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A new electrochemical immunoassay for prion protein based on hybridization chain reaction with hemin/G-quadruplex DNAzyme

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ABSTRACT

In this work, a new electrochemical immunosensor was developed for prion protein assay based on hybridization chain reaction (HCR) with hemin/G-quadruplex DNAzyme for signal amplification. In this amplification system, the hemin/G-quadruplex DNAzyme simultaneously mimicked the biocatalytic functions for H_2O_2 reduction and L-cysteine oxidation. In the presence of L-cysteine, the hemin/G-quadruplex catalyzed the oxidation of L-cysteine to L-cystine. At the same time, H_2O_2 was produced under the oxygen condition. Then, the hemin/G-quadruplex could quickly catalyze the reduction of H_2O_2, mimicking the catalytic performance of horseradish peroxidase (HRP). Under the optimal conditions, the immunosensor showed a wide linear response range from 0.5 pg/mL to 100 ng/mL with the low detection limit of 0.38 pg/mL (3σ). By changing the specific antibody, this strategy could be easily extended to detect the infectious isoform of prion (PrP^{Sc}) and other proteins. Based on its good analytical performance, the developed method shows great potential applications in diagnosis of prion diseases at presymptomatic stage and bioanalysis.

1. Introduction

Prion protein, an important protein which is mainly located in the neuronal cells of the central nervous system in mammals, is thought to be involved in the pathogenesis of transmissible spongiform encephalopathies, such as Creutzfeldt-Jakob disease in humans, bovine spongiform encephalopathy in cattle, and scrapie in sheep [1–3]. 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 [4]. Therefore, it is very important to detect the concentration of PrP^{Sc} in blood. Up to now, several methods for the detection of prion protein have been reported, such as chromatography and mass spectroscopy-based methods [5,6] and immunoassay-based methods [7]. Among these methods, the electrochemical immunosensing technique attracts much attention due to the good specificity, high sensitivity, fast response and miniaturization. However, the concentration of PrP^{Sc} in blood is very low (down to picomolar), resulting in that the accurate detection of prion is difficult. Therefore, the development of signal amplification methods to improve the sensing sensitivity of the electrochemical immunosensors is highly desirable for early diagnostics of prion diseases.

Nowadays, many signal amplification tags and strategies have been used widely for protein detection [8–12]. Among them, hybridization chain reaction (HCR) method is received great attention due to its unique advantages [13,14]. Generally speaking, HCR is an enzyme-free process where a hybridization reaction is triggered by an initiator DNA and leads to the polymerization of oligonucleotides into a long double strand DNA (ds-DNA) structure [15,16]. The HCR-based amplification strategy has high simplicity and sensitivity, and been used widely in various electrochemical biosensors [17–19]. Recently, DNA concatamers were also reported to amplify the response signal in electrochemical protein detection [20,21]. The DNAzyme has higher catalytic activities and is more stable than other enzymes and denatures or redenatures many times without losing the enzyme-like catalytic activity. As one of DNA concatamers, the hemin/G-quadruplex, formed by interacting hemin into a single-strand guanine-rich nucleic acid sequence, could act as an HRP-mimicking enzyme and serves as electrocatalyst and redox mediator [22].

Herein, a new electrochemical immunosensor was constructed for the detection of prion protein based on sandwich-type antigen-antibody reaction (Scheme 1). Due to the big risk of the pathogenicity of PrP^{Sc}, PrP^{C} was taken as the model to verify the feasibility of the developed immunosensor for PrP^{Sc} assay. The AuNPs-modified glassy carbon (GC) electrode was used to immobilize the primary-antibody of prion (Ab1) and AuNPs could enhance the electron transfer properties of the sensor. In this electrochemical immunosensor, the hemin/G-quadruplex served as synergistic pseudobioenzyme system. Under aerobic condition and in the presence of the hemin/G-quadruplex, the catalyzed oxidation of L-

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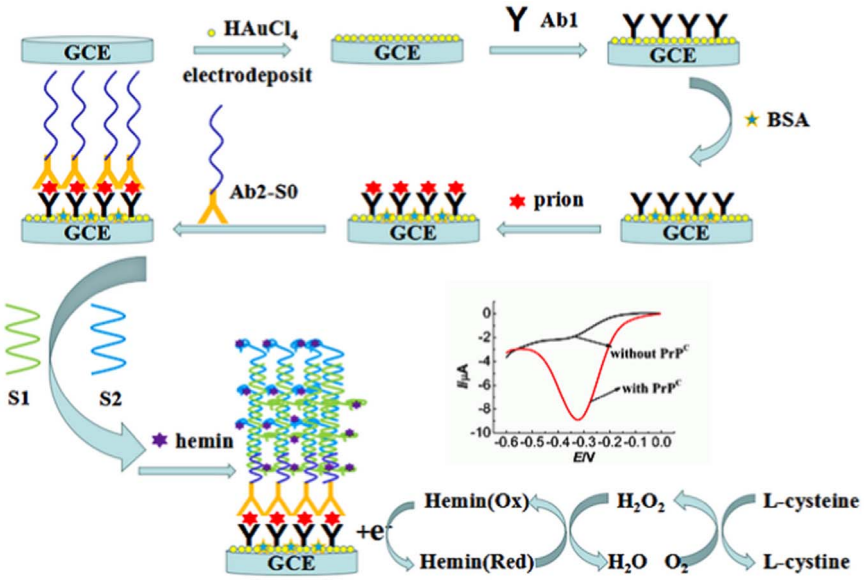

Scheme 1. Schematic illustration of the electrochemical immunosensor based on HCR and hemin/G-quadruplex DNAzyme for signal amplification.

cysteine to L-cystine proceeds with the concomitant generation of H_2O_2 [23]. Then, the hemin/G-quadruplex, a HRP-mimicking DNAzyme, further biocatalyzed the reduction of H_2O_2 to H_2O, at the same time Hemin (Red) was oxidized to Hemin (Ox), which then was reduced to Hemin (Red) electrochemically at the electrode surface [24–31]. By monitoring the reduction current of Hemin (Ox), the concentration of prion protein could be detected sensitively.

