Sensors and Actuators B 259 (2018) 372–379

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Highly active DNAzyme-peptide hybrid structure coupled porous palladium for high-performance electrochemical aptasensing platform

Yongmei Wu, Gaiping Li, Lina Zou, Sheng Lei, Qian Yu, Baoxian Ye*

College of Chemistry and Molecular Engineering, Zhengzhou University, Zhengzhou, 450001, PR China

ARTICLE INFO

Article history:
Received 9 July 2017
Received in revised form
21 November 2017
Accepted 15 December 2017
Available online 16 December 2017

Keywords:
Hemin/G-quadruplex-peptide
DNAzyme activity enhancement
Effective signal amplification
Electrochemical aptasensor
High performance

ABSTRACT

Herein, we developed a high-performance electrochemical aptasensor for the detection of carcinoembryonic antigen (CEA) using highly active peptide-conjugated hemin/G-quadruplex (hGQ-peptide) and porous Pd nanoparticles (PdNPs). Of note, the hGQ-peptide showed an enhanced catalytic activity as compared to the unmodified hGQ, and thus served as efficient electrocatalyst in this biosensor. Porous PdNPs acted as supported matrixes for high immobilization of hGQ-peptide hybrids, aptamer, redox-active toluidine blue (Tb) and alcohol dehydrogenase (ADH), resulting in the formation of ADH/Tb/hGQ-peptide/aptamer/PdNPs as the proposed bioprobes. Upon the sandwich-type specific reaction between aptamer and CEA, the bioprobe was presented on the sensing interface. Highly effective signal amplification could be then achieved by the bioprobe catalyzed cascade enzymatic reaction. Compared with the original hGQ, the hGQ-peptide yielded more than 2-fold improvement in signal amplification because of the peptide-enhanced catalysis. Therefore, the designed aptasensor displayed a wide linear range from 0.0001 to 100 ng mL^{-1} with a lower detection limit of 20 fg mL^{-1} for CEA determination, presenting the better analytical performance as compared to the relevant prior biosensors. This work further expanded the application of hGQ DNAzyme in biosensor. With the good anti-interference ability and selectivity, the proposed electrochemical aptasensor held great promise for ultrasensitive detection of other proteins in clinical diagnosis.

1. Introduction

Highly effective and sensitive detection of protein disease markers plays a pivotal role in early clinical diagnosis and disease prevention [1,2]. Based on the antigen-antibody specific recognition interaction, different immunoassays were exploited for the determination of tumor protein (e.g. carcinoembryonic antigen, a colon/breast cancer-associated marker), such as radioimmunoassay [3], enzyme linked immunosorbent assay [4] and chemiluminescence immunoassay [5]. However, these methods usually require skillful operators and expensive instruments, and suffer from the problem of radiation hazards and consuming time. Besides, these antibody-based methods have several limitations such as low stability at high temperatures, high cost for the production of antibodies. Since the first evolution of aptamers, aptamer-based sensing devices have been actively developed and have arouse great interest in biological detection due to the superiority of aptamer relative to antibody, such as simple synthesis, long-term storage, high specificity and affinity [6–9]. Among these devices, electrochemical aptasensors offer remarkable advantages, including simplicity, oft-impressive sensitivity and inexpensive electrochemical instrument [10], and so they are frequently used to detect protein targets [11–14]. Either low sensitivity or inefficiency can reduce the practical applicability of biosensing tool. For this reason, it is of key importance to improve the sensitivity and efficiency of bioassay, which are determined by signal amplification proficiency of biolabel.

