Biosensors and Bioelectronics 83 (2016) 142–148

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Increased electrocatalyzed performance through hairpin oligonucleotide aptamer-functionalized gold nanorods labels and graphene-streptavidin nanomatrix: Highly selective and sensitive electrochemical biosensor of carcinoembryonic antigen

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ARTICLE INFO

Article history:
Received 29 February 2016
Received in revised form
8 April 2016
Accepted 14 April 2016
Available online 14 April 2016

Keywords:
Hairpin oligonucleotide-functionalized gold nanorods
Horseradish peroxidase
Graphene-streptavidin nanomatrix
Electrochemical biosensor
Carcinoembryonic antigen

ABSTRACT

We report a triplex signal amplification strategy for sensitive biosensing of cancer biomarker by taking advantage of hairpin-shaped oligonucleotide-functionalized gold nanorods (HO-GNRs), graphene and the avidin-biotin reation. The strategy expands electrochemical detection of carcinoembryonic antigen (CEA) by using an aptamer as biosensor's recognition element and HO-GNRs as signal enhancer. To construct this biosensor, the GNR was used as a carrier of horseradish peroxidase (HRP) and HO aptamer with a biotin at the 3′-end and a thiol at the 5′-end, which amplified the electrochemical response because of a large molar ratio of HRP to HO. In the presence of target CEA, the binding reactions of CEA with the loop portions of the HOs caused HOs' loop-stem structure opened and exposed the biotins, and then HRP-GNRs-HO conjugates were captured on graphene and streptavidin modified electrodes via the reaction between the exposed biotins and preimmobilized streptavidins. The accumulation of HRP effectively catalyzed the hydrogen peroxide-mediated oxidation of *o*-phenylenediamine to generate an electrochemical reduction current for CEA detection. Under optimal conditions, the electrochemical biosensor exhibited a wide dynamic range of 5 pg mL^{-1} and 50 ng mL^{-1} toward CEA standards with a low detection limit of 1.5 pg mL^{-1} (signal-to-noise ratio of 3). The proposed biosensor accurately detected CEA concentration in 8 human serum samples from patients with lung diseases, showing excellent correlations with standard chemiluminescence immunoassay. Furthermore, these results of target DNA detection made it abundantly clear that the proposed strategy can also be extended for detection of other relative biomarkers using different functional DNA structures, which shows great prospects in single-nucleotide polymorphisms analysis, biomedical sensing and application for accurate clinical diseases diagnostic.

1. Introduction

Ultrasensitive and highly selective determination of disease-specific protein biomarkers has important significance in many aspects of elaborating the pathogenic mechanism of disease and elucidating the rule of pathological changes, early diagnosis and therapy of disease, development and screening of new drug, etc (Ge et al., 2016). Carcinoembryonic antigen (CEA), as a broad-spectrum tumor marker, is elevated in many malignancies, such as gastric cancer, colorectal cancer, breast cancer, liver cancer and pancreatic cancer. Significant CEA positivity has been reported for reflecting disease progression or regression status, and its levels be identified in monitoring responses to treatment and the early diagnosis of recurrences. Much attention has been focused on the detection of low-abundance CEA using an electrochemical (Zhou et al., 2013; Xue et al., 2015), electrochemiluminescent (Deng et al., 2013), surface-enhanced Raman scattering (Chon et al., 2009),

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http://dx.doi.org/10.1016/j.bios.2016.04.039
0956-5663/

fluorescent (Wang et al., 2014), mass spectrometric (Liu et al., 2011), chemiluminescent (Zong et al., 2012; Wang et al., 2015), or surface plasmon resonance (Springer et al., 2013) techniques. Nevertheless, most of these biosensors use antibody as molecular recognition element and suffer from some intrinsic drawbacks: expensive consumption because of having to use cell lines or animals to produce, poor stability limiting transport and application in harsh conditions (e.g., high temperature or extreme pH), and high molecular weight impeding its ability to penetrate into the deep site of the biosensor, et al. It is very important to explore novel molecular recognition tools for the development of protein biomarkers determination with more promising performances such as low cost, high stability and simplicity. An aptamer is a short, chemically synthesized, single-stranded DNA/RNA oligonucleotide that can fold into a unique secondary or tertiary structures structure that recognizes a specific targets ranging from small molecules to even proteins or cells (Lao et al., 2015). Compared to antibodies, aptamer holds several unique advantages as non-immunogenicity, easier to mass reproducible synthesis, rapid tissue penetration, easy functionalization, faster blood plasma clearance and high stability (Iliuk et al., 2011; Pu et al., 2015). However, there are only few reports on aptamer against CEA protein. In 2010, Tabar and Smith (Tabar and Smith, 2010) firstly selected DNA aptamers against CEA for clinically imaging and diagnosis of cancer cells. Later, Chen and his group developed aptamer-based CEA determination using fluorescent (Lin et al., 2012) and colorimetric (Liang et al., 2014) biosensor. Unfortunately, exploration of the DNA aptamer as electrochemical biosensor's recognition element for CEA determination is still sparse (Shu et al., 2013; Xue et al., 2015). Coupling of the significant advantages of aptamers with high sensitivity, robustness, fast response and the potential for minituarization of electrochemical methods, we postulated that electrochemical detection of CEA in aptasensor may offer a prospective approach for highly sensitive and selective determination of disease-specific protein biomarkers.

