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Application of hydrogel prepared from ferrocene functionalized amino acid in the design of novel electrochemical immunosensing platform

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ABSTRACT

A simple and novel supramolecular hydrogel was prepared from ferrocene (Fc) modified amino acid phenylalanine (F) and utilized as electrochemical immunosensing platform for the detection of human IgG. Scanning electron microscopy (SEM) characterization indicated that the hydrogel is composed of fibrils with diameter around 50–100 nm and length extend to 1 mm. When the prepared hydrogel was reacted with H_2O_2, the Fc moieties on the amino acid was oxidized, leading to the disruption of the hydrogel structure and the decrease of its redox signal, which was characterized in detail by SEM and the electrochemical method. Regarding the redox current decrease upon the reaction of the hydrogel with H_2O_2, the hydrogel modified electrode was utilized as immunosensing interface. After the construction of the immunosensor based on the traditional sandwich protocol with glucose oxidase (GOx) functionalized carbon nanotube (CNT) as detection antibody label, the GOx attached onto electrode surface would catalyze glucose reaction to produce H_2O_2 and cause the decrease of redox current of the electrode. The current change is proportional to the concentration of IgG detected in the range from 0.1 to 100 pg/mL. The high sensitivity, wide linear range and good reproducibility of the immunosensor indicate this immunosensing platform can be easily extended to the detection of other protein biomarkers.

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1. Introduction

The development of supramolecular hydrogels from various organic molecules has received considerable attention recently due to their interesting properties (Appel et al., 2012; Clemente et al., 2012; Gao et al., 2012). Different molecules, such as amino acid derivatives, cholic acid derivatives, carbohydrate and peptides have been reported that can form hydrogels under suitable conditions (Godeau et al., 2009; Liu et al., 2012; Nanda et al., 2012; Pal et al., 2009). The hydrogels prepared from self-assembled amino acid are of special interest because of its biocompatibility and the facile self-assemble process. In addition, the functional groups on the amino acid molecule surface make the chemical and biological modifications of hydrogels be easily performed. Previous research has indicated that after the self-assemble of nanofibrils or tubes from amino acid, the inter-fibril interaction is critical for the formation of the hydrogel (Smith et al., 2008). The porous structure and large number of water molecules contained in the hydrogels make it idea host for different biomolecules. These interesting properties of the hydrogels make them attractive candidates for wide applications including drug delivery, tissue engineering and biosensing (Dahlmann et al., 2013; Dankers et al., 2012; Jang et al., 2012).

Electrochemical immunosensors that prepared based on the specific interaction between antibody and antigen have found wide applications in clinical diagnosis for the detection of different kinds of protein biomarkers (Chikkaveeraiah et al., 2012; Liang et al., 2012; Moreno-Guzman et al., 2012). Compared to other kinds of immunosensors based on fluorescence (Ramanavicius et al., 2007), surface plasma resonance (SPR) (Ko et al., 2009) and quartz crystal microbalance (QCM) (Park et al., 2003), the electrochemical immunosensor has the advantages of high sensitivity, low cost, fast response and simple instrumentation. Different signal amplification strategies have been investigated to increase the sensitivity of the immunosensor and to decrease the detection limit as the concentrations of many protein biomarkers exist in the human fluidics is of ultralow. For example, the most widely utilized signal amplification method is using functionalized nanomaterials as electrochemical label (Ren et al., 2010; Yang et al., 2010; Zhong et al., 2010). With the enhanced loading of signal tags (enzyme, quantum dot etc.) and detection antibodies onto the nanomaterial surface, the sensitivity of the immunosensor can be significantly increased. Other methods include the construction of novel biosensing interface (Song and Yoon, 2009; Svedhem et al., 2001).