Signal amplification strategies that couple the enzyme electrocatalytic label with nanomaterial are currently attracting major attention in amplified quantification of biomarker [15–17]. As an enzyme-mimicking catalyst, hemin/G-quadruplex (hGQ) DNAzyme, which was formed from hemin and a folded G-rich oligonucleotide, exhibited higher thermal stability and was easier to synthesize and label in contrast to its protein counterparts. Besides, it owned these unique features of fast catalytic turnover and reduced nonspecific binding properties, as well as could mimic

* Corresponding author.
E-mail address: yebx@zzu.edu.cn (B. Ye).

https://doi.org/10.1016/j.snb.2017.12.091
0925-4005/

horseradish peroxidase (HRP) and NADH oxidase [18]. Motivated by the listed advantages, many research groups have embarked on the development of diverse hGQ-amplified sensing systems [19–22]. In particular, hGQ-catalyzed pseudobienzyme amplification strategies were appealing in electrochemical bioassays [23–27], wherein the substrate of one enzymatic reaction could be produced in situ by another concurrent reaction, giving rise to the so-called cascade signal amplification [28,29]. However, these methods generally suffered from the issue for limited efficiency due to the inferior catalytic activity of hGQ over the proteinous enzyme. Still, there is a need for constructing a novel hGQ-based electrochemical biosensing platforms to enable the high detection efficiency. More recently, covalently linking cationic peptide to G-quadruplex structure was confirmed to enhance its DNAzyme activity [30]. As such, this provided an exciting opportunity for the design of highly active hGQ-based label. To develop an electrochemical aptasensor based on the enhanced catalytic function of hGQ would greatly promote the efficiency of biosensing tool in clinical detection.

In this work, we modified hGQ with the short polyhistidine peptide and adopted the hybrid structure as efficient elctrocatalytic label. In combination with palladium nanoparticles (PdNPs), which possessed the porous structure and fascinating peroxidase activity, a new electrochemical aptasensing platform was developed (Scheme 1). In this device, due to the larger surface area, porous palladium acted as supported matrix for co-immobilization of the hGQ-peptide hybrid, aptamer, redox-active toluidine blue (Tb) and alcohol dehydrogenase (ADH). Moreover, its electrocatalysis toward hydrogen peroxide further enhanced the sensitivity. Through the specific recognition between aptamer and target protein, the proposed bioprobes (ADH/Tb/hGQ-peptide/aptamer/PdNPs bioconjugates) were captured onto the electrode surface, and then target concentration-related electrochemical signals were obtained. After adding the NAD^+ and ethanol into the working buffer, the co-catalysis of hGQ-peptide, ADH and PdNPs toward substrates led to a significant amplification in electrochemical signal, as discussed in the following text. As a result, the aptasensor could detect carcinoembryonic antigen (CEA) down to 20 fg mL^{-1}. Notably, owing to the improved catalysis of hGQ-peptide, the proposed strategy represented preferable detection results and showed much higher analytical efficiency relative to the previously reported electrochemical methods for CEA detection. Ultimately, the practicality of the aptasensor was validated by assaying CEA in human serum sample, and there was no obvious difference between the results and those obtained from commercial enzyme-linked immunosorbent assay (ELISA), indicating its promising application in clinical detection.

2. Experimental section

2.1. Materials and reagents

Carcinoembryonic antigen (CEA), Potassium tetrachloropalladate(II) (K_2PdCl_4, 99%), gold chloride ($HAuCl_4$), hexanethiol (96%, HT), Sulfosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC), β-nicotinamide adenine dinucleotide hydrate (NAD^+), alcohol dehydrogenase (ADH), hemin and 3,3′,5,5′-tetramethylbenzidine (TMB) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Toluidine blue (Tb), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), N-hydroxy succinimide (NHS), cetyltrimethylammonium chloride (CTAC) and ascorbic acid (AA) were purchased from J&K Scientific Ltd. (Beijing, China). $NaBH_4$, glutaraldehyde (GA) and tris-hydroxymethylaminomethane hydrochloride (Tris-HCl) were purchased from Aladdin Co. Ltd. (Shanghai, China). The peptide (CAAAHHHHHHK) was synthesized by Shanghai Science Peptide Biological Technology Co.; Ltd (Shanghai, China). DNA oligomers used in this study were purchased from Sangon, Biotech Co.; Ltd. (Shanghai, China):

