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A water-dispersible, ferrocene-tagged peptide nanowire for amplified electrochemical immunosensing

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ABSTRACT

A ferrocence (Fc)-tagged peptide comprising the phenylalanine–phenylalanine (Phe–Phe) sequence was synthesized and allowed to self-assemble into uniform nanowires with a diameter of ca. 100 nm and lengths in the range of 5–10 μm. The Fc-tagged peptide nanowire (Fc-PNW) become well dispersed in aqueous solution when coated with poly(diallyldimethylammonium chloride) (PDDA). Gold nanoparticles (AuNPs) and antibody molecules can then be adsorbed onto the Fc-PNW surface. The resultant antibody modified Fc-PNW was explored as a detection probe for sensitive electrochemical immunosensing in a sandwich assay wherein the capture antibody was attached onto a graphene/gold nanoparticle (AuNPs–GN) composite film. The as prepared electrochemical immunosensor possesses a low detection limit (5 fg/mL) for human IgG and a wide linear range encompassing four orders of magnitude (from 10 fg/mL to 100 pg/mL). Such a low detection limit stems from the significant signal amplification by the large number of Fc moieties on the PNW (for a 5-μm-long Fc-PNW, the number of Fc moieties is 5×10^5). The electrochemical immunosensor is also highly selective and the sensor was demonstrated to be amenable to real sample analysis.

1. Introduction

For sensitive detection of trace biomarkers in physiological and clinical samples, methods that resort to signal amplification are commonly employed (Du et al., 2010, 2011; Tang et al., 2012). Perhaps the most widely used signal amplification used for protein detection is the enzyme-linked immunosorbent assay (ELISA) wherein a small number of enzyme (e.g., horseradish peroxidase) molecules attached to the detection antibody help produce chemiluminescent signal (Ochkur et al., 2012; Xiao and Isaacs, 2012). The typical ELISA is of the sandwich type, which utilizes a capture antibody immobilized onto microtiter plates to selectively bind the protein (antigen) target (Ambrosi et al., 2010; Liu et al., 2009). ELISA is sensitive and selective, and can be carried out simultaneously for multiple samples. However, it involves multiple washing and blocking steps that are relatively lengthy and the substrates for the enzymatic amplification reaction are generally carcinogenic. Analytical chemists have made a continuous effort to develop alternative methods that can rival with ELISA for sensitivity and selectivity. For example, attachment of antibody-covered nanoparticles to antigen molecules that had been captured by a quartz crystal microbalance (QCM) leads to a substantially greater mass or signal (Li et al., 2011a; Shen et al., 2011; Su and Li, 2005). Devising a scheme in which an insoluble product is generated from an enzyme–antibody conjugate and subsequently deposited onto the QCM surface also effectively increase the mass loading or signal (Carrigan et al., 2005; Ding et al., 2005). Similarly, nanoparticles or enzymes pre-attached to a detection probe (antibody, ligand, or nucleic acid) have been used in conjunction with surface plasmon resonance (SPR) for ultrasensitive detection of a variety of biomolecules (Mizuta et al., 2010; Tort et al., 2012).

Electrochemical methods are also viable for trace analysis of biological samples, due in large to their high sensitivity, fast response and simple instrumentation (Ahmadi et al., 2011; Chikkaveeraiah et al., 2012). The signal for protein detection based on electrochemistry is mainly from the amperometric current generated from the label on the detection antibody (Moreno-Guzman et al., 2012; Ren et al., 2010; Zhong et al., 2010). Ferrocene (Fc) and its derivatives, because of their high redox activity and stability are one of the most widely utilized electrochemical labels in bioassays (Dai et al., 2007; Mak et al., 2005). However, the signal amplification effect based on single Fc molecule is limited. To solve this issue, different materials, such as gold nanoparticles (AuNPs), magnetic nanoparticles, and dendrimers were selected as hosts to accumulate a large number of Fc moieties for signal amplification (Li et al., 2011b; Teng et al., 2011; Zhuo et al., 2011). Our group has reported Fc-AuNPs as label for the detection of tumor biomarker p53 (Wang et al., 2008). Because each AuNP was decorated with more than 100 Fc molecules, the electrochemical signal was greatly amplified.

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Recently, self-assembled peptide nanostructures have attracted extensive interest due to their good biocompatibility, facile self-assemble process and functional flexibility (Hendler et al., 2007; Reches and Gazit, 2006; Wang et al., 2011). The peptide molecules are highly soluble in organic solvent, but can be rapidly assembled into ordered structures when diluted into aqueous solution (Reches and Gazit, 2003). Especially, the peptide nanostructures based on diphenylalanine (Phe–Phe), the core recognition motif of the amyloid-β (Aβ), have been widely researched due to the connection of Aβ with Alzheimer's disease (AD) (Goerbitz, 2006). Various nanostructures can be formed from Phe–Phe under different assembling conditions, for example, nanotubes, nanowires, and nanospheres (Reches and Gazit, 2004; Ryu and Park, 2009; Yan et al., 2011). The assembled peptide nanostructures can also act as host for the incorporation of other materials (fluorescence dyes, metal nanoparticles, etc.) to form functionalized hybrid nanomaterials (Kim et al., 2011b, 2012; Ryu et al., 2009). These interesting properties of the peptide nanostructures make them widely applied in different areas, such as biosensing, drug delivery and nanofabrication (Kim et al., 2011a; Sedman et al., 2006; Yemini et al., 2005a). For biosensing applications, Gazit and co-workers demonstrated improved electronic conductivity and electrochemical activity of peptide nanotube modified electrode, which offers wide potential for the development of sensors and biosensors with promising analytical performances (Yemini et al., 2005b). However, to the best of our knowledge, no other group has reported the synthesis of Fc tagged peptide nanostructures.