In this work, a novel electrochemical immunosensing platform was proposed based on hydrogel prepared from ferrocene (Fc)

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modified amino acid phenylalanine (F, Fc–F). The hydrogel was prepared with first linking Fc onto F, and then under suitable conditions, the modified Fc–F can be self-assembled into hydrogel that contains a great number of Fc groups and displaying strong redox activity. The hydrogel formation process is rather simple and straightforward. The immunosensor signal transduction strategy was based on the decrease of the redox activity of the hydrogel in the presence of H_2O_2. For the fabrication of immunosensors, human IgG was chosen as a model antigen and GOx modified carbon nanotube was selected as detection anti-human IgG antibody (Ab_2) label (CNT–GOx–Ab_2). With the immobilization of capture anti-human IgG antibody (Ab_1), different concentrations of IgG and then the CNT–GOx–Ab_2 label sequentially onto the hydrogel modified electrode surface, the GOx attached onto the electrode will catalyze glucose reaction to produce H_2O_2 and causing the decrease of the redox signal of the electrode. The decrease of the redox signal was proportional to the concentration of IgG detected. The resulted immunosensor demonstrated high sensitivity, wide linear range and good selectivity. The developed immunosensing platform is simple and versatile, which can be easily extended to the detection of a wide range of analytes.

2. Experimental methods

2.1. Apparatus and reagents

Human IgG and goat anti-human IgG antibody were obtained from Dingguo Biotechnology Co., Ltd. (Beijing, China). Glucose oxidase (GOx, from *Aspergillus niger*, EC 1.1.3.4.150,000 units g^{-1}), poly(diallyldimethylammonium chloride) (PDDA, 20%, w/w in water, MW=200,000–350,000) and chitosan (MW=140,000–220,000) were purchased from Sigma-Aldrich. Multi-walled carbon nanotube (CNT) was obtained from Shenzhen Nanotech Port Co., Ltd. (Shenzhen, China). Dichloromethane (DCM, ACS grade) used for the synthesis was dried and distilled over CaH_2, and stored over molecular sieves. HOBt, HBTU, and H-Phe-OMe · HCl were purchased from GL Biochem (Shanghai, China). Ferrocene monocarboxylic acid (Fc-COOH) was purchased from Xiya Reagent (Chengdu, China). For thin layer chromatography (TLC), glass plates coated with silica gel ($60GF_{254}$) were used. For column chromatography, a column with a width of 2.7 cm (id) and a length of 45 cm was packed 18–22 cm high with 200–300 mesh silica gel (Silicylcye, 230–240 mesh). A 10 mM phosphate buffer solution (PBS) was prepared by mixing NaH_2PO_4 and Na_2HPO_4. All other reagents were of analytical grade and deionized water (MillQ, 18.2 MΩ) was used throughout the study.

All electrochemical measurements were performed on a CHI 650D electrochemical workstation (Shanghai CH Instruments Co., China). A conventional three-electrode system was used for all electrochemical measurements: a glassy carbon electrode (GC, 3 mm in diameter) as the working electrode, a saturated calomel electrode as the reference electrode, and a platinum wire electrode as the counter electrode. UV–vis spectra were recorded on Shimadzu UV-2450 spectrometer (Japan). Scanning electron microscope (SEM) images were obtained from Nova NanoSEM230 (FEI, USA).

2.2. Preparation of the hydrogel

2.2.1. Synthesis of Fc-Phe-OMe

The Fc-COOH (5 mM) and HBTU/HOBt (5.5 mM) were dissolved in DCM (100 mL), Et_3N was added dropwise to activate the carboxyl group for 1 h at 0 °C. H-Phe-OMe HCl (5.5 mL) was then added and the reaction mixture was stirred overnight, followed by washing with saturated aqueous solutions of $NaHCO_3$, HCl (10%), and water, then dried over Na_2SO_4, evaporated to dryness under reduced pressure. The crude product was purified by flash column chromatography (DCM:EtOAc:PE=3:1:5,v/v/v), then evaporated under reduced pressure in a rotovap to a orange oil. The oil was dissolved in DMSO and dried in freeze dryer for overnight, resulting in a orange crystalline.

