Biosensors and Bioelectronics 92 (2017) 1–7

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Label-free immunosensor based on hyperbranched polyester for specific detection of α-fetoprotein

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ARTICLE INFO

Keywords:
Hyperbranched polyester
Electrochemical immunosensor
Ultra-stability
Label-free
α-Fetoprotein

ABSTRACT

A novel label-free immunosensor based on hyperbranched polyester nanoparticles with nitrite groups (HBPE-NO_2), which were synthesized through a simple one-step chemical reaction, was first developed for specific detection of α-fetoprotein (AFP), the tumor marker for liver cancer. The obtained HBPE-NO_2 nanoparticles (NPs) were characterized by the proton nuclear magnetic resonance spectroscopy (^{1}H NMR), X-ray photoelectron spectroscopy (XPS) and X-ray diffraction (XRD). And the fabricated process of immunosensor was investigated by attenuated total reflection Fourier-transform infrared spectra (ATR-FTIR), static water contact angles, scanning electron microscope (SEM), cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The electrochemical performances of the AFP immunosensor were studied. Results indicated the prepared HBPE-NO_2-modified immunosensor showed excellent electrochemical properties and satisfactory accuracy for the detection of AFP of the real clinical samples that attributed to the properties of the HBPE-NO_2 NPs, which had nanosized structure to increase the specific surface area and unique chemical reactivity for loading capacity of protein molecules. Construction of biosensors using the structure and properties of hyperbranched molecules will offer ideal electrode substrates, which provided more possibilities for the design of biosensor.

1. Introduction

Alpha-fetoprotein (AFP), an important tumor marker, is widely used for the diagnosis of patients with germ cell tumors and hepatocellular carcinoma (Alpert et al., 1971; Okuda, 1986; Wang and Xie, 1998), and its concentration is below 20 ng/mL in healthy human serum while much higher in liver cancer patient serum (Xie et al., 2016; Zhou et al., 2015). Therefore, the reliable detection of AFP is significant for the early clinical diagnosis and the long-term treatment (Li et al., 2015). In recent years, numerous novel technologies were developed and applied for AFP detection (Chen et al., 2012; Darwish et al., 2013; Law et al., 2011; Xiao et al., 2014). Electrochemical bioanalytical technique, with a number of virtues such as simple preparation and operation, high sensitivity and selectivity, wide detection range and so forth, has become increasingly important in biological assays (Cui et al., 2016). Particularly, electrochemical label-free immunosensors have been considered to be one of the most promising methods to quantitatively detect AFP owing to their specific advantages, such as low price, low detection limits, fast response, and easy handling (Kavosi et al., 2014).

For the construction of an excellent label-free electrochemical immunosensor, it is crucial that the immobilization of biomolecules onto the electrode surface should be which could ensure the activity of antibody without affected (G.L Wang et al., 2009; J. Wang et al., 2009). Therefore, the electrode substrate should provide a large surface area to immobilize more antibodies and also offer good conductivity in order to assist the electron transfer, as well as stability and without biological toxicity. So how can design and prepare the ideal electrode substrate that can immobilize antibodies sufficiently and effectively for electrochemical immunosensor became the key to this research work.

Hyperbranched polymers (HBP) have attracted more and more attention because of the special architecture and properties that include their three-dimensional shapes, high density of end-groups, nanosized effect, good solubility, etc (Ahmed and Narain, 2012; Magnusson et al., 2000; Karpagam and Guhanathan, 2010; Ding et al., 2012). Further, owing to the multifunctionality in HBP, their properties can be adjusted to a great degree by various chemical modification of the large number of functional end-groups for special

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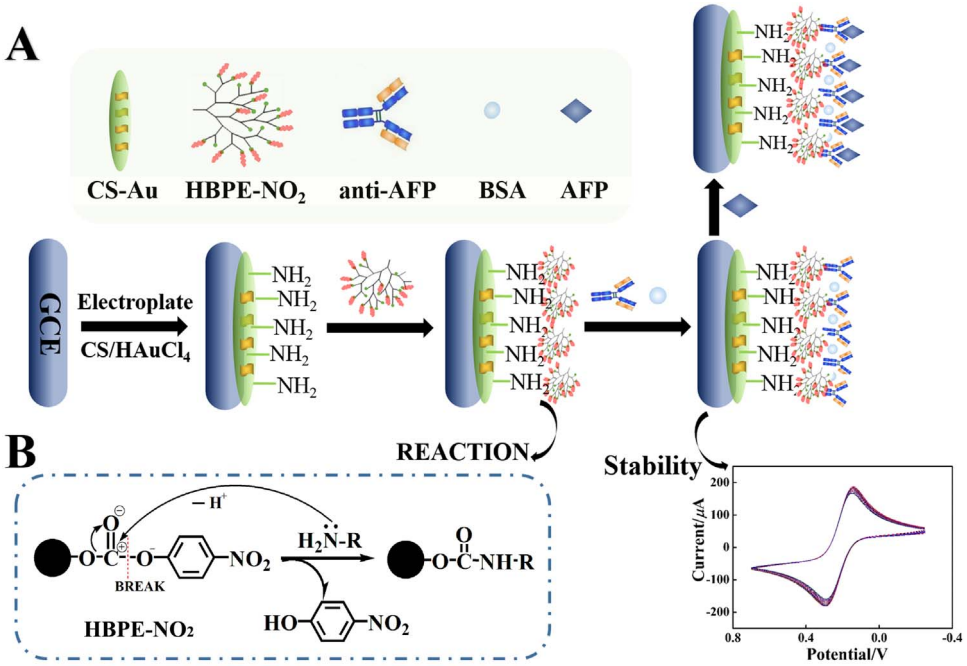
http://dx.doi.org/10.1016/j.bios.2017.01.069
Received 1 December 2016; Received in revised form 23 January 2017; Accepted 31 January 2017
Available online 01 February 2017
0956-5663/