2. Experimental procedures

2.1. Reagents and apparatus

PrP^C protein (full length, sequence: 23-231) was bought from Jena Bioscience (Jena, Germany). Biotinylated secondary-antibody of PrP^C protein (biotin-Ab2) was purchased from Santa Cruz Biotechnology, Inc. (USA). Ab1 of PrP^C protein, BSA and chlorauric acid ($HAuCl_4$) (99.9%) were purchased from Sigma Aldrich (USA). Hemin was purchased from Shanghai Ryon Biological Technology Co., ltd. (Shanghai, China). L-cysteine was bought from Bio Basic Inc. (Toronto, Canada).

Other reagents, such as methanol, KCl, NaCl, $Na_2HPO_4{\cdot}12H_2O$, $NaH_2PO_4.2H_2O$ H_2SO_4, potassium ferrocyanide ($K_4[Fe(CN)_6]$), potassium ferrocyanide ($K_3[Fe(CN)_6]$), were of analytical grade, purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China) and used as received. Normal human serum was purchased from Anyan Inc. (Shanghai, China). Streptavidin and oligonucleotide sequence were obtained from Shanghai Sangon Biological Engineering Technology and Service Co., Ltd. (Shanghai, China). The sequences of oligonucleotides were listed as follows:

S0: 5′-biotin-GGTTGGTGTGGTTGG**AGAAGAAGGTGTTTAAGT**A-3′,

S1: 5′-*AGGGCGGGTGGGT*GTTTAAGTTGGAGAATTGT**ACTTAAACACC TTCTTCT**TGGGT-3′

S2: 5′-TGGGTCAATTCTCCAACTTAAACT**AGAAGAAGGTGTTTAAGT** *TGGGTAGGGCGGG*-3′

All electrochemical measurements were performed on a CHI 660B Electrochemical Workstation (Chenhua Instrument Company of Shanghai, China). A conventional three-electrode cell was used with a GC electrode (3.0 mm) as the working electrode, a platinum wire as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. All the potentials in this paper were referred to SCE.

2.2. Preparation of Ab2-S0 bioconjugation

The Ab2-S0 was prepared through biotin-streptavidin conjugation [32,33]. 40 μL biotinylated oligonucleotide (biotin-S0,10 μM) was mixed with 24 μL streptavidin (16.67 μM) and 16 μL Tris-HCl (20 mM) at 37 °C for 40 min. After cooling down to room temperature, the solution was mixed with 80 μL biotin-labeled Ab2 (5 μM) for 40 min. The mixture was not needed for further purification because of the high-affinity interaction between biotin and streptavidin.

2.3. Fabrication of the BSA/Ab1/AuNPs/GC electrode

The GC electrode was firstly polished to a mirror-like surface with 0.5 and 0.05 μm alumina slurries, followed by ultrasonication in methanol and ultra pure water. Then, the electrode was pretreated by potential cycling in 0.5 M H_2SO_4 aqueous solution between −0.2 and 1.0 V at a scan rate of 100 mV/s for 5 circles, washed with copious amount of ultra pure water and dried under room temperature. After that, the pre-treated electrode was immersed in $HAuCl_4$ solution (24.3 mM) and AuNPs were electrodeposited on the surface of GC electrode at potential of −0.2 V for 60 s [34,35]. The obtained electrode was washed with ultrapure water, dried at room temperature and denoted as AuNPs/GC. For the immobilization of Ab1, the AuNPs/GC electrode was coated 5 μL Ab1 (20 μg/mL) and incubated at 37 °C for 2 h [36,37]. Next, the Ab1/AuNPs/GC electrode was incubated with 5 μL BSA solution (2.5 mg/mL) for 40 min to block the nonspecific binding sites. After washed with ultrapure water, the obtained electrode was labeled as BSA/Ab1/AuNPs/GC electrode.

2.4. Electrochemical detection of PrP^C protein

5 μL PrP^C protein with various concentrations and 5 μL Ab2-S0 (1 μM) were dropped onto the BSA/Ab1/AuNPs/GC electrode and the electrode was incubated at 37 °C for a definite time. Based on the immunoreaction between antibody and PrP^C protein, the sandwich structure between Ab1 (Ab2) and PrP^C formed and the electrode was labeled as Ab2-S0/PrP^C/BSA/Ab1/AuNPs/GC electrode. After washed with ultrapure water, 5 μL mixed solution of S1(1 μM) and S2 (1 μM) was added onto the electrode for HCR. Finally, the obtained electrode was incubated with 10 μL hemin (0.5 mM) for a definite time at room temperature and the electrode was labeled as hemin/G-quadruplex/Ab2-S0/PrP^C/BSA/Ab1/AuNPs/GC electrode. The determination of