2.2.2. Synthesis of Fc-Phe-OH

The Fc-Phe-OMe (3 mM) was dissolved in CH_3OH (30 mL) and mixed with NaOH (15 mL, 1 M) and the reaction mixture was stirred for 2 h, thus allowed the Fc-Phe-OMe to be deprotected from methoxycarbonyl group. The mixture was neutralized by HCl (10%) prior to removing the CH_3OH by evaporation under reduced pressure in a rotovap resulting in the orange suspension. The suspension was dissolved in DCM followed by washing with HCl (10%) and water, then dried over Na_2SO_4, evaporated to dryness under reduced pressure. The crude product was purified by flash column chromatography (DCM:EtOAc:MeOH=9:3:1, v/v/v), then evaporated under reduced pressure in a rotovap to a orange oil. The oil was dissolved in DMSO and dried in freeze dryer for overnight, resulting in an orange needle crystalline. The detailed procedure for the synthesis of Fc-Phe-OH was shown in Scheme 1A.

EI-MS (Negative ion mode) calculated for Fc-Phe-OH ($C_{20}H_{19}FeNO_3$):377.07; found 375.89 $[M–H]^-$. ^1H NMR ($CDCl_3$), δ=7.325 (2H, d, J=6 Hz, CH, Phe) ; 7.281 (3H, m, CH, Phe); 6.223 (1H, d, J=6.7Hz, NH, Phe); 4.950 (1H, d, J=7.2 Hz, α-CH, Phe); 4.613 (1H, s, α-CH, Cp); 4.585 (1H, s, α-CH', Cp); 4.310 (2H, s, β-CH, Cp); 4.061 (5H, s, Cp) and 3.250 (1H, d, J=7.0 Hz, β-CH, Phe).

2.2.3. Preparation of the hydrogel

The lyophilized form of Fc-Phe-OH was dissolved in DMSO at a concentration of 100 mg/mL and used as a stock solution. Then the stock solutions were diluted to a final concentration of 4 mg/mL in PBS (10 mM, pH=7.4) followed by sonication for a few seconds. The suspension turned to clear yellowish hydrogel immediately.

2.3. Synthesis of CNT–GOx–Ab_2

The purchased CNT was first treated in mixture of H_2SO_4 and HNO_3 to shorten the CNT and oxidize the surface of CNT to produce carboxylic groups. The obtained CNT was then dispersed into a 0.5% PDDA aqueous solution containing 0.5 M NaCl to reach a concentration of about 1 mg/mL and stirred for 1 h. Then, the PDDA modified CNT was dispersed into 13 nm colloidal AuNPs and stirred for 30 min to adsorb AuNPs onto CNT surface. Finally, the modified CNT was added into solution containing 1 mg/mL of GOx and 0.1 mg/mL Ab_2 solution. The mixture was gently mixed for 3 h and centrifuged. The obtained bioconjugate (CNT–GOx–Ab_2) was stored at 4 °C before use.

2.4. Fabrication of the immunosensor

To fabricate the immunosensor, 3 μL of hydrogel solution was first added onto electrode surface. After dried, 3 μL of chitosan solution (1%, w/w) was dropped onto the electrode surface. Then, after dried naturally, the electrode was immersed into AuNPs solution for 1 h to adsorb the AuNPs onto electrode surface. Then, 3 μL of the Ab_1 (10 μg/mL) solution was added onto the electrode and incubated for 1 h. After rinsing with PBS solution to remove physically immobilized antibodies, the modified electrode was blocked by incubating the electrode with 1% BSA for 0.5 h. Then, human IgG solution of different concentrations was placed onto the electrode surface and incubated for 1 h. After rinsing with PBS again, finally, the prepared CNT–GOx–Ab_2 solution was added onto the electrode and incubated for another 1 h.

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