Hairpin-shaped oligonucleotides (HO), also called molecular aptamer beacons, consist of a base-paired stem structure and a loop sequence of aptamer with unpaired nucleotides, which holds the advantages of both the generality of an aptamer and the good signal switching capability of molecular beacon (Huang et al., 2014). Besides the merits of linear aptamers, HO also have inherent a target-induced conformation change mechanism that enables the determination of targets without the separation of unbound probes. Furthermore, the thermodynamic stability of the unique stem-loop structure makes HO exceptional linear aptamers with excellent selectivity, which involve HO widely used in biosensor development to achieve low background and high accuracy (Wang et al., 2009). The sensitivities of HO based biosensors have been improved by the combinations of HO with graphene oxide (He et al., 2011), DNAzymes (Zuo et al., 2010), or the modifications of HO with gold nanoparticles (He et al., 2010; Fan et al., 2011), quantum dots (Kim et al., 2004; Zhang et al., 2015). Among different kinds of signal enhancers, gold nanorods (GNRs), which possess distinctive chemical and physical properties as a typical elongated gold nanostructure, have rapidly become powerful base platforms for biosensing, bioimaging and nanomedical applications (Chen et al., 2013). Compared with similarly sized spherical nanoparticles, GNRs exhibit higher absorption, both transverse and longitudinal localized surface plasmon resonances, inherently more sensitive to the local dielectric environment and can convert light into heat through various nonradiative photophysical processes. In addition, GNRs can be easily functionalized via simple thiolate chemistry. However, according to our knowledge, few attentions have been paid to the employment of HO-functionalized GNRs probes for highly selective and sensitive detection of protein biomarkers in an electrochemical biosensing system.

Herein, a new triplex signal amplification electrochemical biosensor by taking advantage of the HO-functionalized GNRs probes, graphene and the avidin-biotin reation was reported for CEA detection. As shown in Scheme 1, the HO-functionalized GNRs probes were prepared by a self-assembling process of a hairpin oligonucleotide modified with a biotin at the 3′-end and a thiol at the 5′-end on the surface of GNR. Then horseradish peroxidase (HRP) was also conjugated to the surface of GNR to prepare HRP-GNRs-HO conjugate, and could easily use as an electrochemical tracing label. Graphene (GR), which presents excellent electron-transfer ability, large surface area, favorable biocompatibility and so on (Xu et al., 2015; Tang et al., 2015), was employed to increase the effective surface area for the loading of streptavidin (SA) on the surface of electrode and accelerate the electron transfer of electroactive species. In the presence of CEA, the binding reactions of target CEA with the loop portions of the HOs on the GNRs surface opened the stem of the HOs and exposed the biotins which were located at the end of HOs. Then the HRP-GNRs-HO conjugates associated with the activated biotins were captured on GR and streptavidin modified electrodes via the reaction between the exposed biotins and preimmobilized streptavidins. The captured HRP effectively catalyzed the hydrogen peroxide-mediated oxidation of *o*-phenylenediamine (oPD) in solution, and an enzymatic generation of 2, 3-diaminophenazine (DAP) was produced as redox probe (Hamilton et al., 1999; Cui et al., 2008), enabling electrochemical detection of CEA. In the absence of CEA, The HO on the GNR surface maintained its hairpin structure, and the biotin groups stayed 'inactive', and the HRP-GNRs-HO conjugates were not captured on the sensor electrode, resulting a very limited background current. The as-proposed triple signal amplification electrochemical biosensor shows great promise in clinical diagnostics for detection of cancer biomarkers, and it is fairly easy to generalize this approach to detect target DNA.

2. Experimental sections

2.1. Reagents

Carcinoembryonic antigen (CEA, from human fluids), tris(2-carboxyethyl)phosphine (TCEP), $NaBH_4$, Cetyltrimetylammonium bromide (CTAB) and streptavidin (SA) were purchased from Sigma-Aldrich. GR was purchased from Sinocarbon Materials Technology Co., Ltd (China). Hydrogen tetrachloroaurate ($HAuCl_4$) was purchased from Alfa Aesar. O-phenylenediamine (oPD) was obtained from Guangcheng Chemical Reagent Co., Ltd (China). Sodium dodecyl sulfate(SDS), $AgNO_3$, horseradish peroxidase (HRP), bovine serum albumin (BSA) were purchased from Sinopharm Chemical Reagent Co., Ltd (China). Deoxyadenosine triphosphate (dATP) and chitosan (CS) were purchased from Aladdin Chemistry Co., Ltd. (China). Clinical serum samples of CEA with different concentrations were provided by the Wuhan Commercial Staff Hospital. All DNA sequences were synthesized and purified by Shanghai Sangon Biotechnology Co., Ltd (Shanghai, China), and their sequences in detail were as follows:

CEA aptamer: 5′-HS-$(CH_2)_6$-CCAC GATA CCAG CTTA TTCA ATTC GTGG-Biotin-3′.

Biotinylated hairpin DNA probe: 5′-HS-$(CH_2)_6$-ACAC GGCA GTTG ATCC TTTG GATA CCCT GGCG TGT-Biotin-3′.

Target DNA: 5′-CCAG GGTA TCCA AAGG ATCA ACTG C-3′.

One base-mismatched DNA: 5′-CCAG GGTA TCCA A**C**GG ATCA ACTG C-3′.

Three base-mismatched DNA: 5′-CCAG GGTA T**GC**G A**C**GG ATCA ACTG C-3′.

DNA oligonucleotide stock solutions were prepared with Tris–HCl buffer (20 mM Tris–HCl, 100 mM NaCl, 5 mM $MgCl_2$, pH = 7.4)