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Electrochemical detection of protein based on hybridization chain reaction-assisted formation of copper nanoparticles

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A B S T R A C T

In this paper, we report an electrochemical method for highly sensitive and specific detection of protein based on hybridization chain reaction (HCR)-assisted formation of copper nanoparticles by using small molecule such as folate-linked DNA as probe. In the presence of target protein, taking folate receptor (FR) as the model protein in this study, its binding with folate can protect the probe DNA from exonuclease I-catalyzed degradation, thus the probe DNA can be immobilized onto the electrode surface through the hybridization with capture DNA, triggering HCR on the electrode surface. Subsequently, copper nanoparticles can be formed on the electrode surface by using long duplex DNA oligomers from HCR as templates. Furthermore, copper ions released from acid-dissolution of copper nanoparticles can catalyze the oxidation of o-phenylenediamine by dissolved oxygen, leading to significant electrochemical responses. As a result, our method can sensitively detect FR in the linear range from 0.01 ng/mL to 100 ng/mL with a detection limit of 3 pg/mL. It can also specifically distinguish the target protein in both buffer and complex serum samples. Since many other proteins can be assayed by changing the corresponding small molecule, this method may be promising for the development of the technique for protein detections.

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1. Introduction

Sensitive and specific detection of protein, especially disease-related biomarkers, has attracted increasing attention in both biomedical research and clinical diagnosis (Jinmyoung et al., 2012; Y. Li et al., 2014; Ozdemir et al., 2013). As an alternative to antibody and aptamer, small molecule that can bind to protein through a special interaction has become an appealing recognition element in biosensing (Fechner et al., 2014; Wang et al., 2013; Zhao et al., 2013), and terminal protection by using small molecule-linked DNA as probe has provided a new insight for protein detection (Cao et al., 2012; Ou et al., 2013; Zhou et al., 2013). Besides the high selectivity from small molecule–protein interaction, well-designed probe DNA can pave the way for signal transduction, acquisition and even amplification, which can greatly improve the flexibility and sensitivity of protein detection. Consequently, different signal amplification techniques and signal readout approaches have been attempted to develop highly specific, sensitive and robust detection for target proteins based on the terminal protection.

DNA amplification techniques are the most commonly-used methods to improve the sensitivity of DNA-based detection. For example, polymerase chain reaction (PCR) that can reliably detect as few as several copies of DNA sequences in biology has been reported to induce significant signal amplification after the target recognition events (Malou and Raoult, 2011; Zhao et al., 2011). Nevertheless, the complex temperature cycling protocol and the need of expensive equipment have obviously restricted the application of PCR. Therefore, several isothermal DNA amplification approaches that can be performed under a constant reaction temperature have been proposed to replace PCR, such as strand displacement amplification (SDA), loop-mediated isothermal amplification (LAMP), rolling circle amplification (RCA), etc. (Deng et al., 2014; Fang et al., 2014; Wong et al., 2014). Recently, an isothermal amplification technique named as hybridization chain reaction (HCR) has aroused great interest, in which an initiator DNA can trigger a cascade of hybridization events to yield the linkage of DNA strands with two stable species of DNA hairpins (Dirks and Pierce, 2004; Wu et al., 2012). Unlike the other isothermal amplification approaches, HCR does not depend on enzymes, which can also exhibit the advantages of low background, mild reaction, and PCR-like sensitivity (Ge et al., 2014; Huang et al., 2011; B. Zhang et al., 2012).

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Nowadays, DNA-templated nanomaterials have attracted great attention in bioanalysis for facile synthesis, excellent optical properties and well biocompatibility. For example, cytosine-rich and guanine-rich oligonucleotides can be efficient templates for the formation of Ag nanoclusters, which have been extensively used for the detection of nucleic acids, small molecules and proteins (Xu et al., 2012; Yeh et al., 2010; M. Zhang et al., 2012; Zhang et al., 2014). Recently, Mokhir et al. reported a method for selective formation of copper nanoparticles (CuNPs) by using double-stranded DNA (dsDNA) as template in the presence of a low concentration of Cu^{2+}, whereas single-stranded DNA (ssDNA) cannot serve as the efficient template (Rotaru et al., 2010). Compared with DNA-templated Ag nanoclusters, dsDNA-templated CuNPs with high fluorescence and good photostability nearly require no specific DNA sequence, thus gaining wider acceptance in bioanalysis (Jia et al., 2012; Zhang et al., 2013a, 2013b). Nonetheless, the application of dsDNA-templated CuNPs is still based on their fluorescent properties, which hardly have association with other characterization techniques so far.

Similar to fluorescence technique, electrochemical technique is another widely-used characterization technology in the field of bioanalysis, which can exhibit several noticeable advantages, including simple operation, rapid response, low cost, high sensitivity and good selectivity (H. Li et al., 2014; Zhao et al., 2014; Zhu et al., 2014). Meanwhile, due to the superiority in the determination of metal ions, electrochemical technique has been successfully applied in the characterization of nanoparticle labels, such as gold nanoparticles, Ag nanoparticles and quantum dots (Chu et al., 2005; T. Li et al., 2010; X. Li et al., 2010). Large amounts of metal ions released from the dissolution of nanoparticles can be monitored by using electrochemical techniques with ultra-high sensitivity, resulting in obvious signal amplification for the detection of many kinds of biomolecules. Therefore, by taking the advantages of electrochemical techniques in tracing metal nanoparticles, we have herein proposed a highly sensitive and selective method for protein detection based on CuNPs that are formed by using HCR-assisted dsDNA as template and characterized by electrochemical techniques via the combination with Cu^{2+}-catalyzed oxidation of o-phenylenediamine (OPD). On the other hand, we have employed a small molecule instead of the traditional antibody or aptamer to recognize the target protein, which can offer a wider range of choices for recognition elements in protein detection, especially when the target protein has no corresponding antibody or aptamer.

