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Bio-bar-code-based photoelectrochemical immunoassay for sensitive detection of prostate-specific antigen using rolling circle amplification and enzymatic biocatalytic precipitation

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ABSTRACT

Methods based on photoelectrochemistry have been developed for immunoassay, but most involve in a low sensitivity and a relatively narrow detectable range. Herein a new bio-bar-code-based split-type photoelectrochemical (PEC) immunoassay was designed for sensitive detection of prostate-specific antigen (PSA), coupling rolling circle amplification (RCA) with enzymatic biocatalytic precipitation. The bio-bar-code-based immunoreaction was carried out on monoclonal anti-PSA antibody (mAb_1)-coated microplate using primer DNA and polyclonal anti-PSA antibody-conjugated gold nanoparticle (pDNA-AuNP-pAb_2) with a sandwich-type assay format. Accompanying the immunocomplex, the labeled primer DNA on gold nanoparticle readily triggered RCA reaction in the presence of padlock probe/dNTPs/ligase/polymerase. The RCA product with a long single-stranded DNA could cause the formation of numerous hemin/G-quadruplex-based DNAzyme concatamers. With the assistance of nicking endonuclease, DNAzyme concatamers were dissociated from gold nanoparticle, which catalyzed the precipitation of 4-chloro-1-naphthol in the presence of H_2O_2 onto CdS nanorods-coated electrode (as the photoanode for the generated holes). The formed insoluble precipitate inhibited the electron transfer from the solution to CdS nanorods-modified electrode by using ascorbic acid as the electron donor. Under the optimum conditions, the photocurrent of the modified electrode decreased with the increasing of PSA concentration. A detectable concentration for target PSA with this system could be achieved as low as 1.8 pg mL^{-1}. In addition, our strategy also showed good reproducibility, high specificity and accuracy matched well with commercial PSA ELISA kits for real sample analysis. These remarkable properties revealed that the developed PEC immunoassay has great potential as a useful tool for the detection of PSA in practical application.

1. Introduction

Along with improvement of the people's life quality and the development of science and technology, people's health awareness is increasing gradually, which has greatly stimulated interest of researchers to explore various analytical methods for the highly selective detection of low-concentration analyte. Photoelectrochemical (PEC) sensor (as a new analytical technique on the basis of photochemistry and electrochemistry) has developed rapidly in recent years to monitor different analytes, *e.g.*, proteins (J. Wang et al., 2017; X. Wang et al., 2017), antibiotics (Yan et al., 2015), nucleic acids (Wu et al., 2013) and heavy metals (Zang et al., 2014). Derived from the traditional electrochemical methods, the PEC method has the merits of simple operation, good portability and short response time. Due to different energy forms of exciter (light) and detector (electricity), the PEC analysis platform has an excellent sensitivity and a low background signal. Despite some significant results, the detectable sensitivity still falls short of the practical requirements and large-scale applications.

Generally, the development of highly efficient PEC sensing systems depends on at least three concerns: i) biomolecular immobilization in detection scheme, ii) the signal amplification and iii) photosensitive materials. The first key point is to design a feasible detection protocol since the PEC detection system involves the light illumination as well as the strong oxidation characteristics of photogenerated holes in photoactive materials (Shi et al., 2016; Wang et al., 2014; Wen and Ju, 2016; Zang et al., 2014), which inevitably causes the damage of the immobilized biomolecules on the electrode (especially using UV light) (Zhao et al., 2012; Yan et al., 2015; Ma et al., 2015; Zhang et al., 2016). In this case, design of biomolecular immobilization in the PEC system becomes more and more important. An overwhelming strategy is to

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separate PEC measurement from the biomolecular reaction (*e.g.*, immunoreaction). The emergence of split-type detection mode provides a new idea for development of PEC sensing platform. Yamamoto et al. (1995) described a split-type flow cell of a polarized spectrophotometric detector in HPLC for colored amino acid-copper(II) complexes. Zhuang et al. (2018, 2015) devised two split-type PEC immunosensing systems for sensitive monitoring of disease biomarkers. Such a split-type detection scheme by immobilizing biomolecules in the reaction cell and measuring in PEC cell can efficiently avoid the damage of biomolecules.

