last, the obtained bare C-dots solution was kept at 4 °C until use. For modification of the electrode, 10 μL of 1 mg/mL homogeneous C-dots solution after ultrasonication was dropped onto the electrochemical cleaned GCE (Wang et al., 2012a, 2012b) and allowed to dry in ambient air for 24 h, and then rinsed with distilled water to get the C-dots/GCE. Then, the C-dots/GCE were immersed in 0.1 M KNO_3 solution containing 0.2 g/L $HAuCl_4$ and then conducted for 60 s at −0.26 V (vs. SCE) with electrochemical working station for the electrochemical deposition of AuNPs. After that the AuNPs/C-dots/GCE was obtained.

2.3. Preparation of the GOx modified DNA sequence

For the preparation of the MB and GOx dully labeled sequence, MB–S1 with the MB labeled at the base T near the 5′ end and the biotin labeled at 3′ end was designed. The introduction of MB in oligonucleotides S1 was completed by Sangon. We needed to further tether GOx at 3′ end by the interaction of biotin–streptavidin (Wang et al., 2012a, 2012b). Briefly, 100 μL GOx-avidin (30 μM) was reacted with the 3′-biotin-moiety of the S1 (30 μM) for 2 h in 0.1 M PBS (pH 7.4) at room temperature, followed by the separation of dialysis to obtain GOx-MB dully labeled sequence (GOx–MB–S1). For the control assays, the introduction of GOx in oligonucleotides S1 without MB label (GOx–S1) was obtained under the same processes except for replacing MB–S1 with S1.

2.4. Assembly of the GOx–MB–S1 on the electrode

The immobilization of the GOx–MB–S1 was performed following the dropping method. The AuNPs/C-dots/GCE was immersed into 30 μL Tris–HCl buffer (10 mM, pH 7.4) containing 1.0 μM GOx–MB–S1 probe and 1.0 M NaCl in humidified chamber. The self-assembly through the thiol group of S1 and Au atoms was proceeded for 2 h. Then the electrode was incubated in 1 mM PBS (pH 7.4) with 5% BSA for 1 h to eliminate the nonspecific-bonded DNA. After being thoroughly rinsed with the washing buffer (10 mM PBS, pH 7.4, 100 mM NaCl), GOx–MB–S1/AuNPs/C-dots/GCE was completed and ready for further use. The sensor obtained was found to maintain the activity for more than one week if stored at 4 °C in the PBS (pH 7.4) that included 50 mM NaCl. Under the same reaction condition, GOx–S1 modified electrode (GOx–S1/AuNPs/C-dots/GCE), MB–S1 without GOx modified electrode (MB–S1/AuNPs/C-dots/GCE) and GOx–MB-S1 modified on AuNPs/GCE (GOx–MB–S1/AuNPs/GCE) were constructed for control experiments.

2.5. Electrochemical detection of glucose and Ag^+

To form an electrical contacted enzyme biosensor for Ag^+, we immersed GOx–MB–S1/AuNPs/C-dots/GCE in the PBS (pH 7.4) containing 1 μM Ag^+ ions and 10 mM NaCl at 32 °C for 50 min. After the self-hybridization of the GOx–MB–S1 in the presence of Ag^+, the electrical contacted enzyme biosensor was obtained which we name as GOx–MB–S1–Ag^+–duplex/AuNPs/C-dots/GCE. For the control experiment, MB-S1/AuNPs/C-dots/GCE, GOx–S1/AuNPs/C-dots/GCE