Biosensors and Bioelectronics 91 (2017) 246–252

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A novel immunosensing platform for highly sensitive prostate specific antigen detection based on dual-quenching of photocurrent from CdSe sensitized TiO_2 electrode by gold nanoparticles decorated polydopamine nanospheres

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ARTICLE INFO

Keywords:
Dual quenching strategy
AuNPs-Dpa-melanin CNSs
Photoelectrochemical biosensor
Exciton energy transfer
Steric hindrance effect
Prostate specific antigen

ABSTRACT

Herein, a novel photoelectrochemical (PEC) immunosensing platform for highly sensitive detection of prostate specific antigen (PSA) was constructed based on dual-quenching of photocurrent from CdSe sensitized TiO_2 electrode by gold nanoparticles decorated dopamine–melanin nanospheres (AuNPs-Dpa-melanin CNSs). In this proposal, CdSe sensitized TiO_2 was used as photoelectrochemical matrix and the functional AuNPs-Dpa-melanin CNSs were used as signal quenching element. The dual quenching of the gold nanoparticles decorated Dpa-melanin CNSs to the CdSe sensitized TiO_2 was achieved as follows: (i) the strong energy transfer between the CdSe quantum dots (QDs) and Au NPs diminishes the photocurrent signal of CdSe QDs; (ii) the steric hindrance of AuNPs-Dpa-melanin CNSs partly obstructs the diffusion of the electron donor, i.e. ascorbic acid, to the surface of photoelectrode, which make the depleting efficiency of the photogenerated holes decrease, leading to a declined photocurrent intensity. On the basis of the dual quenching effect of AuNPs-Dpa-melanin CNSs, a competitive immunosensing platform for PSA was designed upon the specific binding of anti-PSA to PSA and PSA functionalized AuNPs-Dpa-melanin CNSs conjugates. This proposed immunosensor possesses wide linear range from 1.0×10^{-11} g mL^{-1} to 1.0×10^{-7} g mL^{-1} with the detection limit of 2.7 pg mL^{-1}. Moreover, the applicability of the present method was demonstrated in the determination of PSA in human serum. The strategy creates new paradigms for PSA and other tumor markers detection and demonstrates high sensitivity, good specificity, and satisfied applicability in complex biological samples.

1. Introduction

Photoelectrochemical (PEC) technique, with features such as simple devices, low cost and simple operation, and high sensitivity, is becoming an innovative and powerful analytical method for bioanalysis. To date, PEC technique has been applied for the determination various target analytes including DNA, protein, metal ions and cells (Zhao et al., 2014a, 2014b, 2015, 2016). The consistent detection principle of PEC biosensors is that the photocurrent change could be produced by the biological interactions between the various recognition elements and their corresponding targets. The photocurrent changes in the detection process often relay on the steric hindrance effects (Wang et al., 2009), the consumption/generation of coreactant based on enzymatic reactions (Cao et al., 2015; Wang et al., 2014b) and the Förster resonance energy transfer (FRET) between semiconductor nanocrystals and metallic nanoparticles (Shen et al., 2015; Xu et al., 2016; Zhao et al., 2012). Among various methods, the signal quenching strategies via the consumption of the coreactant, steric hindrance effect, or exciton energy transfer (EET) have been usually utilized for the construction of PEC biosensors. For example, Wang et al. developed a label-free PEC immunoassay for α-fetoprotein detection based on the steric hindrance of the immunocomplex for ascorbic acid to the surface of CdS (Wang et al., 2009). By monitoring the formation or consumption of hydrogen peroxide as coreactant, quantum dots functionalized porous ZnO nanosheets-based PEC platform has been designed for DNA detection (Wang et al., 2014b). Based on the strong EET effect between Au NPs and CdSe quantum dots (QDs), a highly sensitive DNA methyltransferase activity and inhibitor screening PEC

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http://dx.doi.org/10.1016/j.bios.2016.12.043
Received 9 October 2016; Received in revised form 3 December 2016; Accepted 16 December 2016
Available online 18 December 2016
0956-5663/

assay has been developed (Shen et al., 2015). Summarized from these examples, single signal change path was always adopted. It would be deduced that the combination of two or more these signal change paths might yield synergetic effect to the PEC system, which might greatly improve the detection sensitivity.

Dopamine-melanin colloidal nanospheres (Dpa-melanin CNSs) with the advantages of excellent compatibility and biodegradability are appealing materials for the construction of sensing interface due to its rich surface-functional groups (–OH, $–NH_2$) for easily conjugation with some interesting molecules. Up to now, the Dpa-melanin CNSs have been exploited in the field of fluorescent (Xie et al., 2015), chemiluminescent (Wang et al., 2016), electrochemical (Wang et al., 2014a), and ECL (Liu et al., 2014) detection. In our previous work, Dpa-melanin CNSs immobilized with $Ru(bpy)_3^{2+}$ were used as an ECL tag for sensitive thrombin detection (Liu et al., 2014). The Dpa-melanin with large surface area could enrich a large number of luminophore reagent such as $Ru(bpy)_3^{2+}$ on its surface and thus could produce a strong ECL response. Inspired by this ingenious idea, a large amount of gold nanoparticles (AuNPs) could be also immobilized on the surface of Dpa-melanin CNSs. Compared with the bioprobes that one bioprobe labeled with one single AuNP (Shen et al., 2015), the AuNPs coated Dpa-melanin CNSs would become more efficient energy acceptors in the EET of the PEC system. How about the AuNPs coated Dpa-melanin CNSs working in the PEC assay?