The signal amplification is another important factor in PEC sensing systems for achieving low limits of detection and quantification. Routine approaches are usually adopted *via* controlling the orientation and density of biomolecules immobilized on the substrates (*e.g.*, microplate and magnetic beads) (Chen et al., 2014; Li et al., 2017; Wang et al., 2015). Unfavorably, the conformational freedom of the immobilized biomolecules (*i.e.*, their structural configuration) is limited because of the steric-hindrance effect, thus resulting in reduction of the bonding efficiency and rate between the biological probes and the targets (Yu and Lai, 2013). In this regard, improvement of the signal molecules in the detection mode is extremely urgent. Rolling circle amplification (RCA) is a simple and efficient isothermal nucleic-acid signal amplification method mediated by DNA polymerases, in which a long single-stranded DNA containing numerous complementary copies of the short circular template can be synthesized (Ye et al., 2014; Kong et al., 2016). Since the discovery of RCA in the mid-1990s, the power, simplicity and versatility of the DNA amplification technique have made it an attractive tool for biomedical research and nanobiotechnology (Zhao et al., 2008). Typically, the amplification process is carried out in aqueous solution, on the surface of solid supports or even in a sophisticated biological environment, and does not require specialized equipment. Importantly, the function of the RCA products can be manipulated by designing the circular template sequence in a very predictable manner to meet the needs of specific applications (Niu et al., 2016; Cao et al., 2017; Deng et al., 2017; Zhu et al., 2016; Wen et al., 2012), especially to form hemin/G-quadruplex-based DNAzyme (Tang et al., 2012; Dong et al., 2013). To the best of our knowledge, there are few reports focusing on the development of RCA-based PEC immunoassays. To this end, our motivation of this study is to design a split-type PEC immunoassay by coupling with RCA-based formation of hemin/G-quadruplex-based DNAzyme concatamers (note: RCA product is a concatamer containing tens to hundreds of tandem repeats that are complementary to the circular template (Ali et al., 2014)).

As the signal-generation tag, cadmium sulfide (CdS; an outstanding narrow band gap of ~2.4 eV because of the requirement of a low excited energy according to the equation: $E = h\nu$) (Harakeh et al., 2008) photosensitive semiconductor has been widely studied in the past years owing to its excellent light response. However, the presence of bulk recombination leads to the severe photocorrosion (Zhang et al., 2017a). To decrease the bulk recombination, researchers have made great efforts for the synthesis of CdS nanocrystals with different morphologies (*e.g.,* nanorods, nanowires, quantum dots and nanoparticles). Typically, CdS nanorods increase the PEC activity by reducing the radial transport distance of the charge carriers and expanding the surface area for the reaction (Vaquero et al., 2017). Prostate-specific antigen (PSA) is a protein produced by normal prostate cells. The normal levels of PSA in the blood serum of healthy males are maintained at a low level (< 4 ng mL^{-1}), while rising levels are associated with prostate cancer. Herein, we design a new bio-bar-code-based immunoassay method for photoelectrochemical (PEC) detection of target PSA on CdS nanorods-modified electrode, coupling with rolling circle amplification and hemin/G-quadruplex-based DNAzyme concatamers (Scheme 1). The immunoreaction and rolling circle amplification are carried out on a microtiter well. In the presence of target PSA, the immobilized capture antibody (mAb_1) on the microplate captures the biofunctionalized gold nanoparticle with the primer DNA and detection antibody (pDNA-AuNP-pAb_2). Introduction of primer DNA on the gold nanoparticle ensures the progression of RCA reaction. Accompanying with the formation of DNAzyme concatamers, the formed double-stranded DNA is cleaved *via* Nt.BbvCI (note: Nt.BbvCI is a nicking endonuclease that cleaves only one strand of DNA on a double-stranded DNA substrate) to release the DNAzyme concatamers. Upon addition of H_2O_2, the dissociated DNAzyme concatamers can catalyze 4-chloro-1-naphthol into an insoluble benzo-4-chlorohexadienone product, and coat the surface of CdS nanorods-modified electrode, thus inhibiting the electron transfer. In this case, the photocurrent of the modified electrode decreases using ascorbic acid as the electron donor.

