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A label-free and cascaded dual-signaling amplified electrochemical aptasensing platform for sensitive prion assay

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ABSTRACT

Prion proteins, as an important biomarker of prion disease, are responsible for the transmissible spongiform encephalopathies (a group of fatal neurodegenerative diseases). Hence, the sensitive detection of prion protein is very essential for biological studies and medical diagnostics. In this paper, a novel label-free and cascaded dual-signaling amplified electrochemical strategy was developed for sensitive and selective analysis of cellular prion protein (PrP^C). The recognition elements included double-stranded DNA consisted of PrP^C-binding aptamer (DNA1) and its partially complementary DNA (DNA2), and ordered mesoporous carbon probe (OMCP) fabricated by sealing the electroactive ferrocenecarboxylic acid (Fc) into its inner pores and then using single-stranded DNA (DNA3) as the gatekeeper. In the presence of PrP^C, DNA1 could bind the target protein and free DNA2. More importantly, DNA2 could hybridize with DNA3 to form a rigid duplex DNA and thus triggered the exonuclease III (Exo III) cleavage process to realize the DNA2 recycling, accompanied by opening more biogates and releasing more Fc. The released Fc could be further used as a competitive guest of β-cyclodextrin (β-CD) to displace the Rhodamine B (RhB) on the electrode. As a result, an amplified oxidation peak current of Fc (RhB) increased (decreased) with the increase of PrP^C concentration. When "$\Delta I = \Delta I_{Fc} + |\Delta I_{RhB}|$" ($\Delta I_{Fc}$ and ΔI_{RhB} were the change values of the oxidation peak currents of Fc and RhB, respectively.) was used as the response signal for quantitative determination of PrP^C, the detection limit was 7.6 fM (3σ), which was much lower than that of the most reported methods for PrP^C assay. This strategy provided a simple and sensitive approach for the detection of PrP^C and has a great potential for bioanalysis, disease diagnostics, and clinical biomedicine applications.

1. Introduction

Prion diseases are a group of fatal neurodegenerative diseases that include Creutzfeldt–Jakob disease (CJD) in humans, and bovine spongiform encephalopathy (BSE) and scrapie in animals. According to the protein-only hypothesis, conformational change from normal cellular form (PrP^C) into its infectious isoform (PrP^{Sc}) is a crucial step in prion propagation (Macedo et al., 2015). As the concentration of PrP^{Sc} is down to picomolar in the blood (Panigaj et al., 2011), the main problem for diagnosis of prion diseases at pre-symptomatic stage is how to detect the minute quantities of PrP^{Sc} in complex biological systems (Xiao et al., 2013). Till now, various analytical techniques have been reported for the detection of prion protein, such as chromatography and mass spectroscopy-based methods (Gielbert et al., 2013; Sturm et al., 2012) and immunoassay-based methods (Smith and Greenlee, 2014). However, chromatography and mass spectroscopy-based methods need expensive instruments, require skilled personnel for analysis and interpretation, and lack on-site applicability. Immunoassay-based methods are cumbered for the widespread use of rather expensive antibody. Thus, it is of great significance to develop a sensitive and cost-effective strategy for prion assay.

Electrochemical aptamer-based (E-AB) method has received great interests because of the simplicity, fast response, relatively low cost, high sensitivity and low power requirement (Yu et al., 2016; Zhu et al., 2014). Hianik et al. developed a "signal-on" E-AB sensor based on multi-walled carbon nanotubes (MWCNTs) with immobilized aptamers for detection of human recombinant PrP^C (Hianik et al., 2009), which was very simple and avoided labeling processes. Korri–Youssoufi et al. designed "signal-off" E-AB sensors for prion assay based on a new platform for connecting redox markers and aptamers (Miodek et al., 2013a, 2014). Those methods were based on one signal, either signal-on or signal-off. Recently, to improve the sensitivity, a dual-signaling E-AB strategy for prion

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assay has been developed in our group by coupling signal-on and signal-off strategies (Yu et al., 2015). However, it was still based on redox probe-labeled DNA strand which potentially increased the assay cost. Hence, a label-free dual-signaling electrochemical assay of prion protein is highly desired.

On the other hand, in order to amplify the detection signal, various nanomaterials, such as carbon nanotubes (Yuan et al., 2014), graphene (Wei et al., 2014; Zhou et al., 2015), mesoporous silica nanoparticles (Ren et al., 2014; Zhang et al., 2013), magnetic beads (Wang et al., 2015a), gold nanoparticles (Zhou et al., 2013), quantum dots (Yan et al., 2015a), have been applied to develop highly sensitive biosensors. Among these materials, ordered mesoporous carbon (OMC) has aroused great research interest recently due to its high specific surface areas, regular and tunable pore sizes, large pore volumes, as well as stable and interconnected frameworks with active pore surface for easy functionalization (Ghasemi et al., 2015; Nickel et al., 2015). More importantly, due to its unique mesoporous structures, OMC materials are superior in repelling against interfering large-molecular-weight proteins due to the size-exclusive effect, which makes them suitable for bioanalysis (Wang et al., 2015b). However, most of electrochemical biosensors just made use of OMC to modify the electrode for OMC can provide many favorable sites for the electron transfer and exhibit excellent catalytic activity (Yang et al., 2015). The application of OMC in E-AB sensor to construct stimuli-responsive OMC probe (OMCP) for target-responsive cargo release has not been reported.