CEA aptamer-1 (Apt1): 3′- NH_2-$(CH_2)_6$-ATACCAGCTTATTCAATT-5′

CEA aptamer-2: (Apt2): 3′-NH_2-AGGGGGTGAAGGGATACCC-5′

G-quadruplex sequence (GQ): 5′-GTGGGTAGGGCGGGTTGGA$_6$—NH_2-3′

Phosphate buffered solution (PBS, pH 7.4) was prepared with 0.1 M Na_2HPO_4 and 0.1 M NaH_2PO_4 (0.1 M KCl as the supporting electrolyte). Tris-HCl buffer (20 mM, pH 7.4) was prepared with 140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$ and 1 mM $MgCl_2$. Conjugation buffer (pH 7.3) was prepared with 500 mM NaCl and 100 mM Na_2HPO_4— NaH_2PO_4. Unless specified otherwise, all chemicals were analytical grade and used without further purification. Ultrapure water coming from a Millipore system was used throughout the experiments.

2.2. Apparatus and experimental measurements

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were carried out with a CHI 650A electrochemical workstation (Shanghai Chenhua Instrument, China). All electrochemical measurements were implement with a three-electrode system including the modified glassy carbon electrode (diameter: 4 mm) as working electrode, a platinum wire as the counter electrode and an Ag/AgCl electrode as the reference electrode, respectively. A USA FEI, M3000 transmission electron microscope (TEM) was used to explore the surface morphology of nanomaterials. The pH measurement was performed on a PHS-3C precision pH meter (Shanghai, China). CV experiments were performed in 2 mL of $[Fe(CN)_6]^{3-/4-}$ (5 mM, pH 7.4) with the potential scans between −0.2 to 0.7 V at the scan rate of 50 mV s^{-1}. DPV measurements were carried out in 2 mL PBS (0.1 M, pH 7.4) containing NAD^+ (0.25 mM) and 90 μL absolute ethanol. The DPV parameters were listed as followed: the applied potential ranging from 0.0 V to −0.5 V (vs. Ag/AgCl), scan rate of 50 mV s^{-1}, the modulation amplitude of 50 mV, pulse width of 0.05 s and sample width of 0.0167 s. XRD patterns were record on the X' Pert PRO X-ray diffractiometer with Cu Kα radiation source (λ = 1.5406 Å).

2.3. Preparation of porous PdNPs and hGQ-peptide hybrid

Porous PdNPs were synthesized according to ref [31]. 50 μL of K_2PdCl_4 (0.01 M) solution was mixed with 1.95 mL of CTAC solution (0.1 M). The freshly prepared $NaBH_4$ (120 μL, 0.01 M) in ice solution was added to above miscible liquids and was stirred vigorously. The resulting solution rested for 2 h and then 0.5 mL of the solution was mixed with 194 mL of CTAC (45 mM) and 6 mL of K_2PdCl_4 (0.01 M). Then, 2 mL of AA (0.1 M) was injected into the mixture with gentle inverse stirring for 10 s. The reaction solution was allowed to proceed unperturbed for 6 h at room temperature and then was centrifuged and washed three times. The porous PdNPs were redispersed in 2 mL PBS (pH 7.4) for further use.

According to the previous method with a little modification [30], we prepared the hGQ-peptide hybrid as follows: Briefly, sulfo-SMCC (2 mg) was added to 200 μL of DNAzyme (100 μM) in conjugation buffer. The solution was stirred for 2 h under agitations at room temperature, followed by the centrifugation and washing with conjugation buffer for six times by an Amicon–3 K ultrafilter (3 kDa cutoff) to remove the excess sulfo-SMCC. Whereafter, peptide (1.0 mg) was added to this above solution and kept stirring for 48 h at room temperature. The cysteine-terminated polyhistidine peptide could be conjugated to the sulfo-SMCC-activated G-quadruplex through Michael addition reaction. Then, washing