Biosensors and Bioelectronics 99 (2018) 28–33

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A high-sensitivity electrochemical aptasensor of carcinoembryonic antigen based on graphene quantum dots-ionic liquid-nafion nanomatrix and DNAzyme-assisted signal amplification strategy

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ARTICLE INFO

Keywords:
Graphene quantum dots
DNAzyme
Ionic liquid
Electrochemical aptasensor
Carcinoembryonic antigen

ABSTRACT

In this work, we have developed an electrochemical aptasensor for high-sensitivity determination of carcinoembryonic antigen (CEA) based on lead ion (Pb^{2+})-dependent DNAzyme-assisted signal amplification and graphene quantum dot-ionic liquid-nafion (GQDs-IL-NF) composite film. We designed hairpin DNA containing CEA-specific aptamers and DNAzyme chains. In the presence of CEA, hairpin DNA recognized the target and performed a DNAzyme-assisted signal amplification reaction to yield a large number of single-stranded DNA. The GQDs-IL-NF composite film was immobilized on the glassy carbon electrode for the interaction with single-stranded DNA through noncovalent π-π stacking interaction. Therefore, the methylene blue-labeled substrate DNA (MB-substrate) was fixed on the electrode and exhibited an initial electrochemical signal. Under optimal conditions, the response current change was proportional to the concentration of CEA, demonstrating a wide linear range from 0.5 fg mL^{-1} to 0.5 ng mL^{-1}, with a low detection limit of 0.34 fg mL^{-1}. Furthermore, the proposed aptasensor was successfully applied in determining CEA in serum samples, showing its superior prospects in clinical diagnosis.

1. Introduction

Cancer is one of modern society's greatest challenges in terms of deadly diseases. As such, there is extensive research into this class of diseases. Among the many areas of study, the detection of protein biomarkers has very important significance in exploring early diagnosis, elaborating the rule of pathological changes, and so on. Carcinoembryonic antigen (CEA) is a glycoprotein formed by many malignant tissues that can reflect the existence of a wide variety of tumors (Wen et al., 2016). CEA is also one of the most promising tumor markers for diagnosing cancer, monitoring and prognosis estimation, and can indicate the curative effect of various cancers (e.g. colorectal cancer, breast cancer and lung cancer) (Miao et al., 2016). Therefore, developing a convenient, rapid and sensitive analytical method for CEA detection in serum is of great importance. The level of CEA is often found in abnormally high concentrations in the serum of patients with malignancies, particularly epithelial tumors. A CEA level of 5.0 ng mL^{-1} in human serum is commonly used cutoff point for distinguishing abnormal from normal level. Serial measurement of CEA levels in patients with colorectal cancer can monitor different stages of disease, and detect recurrences early after surgery (Fletcher, 1986). However, the concentration of CEA in human serum is very low, especially in the initial stage of cancer progression. Therefore, it is necessary to establish a highly sensitive method for protein detection (Chang et al., 2010). In particular, enzyme-based signal amplification strategy has attracted a lot of interest in biosensing due to its high specificity, sensitivity and effective catalytic activity (Bao et al., 2015; Ge et al., 2016). Although promising, the use of protein enzymatic signal amplification is limited due to the high cost and poor enzyme stability.

In recent years, DNAzyme has received great attention as a new type of signal amplification recognition tool in the development of protein detection (Liu et al., 2016; Xia et al., 2015). It is a single-stranded oligodeoxynucleotide capable of catalyzing a specific chemical

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http://dx.doi.org/10.1016/j.bios.2017.07.036
Received 26 March 2017; Received in revised form 17 June 2017; Accepted 13 July 2017
Available online 15 July 2017
0956-5663/

reaction (Shen et al., 2016; Zhang et al., 2017). Compared to protein enzyme, it benefits from lower cost, better thermal stability and ease of synthesis (Wang et al., 2016, 2015). Since the DNAzyme can be complementary to a specific substrate sequence, the substrate chain is cleaved into two fragments when metal ions are present as cofactors (Cui et al., 2015; Shi et al., 2016; Xiao et al., 2007). In addition, the metal ion-dependent DNAzyme can undergo multiple conversions without loss of binding capacity and activity. Thus it can be subjected to a target cycling signal amplification reaction (Yang et al., 2016; Zhang et al., 2015). DNAzyme signal amplification strategy provides a good method for high-sensitivity determination of proteins through the target recirculation process.

Carbon materials modified electrodes have been widely used as electrochemical sensors. Graphene quantum dots (GQDs), as the most dazzling member of the family of carbon materials, have ignited great attention in the field of modified electrodes (Hu et al., 2016; J. Li et al., 2016; L. Wang et al., 2013; X. Wang et al., 2013). GQDs, as a type of zero-dimensional nanomaterial, are widely used in electrochemical detection, because of their high thermal conductivity, large surface area and high mobility of charge carriers (Wang et al., 2014, 2017; Zheng et al., 2015). In addition, GQDs also have some features of graphene, which can combine with single-stranded DNA through the π-π stacking. But they are unable to combine with rigid double-stranded DNA (Chau et al., 2016; He et al., 2011; Zhu et al., 2013). Nevertheless, GQDs have great solubility in aqueous solution, so they are not easily fixed on the surface of electrode. Nafion (NF), as a sulfonated ion exchange polymer, is a good choice for an excellent electrode matrix material due to its distinct electrochemical properties such as chemical inertness, outstanding antifouling capacity, great permeability to cations and good biocompatibility (Chaiyo et al., 2016; Gong et al., 2017;). In addition, NF can fix the carbon nanomaterial on the surface of electrode because of its hydrophobicity. It can also prevent the aggregation between the carbon nanomaterials (Xie et al., 2017; Zarei and Helli, 2015). However, pure NF film suffers from low conductivity which hinders electron transport (Y. Li et al., 2016; X. Zhang et al., 2016). A conductive material is often used in combination with NF to overcome this shortcoming. As an electrochemical material with excellent ionic conductivity and wide electrochemical window, ionic liquid (IL) is widely used as an electrode modifier in the manufacture of sensors (Chen et al., 2013; L. Wang et al., 2013; X. Wang et al., 2013; H. Zhang et al., 2016). The integration of GQDs, IL and NF could elicit synergistic effects in the electrochemical applications.