2. Experimental

2.1. Chemicals and materials

Folate receptor (FR) was purchased from Sino Biological Inc. Exonuclease I (Exo I) was purchased from New England Biolabs. Bovine serum albumin (BSA), ovalbumin (OVA), fetal calf serum and $CuSO_4$ were purchased from Dingguo Biotech. Co. Tris(2-carboxyethyl)-phosphine hydrochloride (TCEP), mercaptohexanol (MCH), OPD and thrombin were from Sigma Aldrich Chemical Co. DNA oligonucleotides were synthesized by TAKARA Biotechnology (Dalian, China) and the sequences are as follows:

Folate-linked probe DNA: 5′-AAATTGAGCTGCAGAATGGGATCG GTACACTG CG-NH-folate-3′;

Capture DNA: 5′-SH-AAACGCAGTGTACCGATCC-3′;

H1: 5′-CATTCTGCAGCTCAACAAACTTTGAGCTGC-3′;

H2: 5′-TTGAGCTGCAGAATGGCAGCTCAAAGTTTG-3′.

The buffer solutions used in this work are as follows. DNA immobilization buffer: 10 mM Tris–HCl, 1 mM EDTA, 10 mM TCEP, and 0.1 M NaCl (pH 7.4); hybridization buffer: 50 mM sodium phosphate buffer containing 0.5 M NaCl (pH 6.8); 4 × Exo I Buffer: 20 mM Tris–HCl, 3 mM $MgCl_2$, 50 mM NaCl (pH 7.4); washing buffer: 10 mM Tris–HCl, 1 mM EDTA; copper nanoparticle formation buffer: 20 mM MOPS, 300 mM NaCl (pH 7.5); buffer for electrochemical impedance spectroscopy (EIS): 0.1 M PBS (pH 6.8) containing 5 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl. All the buffers were prepared with double-distilled water, which was purified with a Milli-Q purification system to a specific resistance of 18 M Ω cm.

2.2. Preparation of capture DNA modified gold electrode

Capture probe was immobilized onto a gold electrode surface via gold-sulfur interactions. The substrate gold electrode was first cleaned with piranha solution (H_2SO_4:H_2O_2=3:1) for 5 min followed by rinsing with double-distilled water. Then, the electrode was polished on silk with 1 μm, 0.3 μm and 0.05 μm alumina slurry in sequence. Afterward, the electrode was thoroughly ultrasonicated in both ethanol and double-distilled water for 5 min, respectively. Finally, the electrode was electrochemically cleaned to remove any remaining impurities in 0.5 M H_2SO_4. After being dried with nitrogen, the electrode was immediately used for capture DNA immobilization through incubation with 0.5 μM capture DNA for 16 h, followed by treatment with 1 mM MCH for 1 h to obtain well-aligned DNA monolayers.

2.3. HCR-assisted formation of copper nanoparticles on the electrode surface

Prior to use, all the hairpin oligonucleotides (H1 and H2) were heated to 95 °C for 2 min and then cooled to room temperature for 1 h. After incubating with desired concentration of FR at the room temperature for 2 h, 100 nM folate-linked probe DNA was incubated with 0.4 U/L Exo I at 37 °C for 0.5 h, which was terminated by being heated to 80 °C for 20 min. Then, the resulting solution was incubated with the capture DNA modified electrode at room temperature for 2 h. After being thoroughly rinsed with double-distilled water, the electrode was immersed in a hybridization buffer containing 150 nM H1 and 150 nM H2 for 2 h. After then, the electrode was dipped in a solution containing 200 μM Cu^{2+} and 100 mM ascorbic acid at the room temperature for 15 min to form copper nanoparticles. Finally, the electrode was thoroughly rinsed with washing buffer to remove the unspecific adsorption of Cu^{2+}. For the control experiments, the folate-linked probe DNA was firstly incubated with the control protein (1 mg/mL OVA, BSA or thrombin) at the room temperature for 2 h instead of FR.

2.4. Electrochemical measurements

CuNPs-coated electrode was firstly dipped into 1 mL 0.5 M HNO_3 for 1 h at the room temperature to dissolve CuNPs on the electrode surface. After diluting by 3 mL PBS, the resulting solution was incubated with 1 mg/mL OPD in 80 °C water bath for 15 min to oxidize OPD. Electrochemical measurements were carried out on a model 660c Electrochemical Analyzer (CH Instruments) with a conventional three-electrode system. The three-electrode system consisted of a gold electrode as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum wire as the counter electrode. All the electrolytes were thoroughly deoxygenated by bubbling high-purity nitrogen through the solutions for at least 10 min before the measurements, and a stream of nitrogen was blown gently across the surface of the solution in order to maintain the solution anaerobic throughout all the experiments.

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