2. Experimental

2.1. Chemicals and reagents

PSA standards were acquired from Biocell Biotechnol. Inc. (Zhengzhou, China). Monoclonal anti-PSA capture antibody (A45180, designated as mAb_1) and monoclonal anti-PSA detection antibody (A45190, designated as pAb_2) were the products of BiosPacific, Inc. (CA, USA). All high-binding polystyrene 96-well microplates were obtained from Greiner Bio-One (Frickenhausen, 705071, Germany). Gold nanoparticles with an average diameter of 16 nm were prepared according to our previous report (Zhang et al., 2012). T4 DNA ligase and Phi29 DNA polymerase were obtained from Thermo Fisher Scientific Inc. (Shanghai, China). Nt.BbvCI was acquired from New England Biolab (Beijing, China). Oligonucleotides and dNTPs were purchased from Sangon Biotech. Co., Ltd. (Shanghai, China). Bovine serum albumin (BSA) was acquired from Dingguo Biotechnol. Co., Ltd. (Beijing, China). Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) and 4-chloro-1-naphthol (4-CN) were obtained from Sigma-Aldrich (USA). Cadmium nitrate tetrahydrate [$Cd(NO_3)_2 \cdot 4H_2O$], thiourea, ethlylenediamine, 3,3′,5,5′-Tetramethylbenzidine (TMB) and ascorbic acid (AA) were purchased from Sinopharm Chem. Re. Co., Ltd. (Shanghai, China). All reagents (analytical grade) were used as received without further purification. Ultrapure water obtained from a Millipore water purification system (18.2 MΩ cm^{-1}, Milli-Q, Millipore). The sequences of oligonucleotides used in this work:

Primer DNA (pDNA): 5′-SH-TTAAT CCTCA GCTTA TTTGA GGTAG TAGGT TGTAT AGTT-3′
Linear padlock DNA: 5′-phosphate-CTACT ACCTC AAAAA CATCC CAACC CGCCC TACCC ACGCA ACTAT ACAAC-3′
Cleaved DNA: 5′-AAGCT GAGGA TT-3′
G-rich DNA: 5′-SH-TTAAT CCTCA GCTTA TTGGG TAGGG CGGGT TGGGA TGTT-3′

Buffer solutions were used as follows:

DNA ligation buffer: 40 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM dithiothreitol (DTT), 0.5 mM ATP, pH 7.8
Reaction buffer: 33 mM Tris-HAc, 10 mM $Mg(Ac)_2$, 66 mM KAc, 0.1% (v/v) Tween 20, 1.0 mM DTT, pH 7.9
CutSmart buffer: 50 mM KAc, 20 mM Tris-HAc, 10 mM $Mg(Ac)_2$, 100 μg mL^{-1} BSA, pH 7.9
Phosphate buffered saline (PBS, 10 mM, pH 7.4): 2.9 g $Na_2HPO_4 \cdot 12H_2O$, 0.24 g KH_2PO_4, 0.2 g KCl, 8.0 g NaCl
Washing buffer: 10 mM PBS, 0.05% Tween 20, pH 7.4
Blocking buffer: 10 mM PBS, 1.0 wt% BSA, pH 7.4

2.2. Fabrication of CdS nanorods-based photoanode

CdS nanorods were synthesized by using a hydrothermal method according to the literature with minor modification (Yin et al., 2016). Generally, $Cd(NO_3)_2 \cdot 4H_2O$ (8.1 mmol) was initially added into 40-mL ethlylenediamine to form a homogeneous solution under sonication and

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