Herein, a novel competitive PEC immunosensor for protein detection was developed based on dual signal quenching strategy. The Dpa-melanin CNSs were facilely synthesized from dopamine hydrochloride in an alkaline environment and the AuNPs-Dpa-melanin CNSs were prepared by *in situ* growth of AuNPs on Dpa-melanin CNSs. To fabricate the immunosensor, TiO_2, PDDA, CdSe QDs, antibody (Ab), were stepwise assembled on an indium tin oxide (ITO) electrode as depicted in Scheme 1. Prostate specific antigen (PSA), a valuable premier tumor marker for detecting early stage prostate cancer or other prostate disorders, was used as a model protein in this work. In the absence of PSA, the PSA-AuNPs-Dpa-melanin CNSs conjugates specifically bound with Ab specific for PSA on the electrode. The bound of PSA-AuNPs-Dpa-melanin CNSs could induce an obvious decrease of photocurrent signal by both the EET effect between CdSe QDs and AuNPs and the steric hindrance of Dpa-melanin CNSs. In the presence of PSA, the competitive interaction of PSA and PSA-AuNPs-Dpa-melanin CNSs with the Ab on the electrode was occurred. In this state, the amount of PSA-AuNPs-Dpa-melanin CNSs conjugates bound on the electrode decreased, producing an increased photocurrent signal compared to the electrode only with PSA-AuNPs-Dpa-melanin CNSs incubation. Under the optimum conditions, the immunosensor exhibits excellent performance for PSA detection. Furthermore, the application of the proposed sensing strategy was demonstrated by determination the concentration of PSA in complex biological samples.

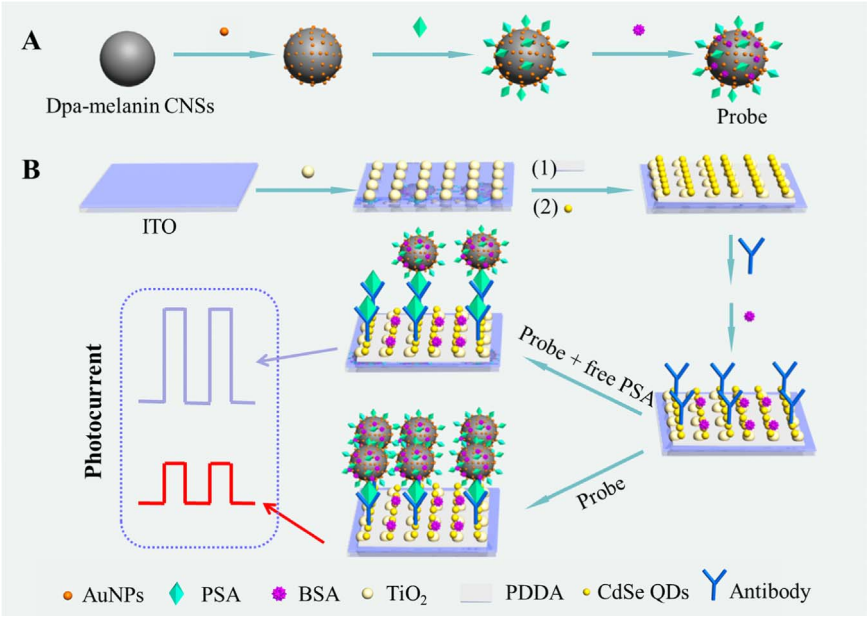
2. Experimental

2.1. Materials and reagents

Prostate specific antigen (PSA, L2C001) standards and mouse monoclonal PSA antibody (clone L1C00401, capture antibody, Ab) were purchased from Shanghai Linc-Bio Science Co., Ltd. (Shanghai, China). TiO_2 powder was from the Degussa Co. (P25, Germany). Hyrogen tetra chloroaurate(III) trihydrate ($HAuCl_4·3H_2O$), ascorbic acid (AA), cadmium chloride ($CdCl_2·2.5H_2O$) and sodium borohydride ($NaBH_4$) were obtained from Shanghai Reagent Company (Shanghai, China). Human serum albumin (HSA), human immunoglobulin (hIgG), bovine serum albumin (BSA), and thrombin (TB) were from Shanghai Solarbio Bioscience & Technology Co., Ltd. (Seebio Biotechnology). Poly(diallyldimethylammonium chloride) (PDDA; 20%, w/w in water, MW=200,000–350,000), selenium powder (Se), thioglycolic acid (TGA), *N*-(3-Dimethylaminopropyl)-*N*-ethyl-carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich. Other chemicals were of analytical reagent grade and used as received. All aqueous solutions were prepared using pure water (18.2 MΩ·cm, ultrapure water system from Aike Water Treatment Solution Provider, China).

2.2. Apparatus

PEC signal was detected by the home-made PEC system equipped with a 500 W Xe lamp. Photocurrent was measured on a RST5200 electrochemical workstation (Zhengzhou Shiruisi Technology Co., Ltd., China) with a three-electrode system: a modified ITO electrode with a geometrical area of 0.25 cm^2 as the working electrode, a Pt wire as the auxiliary electrode and a saturated Ag/AgCl electrode as the reference electrode. A 0.01 M phosphate buffer solution (PBS, pH 7.4) containing 0.1 M AA was used as the electrolyte for photocurrent measurements.



Scheme 1. (A) The preparation of the probe. (B) The fabrication procedure of the immunosensor.