In this work, considering the above mentioned advantages of peptide nanostructures and Fc, Fc tagged peptide nanowire (Fc-PNW) was synthesized by self-assembling the Fc modified Phe–Phe molecules (Fc-Phe–Phe). The Fc-PNW was made water soluble with the wrapping of a polyelectrolyte layer onto its surface and then utilized as label for the fabrication of electrochemical immunosensors to detect human IgG (Liu et al., 2010). For Fc-PNW with a diameter of 100 nm and a length of 5 μm, the number of Fc moieties on Fc-PNW surface was calculated to be 5×10^5, which could significantly enhance the immunosensor signal. To construct the sensor platform for the immobilization of capture antibodies (Ab_1), gold nanoparticles (AuNPs) modified graphene (GN) hybrids (AuNPs–GN) was prepared. The biocompatible microenvironment provided by AuNPs–GN could maintain the activity of the antibodies, while its good conductivity could enhance the electrochemical signal of Fc (Wang et al., 2012). Combing the merits of Fc-PNW and AuNPs–GN, the fabricated immunosensor exhibit high sensitivity, wide linear range and ultralow detection limit toward human IgG detection.

2. Materials and methods

2.1. Apparatus and reagents

Human IgG and goat anti-human IgG antibody were obtained from Dingguo Biotechnology Co., Ltd. (Beijing, China). 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 (St. Louis, MO). Dichloromethane (DCM; ACS grade) was stored with molecular sieves, dried over CaH_2 and distilled before the synthesis. The common reagents used for peptide synthesis include 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), hydroxybenzotriazole (HOBt), Boc-Phe-COOH and H-Phe-OMe · HCl, which are obtained from GL Biochem. (Shanghai, China). Ferrocene monocarboxylic acid (Fc-COOH) was synthesized as described in the literature (Barisic et al., 2002). For thin layer chromatography (TLC), we used glass plates coated with silica gel (60 GF_{254}), while for column chromatography, we packed 18–22 cm of 200–300 mesh silica gel (Silicylcye, 230–240 mesh) into a 2.7-cm-wide and 45-cm-long glass tube. Phosphate buffered solution (PBS, 0.1 M) was prepared by mixing 0.1 M NaH_2PO_4 and Na_2HPO_4. All other reagents were of analytical grade and prepared with deionized water (MillQ, 18.2 MΩ).

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

2.2. Synthesis of Fc-Phe–Phe-OMe

Boc-Phe-COOH (4 mM) and HBTU/HOBt (4.4 mM) were dissolved in DCM (50 mL), and Et_3N was then added dropwise to activate the carboxyl group for 1 h at 0 °C. After that, H-Phe-OMe · HCl (4.4 mL) was added and the reaction mixture was stirred overnight. Upon washing with saturated aqueous solutions of $NaHCO_3$, HCl (10%), and water, the crude product was dried over Na_2SO_4 under reduced pressure. The crude product was purified by flash column chromatography (DCM: EtOAc=2:1, v/v), then evaporated under reduced pressure in a rotovap to a white oil. The oil was then dissolved in DMSO and dried in freeze dryer for overnight, resulting in a white crystalline. The resulting crystalline was dissolved in the mixture of DCM (20 mL) and TFA(10 mL) and stirred for 30 min, then CH_2Cl_2 and TFA were subsequently removed. The resulting H-Phe–Phe-OMe was treated with Et_3N in CH_2Cl_2 (2 mL, pH~8). The solution was diluted in DCM (20 mL) and mixed with Fc-OBt (2.2 mM) that was obtained by using the standard HBTU/HOBt method in solution of activating Fc-OH. The reaction mixture was then stirred for 1 h, followed by washing with saturated aqueous solutions of $NaHCO_3$, HCl (10%), and water, then dried. The crude product was purified by flash column chromatography (DCM:EtOAc=3:1, v/v), then evaporated under reduced pressure in a rotovap to a yellow oil. The oil was dissolved in DMSO and dried in freeze dryer for overnight, resulting in a yellow needle crystalline, Fc-Phe–Phe-OMe. The detailed procedure for the synthesis of Fc-Phe–Phe-OMe is shown in Fig. 1A.

2.3. Formation of water-dispersible Fc-PNW coated with antibody (Fc-PNW-Ab_2)

Fc-Phe–Phe-OMe was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol to produce a 100 mg/mL stock solution. Aliquots of the stock solution were diluted with methanol to a concentration of 2 mg/mL. Fc-PNW was produced upon evaporation of the solvent at room temperature. The above synthesized Fc-PNW was dispersed into aqueous PDDA solution (1%, w/w) to reach a concentration of 1 mg/mL and stirred gently for 1 h. Residual PDDA polymer was removed by centrifuge, and the modified nanowires were washed twice with PBS. Then, the PDDA-coated Fc-PNW was dispersed into colloidal AuNPs (diameter=13 nm) solution and stirred for another 1 h. The detailed procedure for the synthesis of AuNPs was described in Supporting Information. After centrifuging, the Fc-PNW decorated with AuNPs was mixed with the detection antibody (Ab_2, 10 μg/mL) for 3 h. The free antibody was separated, and the obtained bioconjugate Fc-PNW-Ab_2 was washed with buffer and stored at 4 °C before use. Fig. 1B displays the procedure for the preparation of Fc-PNW-Ab_2.