Scheme 1. (A) Schematic illustration of the fabrication process of the AFP biosensor; (B) The nucleophilic substitution reaction mechanism between HBPE-NO_2 NPs and primary amines (-NH_2).

purposes, which made the HBP have been used extensively in diverse fields, such as coatings, additives, biomedical fields, and photoelectric materials (Gao and Yan, 2004; Seiler et al., 2004; Hong et al., 2008; Zagar et al., 2006; Kontoyianni et al., 2008; Sun et al., 2014).

In this case, *p*-nitrophenyl oxycarbonyl group-terminated hyperbranched polyester (HBPE-NO_2), obtained from hyperbranched polyester with terminal hydroxyl groups (HBPE-OH) that modified by *p*-nitrophenyl chloroformate containing active esters group (-O-COO-), which can react with primary amine (-NH_2) of chitosan (CS) and anti-AFP in situ, respectively. Based on this, a novel electrochemical immunoassay biosensor was proposed and its biosensing performance was investigated.

2. Experimental section

2.1. Synthesis of HBPE-NO_2 nanoparticles (NPs)

As shown in Scheme S1, HBPE-NO_2 was synthesized through one-step chemical reaction. 1.0 g HBPE-OH (aliphatic hyperbranched polyester with terminal -OH group) (Scheme S1A) was dissolved in anhydrous tetrahydrofuran (THF, 100 mL), then 10 mL anhydrous THF containing *p*-nitrophenyl chloroformate (2.0 g, 9.9 mmol) was added dropwise with the presence of an excess of triethylamine (TEA, 1.5 mL). And the mixture was allowed to continue to react about 12 h with stirring at room temperature (Scheme S1B). After the reaction, a yellowish opacity solution was formed finally (Hu and Ji, 2011), and then the resulting solution was filtered and concentrated to about 10 mL through rotary evaporation. The final product was obtained by precipitation from diethyl ether, then redissolved in THF and reprecipitated from diethyl ether again, the reprecipitation procedure was repeated three times to remove the residual *p*-nitrophenyl chloroformate and dried in a vacuum.

2.2. Fabrication of the HBPE-NO_2 NPs modified biosensor

Glassy carbon electrode (GCE) was used as base electrode. Prior to modification, it was polished to a mirror finish using 0.3 and 0.05 μm alumina slurry, respectively, and rinsed thoroughly with distilled water between each polishing step (Sun et al., 2013). After that, the mirror-like electrode was sonicated in pure ethanol and distilled water about 5 min, respectively, and allowed to air-dry at room temperature.

The preparation procedure of AFP biosensor was shown in Scheme 1. Firstly, the GCE was electrodeposited with CS and hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$, 99.9%) mixture containing 2 mL of 1 g/L CS (1% acetic acid as solvent) and 2 mL of 500 mg/L $HAuCl_4$ solution by applying a constant potential of −1.5 V for 180 s, then the modified GCE were rinsed with distilled water to obtain the CS-Au NPs modified electrode (CS-Au/GCE) (Sun et al., 2014). The CS-Au/GCE was soaked in 8 mL of 0.1 mg/mL HBPE-NO_2 solution (dispersed in distilled water and sonicated well) for 15 min and then rinsed with distilled water to remove the unreacted HBPE-NO_2 (Scheme 1A). Thus, the HBPE-NO_2 NPs were immobilized by electrostatic absorption and in situ chemical reaction (Scheme 1B), and the obtained (HBPE-NO_2)/CS-Au/GCE was stored at 4 °C before use. *p*-Nitrophenol, the byproduct of the chemical reaction could be eliminated simply by immersion in distilled water mentioned above, which was a necessary step to ensure the activity of anti-AFP that will be grafted next step was not affected by the byproduct.

Next, AFP/anti-AFP/(HBPE-NO_2)/CS-Au/GCE biosensor was obtained easily by a three-step modified process. Firstly, the prepared (HBPE-NO_2)/CS-Au/GCE was immersed into 10 mL solution of anti-AFP (200 ng/mL, PBS, pH 7.4) for 15 min, then the modified GCE was drawn out and rinsed thoroughly with PBS subsequently, and air-dry at room temperature.

And then, the anti-AFP/(HBPE-NO_2)/CS-Au/GCE was blocked the possible remaining active sites against the nonspecific adsorption by 2% bovine serum albumin (BSA) for 1 h. Finally, the modified electrodes were incubated in 10 mL AFP standard solutions of various concentrations (0.1 mol/L PBS, pH 7.4) for 15 min, followed by washing with PBS. Thus, the resulting AFP/anti-AFP/(HBPE-NO_2)/CS-Au/GCE was recorded to produce the detection signal corresponding to the presence of an analyte, and the obtained AFP/anti-AFP/(HBPE-NO_2)/CS-Au/GCE was stored at 4 °C before use.

2.3. Electrochemical measurements

Cyclic voltammetry (CV) was performed over the potentials between −0.2 V~0.7 V. The differential pulse voltammetry (DPV) was