In this paper, we developed a novel electrochemical aptasensor based on Pb^{2+}-dependent DNAzyme assisted signal amplification and GQDs-IL-NF composite film for detection of CEA. With good conductivity, excellent biocompatibility and low toxicity, GQDs-IL-NF composite films were employed as the carrier for DNA immobilization through non-covalent π-π stacking interaction. Due to Pb^{2+}-dependent DNAzyme assisted target recycling signal amplification, it provided high electrochemical response signals. The designed aptamer sensor for CEA detection showed high selectivity, good reproducibility and acceptable stability. Herein, we proposed a promising method for the determination of CEA with intriguing prospects for clinical applications.

2. Experimental

2.1. Materials and reagents

Citric acid monohydrate, Nafion (5%, v/v) and ionic liquid 1-butyl-2,3-dimethylimidazolium tetrafluoroborate were obtained from Sigma Aldrich (USA). Saturated solutions of sodium hydroxide (NaOH), sodium chloride (NaCl), magnesium chloride ($MgCl_2$), and Lead nitrate were obtained from Aladdin Chemical Reagent (China). Carcinoembryonic antigen (CEA), bovine serum albumin (BSA), mucin 1 protein (MUC1) and prostate specific antigen (PSA) were purchased from Linc-Bio Science Co., Ltd (China). 10 mM phosphate buffered saline (PBS, pH 7.4) was obtained using Na_2HPO_4 and KH_2PO_4 and used as supporting electrolyte. 20 mM Tris-HCl (pH 7.4) containing 5 mM $MgCl_2$, 140 mM NaCl was used to dissolve DNA. All buffer solutions were prepared with ultra-pure water (18.25 MΩ cm) from an Aquapro water purification system (China).

Oligonucleotides were obtained from Shanghai Sangon Biotechnology Co., Ltd. (China). The sequence of oligonucleotides are as follows:

Hairpin DNA (HD): 5′- CAT CTC TTC TCC GAG CCG GTC GAA ATA GTG AGT ATA CCA GCT TAT TCA ATT AAG AGA TG-3′;
Substrate Chain: 5′- ACT CAC TAT rA GGA AGA GATG-MB-3′;
Auxiliary DNA (AD): 5′- CAT CTC TTCC-3′.

2.2. Apparatus

Electrochemical experiments were implemented using a CHI660C electrochemical system (Shanghai Chenhua Instrument Co., Ltd., China). In this experiment, three-electrode electrochemical system was used with the platinum as the auxiliary electrode, a saturated calomel electrode (SCE) as the reference electrode, and GQDs-IL-NF composite film modified glassy carbon electrode (GCE) as working electrode. Transmission electron microscopic (TEM) was performed on a FEI Tecnai G20 (USA). Dynamic light scattering (DLS) experiment was recorded by Malvern ZS90 (UK).

2.3. Fabrication of the electrochemical biosensor

The preparation procedure of aptamer signal probes is shown in Fig. 1. First, the hairpin DNA was heated to 95 °C for 5 min in a water bath, and lentamente cooled to room temperature to form a stem-loop DNA structure and stored at 4 °C. To detect CEA, the hairpin DNA (10 μL, 1 μM) was pretreated at 37 °C in a water bath for 1 h and then 10 μL of different concentrations of CEA were added. The mixture was incubated in a 37 °C for 1 h. Cleavage reaction was performed by adding of the MB-substrate (5 μL, 3 μM) and pb^{2+} (15 μL, 10 μM) to the above solution (50 μL) and incubated at 37 °C for 1 h.

2.4. Fabrication of GQDs -IL-NF modified electrode

The GQDs were synthesized from pyrolyzing citric acid following the previously reported procedure (Dong et al., 2012). The obtained solutions were stored at 4 °C. The manufacturing procedure for the GQDs-IL-NF modified GCE is briefly described as follow. First, a bare GCE was polished by 0.05 μm alumina slurry. And the GCE was sequentially sonicated with ultra-pure ethanol and water for 5 min to remove the remaining alumina powder and then the cleaned GCE was allowed to dry at 25 °C. 2 mL of 0.2 wt% Nafion, 100 μL of 1% ionic liquid, and 1 mL 1 mg/mL GQDs solution were mixed homogenously and sonicated for 30 min. Finally, 5 μL of the GQDs-IL-NF composite was drop-coated onto the surface of the cleaned GCE and dried at room temperature.

2.5. Preparation of electrochemical aptasensor

The GQDs-IL-NF nanocomposite film modified GCE was immersed in aptamer solution and incubated at 25 °C for 12 h. It was washed in buffer solution to remove the physically adsorbed aptamers. Finally, the electrodes were incubated in 2 μM of auxiliary DNA for 80 min and washed with PBS solution after fabrication. Electrochemical behaviors of the each step modification of the aptamer sensor were studied by electrochemical impedance spectroscopy (EIS) and cyclic voltammograms (CV).