Herein, we developed a label-free and three-level cascaded dual-signaling amplified E-AB sensor for PrP^C assay. It was based on smart OMC probe, exonuclease III (Exo III)-assisted signal amplification and competitive cyclodextrin–guest interaction (Scheme 1). Although several dual-signaling amplified DNA-based electrochemical sensors have been reported either for single- or multi-targets assay (Bao et al., 2015; Xiong et al., 2015; Liang et al., 2014; Yan et al., 2015b, 2016), to the best of our knowledge, there was no label-free and three-level cascaded dual-signaling amplified E-AB sensor for robust detection of one specific aptamer-binding target. Compared with the reported homogeneous electrochemical immunoassay by combining target-induced proximity hybridization with a mesoporous silica nanoprobe (Ren et al., 2014), this work doesn't need to use complex and expensive DNA-labeled antibodies. Also, unlike nicking endonuclease, which requires a specific recognition site, Exo III does not require any specific recognition sequence and provides a more versatile platform for amplified detection (Xiong et al., 2015). As shown in Scheme 1, due to the flexible binding properties of single strand DNA (Zhang et al., 2013), DNA3 was used as the biogate to seal ferrocenecarboxylic acid (Fc) in the mesopores of OMC materials. For signal amplification, the label-free and three-level cascaded dual-signaling amplification strategy was developed: (I) OMCP as the first-level signal enhancer (After the specific interaction between PrP^C and its aptamer, DNA2 could be released and hybridize with DNA3 to form a DNA2–DNA3 duplex, thus opening the biogate and releasing a few Fc molecules); (II) the Exo III cleavage process resulted in the dissociation of DNA2–DNA3 duplex and the recycle of DNA2, thereby opening more DNA biogates and releasing more Fc molecules; (III) based on competitive host–guest interaction, the released Fc molecule, as a competitive guest of β-cyclodextrin (β-CD), can displace Rhodamine B (RhB) due to its higher hydrophobic property, thus easily realizing the dual-signaling amplification. This method allows us to detect PrP^C down to the femtomolar level, showing high sensitivity and good specificity and could be extended to detect other proteins by changing the specific aptamers.

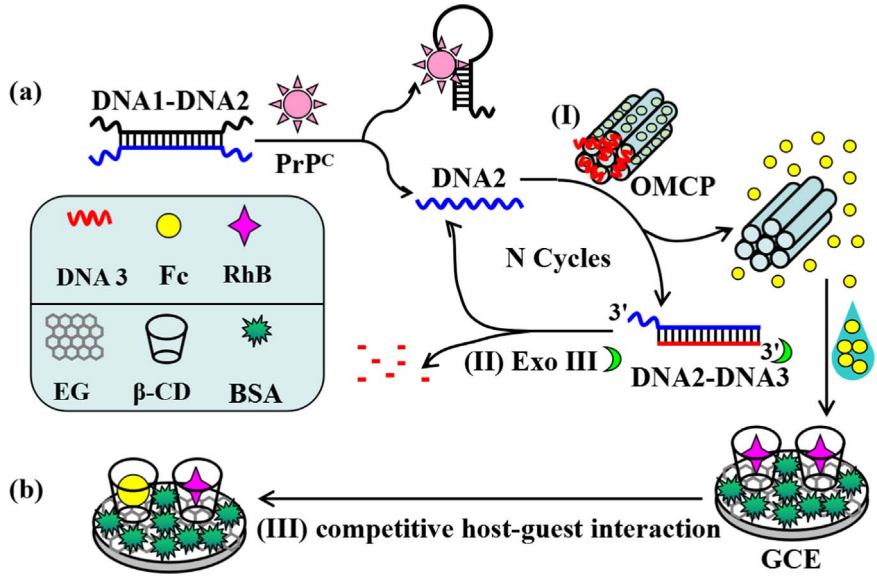
2. Experimental section

2.1. Materials

The native human cellular prion protein, PrP^C (Human PrP^C (23–231), molecular weight 25.16 kDa) was supplied by Jena Bioscience (Jena, Germany). DNA1 and DNA2 were purchased from Takara Biotechnology Co., Ltd. (Dalian, China). DNA3 were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). The sequences of the oligonucleotides were listed as follows:

DNA1 (PrP^C–binding aptamer),
5′–CGGTGGGGCAATTTCTCCTACTGAAAA–3′;
DNA2, 5′–TCTGACAGGAGAAATTGCCCCGAAA–3′;
DNA3 (biogate), 5′–ATTTCTCCTGTCAGA–3′.

In the sequence of DNA1, the underlined portion is the core region of PrP^C-binding aptamer (Zhuang et al., 2013). The aptamer sequence used in this work was selected by Takemura et al., which has a stem-loop like structure (Takemura et al., 2006). In order to get DNA1–DNA2 hybrid, 20 μL of 0.2 μM DNA1 and 20 μL of 0.2 μM DNA2 were initially mixed in 20 mM Tris–HCl buffer (pH



Scheme 1. Schematic illustration of the label-free and cascaded dual-signaling amplified electrochemical strategy for PrP^C detection.