Biosensors and Bioelectronics 101 (2018) 174–180

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

An aptasensor based on heparin-mimicking hyperbranched polyester with anti-biofouling interface for sensitive thrombin detection

Yanlian Niu, Meilin Chu, Ping Xu, Shuangshuang Meng, Qian Zhou, Wenbo Zhao*, Bo Zhao, Jian Shen*

National and Local Joint Engineering Research Center of Biomedical Functional Materials, School of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210023, China

ARTICLE INFO

Keywords:
Heparin-mimicking
Hyperbranched polyester
Whole blood
Aptasensor
Thrombin

ABSTRACT

In this paper, novel heparin-mimicking hyperbranched polyester nanoparticles (HBPE-SO_3 NPs) with abundant of sulfonated acid functional groups were synthesized, and their antithrombogenicities were further evaluated. Further, a label-free electrochemical aptamer biosensor (aptasensor) based on HBPE-SO_3 NPs modified electrode was developed for thrombin (TB) detection in whole blood. Meanwhile, the anti-biofouling properties of different modified electrodes were studied by whole blood and platelet adhesion test, hemolysis assay and morphological changes of red blood cells *in vitro*. Besides, the thrombin-binding aptamer was selected as receptor for the proposed aptasensor, which has excellent binding affinity and selectivity for TB. When binding to TB, the electron transfer taking place at the modified electrode interface was inhibited that can attribute to the stereohindrance effect, resulting in the decreased current response. This aptasensor showed excellent electrochemical properties with a wide detection range and a low detection limit of 0.031 pM (S/N = 3), and provided high selectivity, long-term stability and good reproducibility. Finally, the sensitively detection of TB in whole blood samples directly was achieved by this aptasensor we proposed, which suggested its great potential for TB detection in the clinic.

1. Introduction

Hyperbranched polymers (HBPs) have three-dimensional topological structures and series of particular properties including high density of functional groups, intramolecular cavities, nano-sized effect and so on, which can be attributed to the highly branched globular and dendritic molecular architectures (Ding et al., 2012). In consequence, HBPs exhibit wide applications in a variety of areas, for instance, nanotechnology, biomedical, additives and composite materials (Wu et al., 2015). Our group reported novel new hyperbranched zwitterionic structures-modified bare metal stents with good biocompatibility, which had enormous potential in inhibit thrombosis, minimize restenosis of coronary artery diseases and other biological applications (Wang et al., 2013a, 2013b, 2014). Furthermore, the great development in synthetic strategies and easy to modify give rise to diverse HBPs with desirable functional properties (Zheng et al., 2015; Yu et al., 2010). Brooks had synthesized and characterized series of hyperbranched polyglycerols that functionalized with different ligands, and obtained the functional polymer with promoting hemostasis properties *via* precisely controlled the proportions of different ligands (Wen et al., 2016).

Based on the crucial roles of thrombin (TB) in coagulation cascade, hemostasis and thrombosis, of which the detection and quantification of TB in biological serum or other complex samples are great of significance for clinical diagnosis applications (Shen et al., 2017; Xu et al., 2015). Electrochemical bioanalytical method, with lots of merits such as simple preparation and operation, high sensitivity, great selectivity and anti-interference performance, wide detection range and so forth (Cui et al., 2016), was widely applied in concentration detection of TB. Up to now, however, the most of reported electrochemical methods of TB concentration detection were mainly performed in serum samples (Shen et al., 2017; Xu et al., 2015; Xiao et al., 2005; Sun et al., 2014; Gao et al., 2015). Generally, the serum samples were obtained by factitious operations, especially additional centrifugal treatment, accordingly, both factitious and long treating time will introduce inevitable errors for the TB detection. Thus, it's great significance of designing and preparing an electrochemical biosensor that can be used in whole blood directly.

When the conventional electrodes as foreign materials contact with blood, the coagulation cascade reaction will occur correspondingly. This will lead to the biological pollution of blood components that

* Corresponding authors.
E-mail addresses: zhaowenbo@njnu.edu.cn (W. Zhao), jshen@njnu.edu.cn (J. Shen).

http://dx.doi.org/10.1016/j.bios.2017.10.037
Received 20 August 2017; Received in revised form 14 October 2017; Accepted 16 October 2017
Available online 18 October 2017

coating on the electrode surface (Huang et al., 2016). Therefore, the anticoagulant or anti-biofouling property of the electrode substrates is the key problem to solve for biosensor that used in the whole blood directly (Huang et al., 2014).

Heparin has always been regarded as an injectable anticoagulant in clinical (He et al., 2017; Paluck et al., 2016). Some research groups have demonstrated that the excellent anticoagulant property of heparin can be attributed to the abundant of functional groups (OSO_3^-, $NHSO_3^-$ and COO- groups) in the molecular architecture (Wang et al., 2015; Tamada et al., 2002), which gave heparin a total negative net charge (Capila and Linhardt, 2002). Herein, based on the abundant terminal groups and the nano-sized effects of HBPs, a novel of heparin-mimicking hyperbranched polyester nanoparticles (HBPE-SO_3 NPs) with controlled structure, sulfation and purity were synthesized, and their blood compatibilities were evaluated *in vitro*. Additionally, a label-free aptamer biosensor (aptasensor) based on HBPE-SO_3 NPs modified electrode was designed and developed for sensitive detection of TB *via* the layer-by-layer self-assembly methods. And the fabricated process of aptasensor, the electrochemical performance and the real samples measurements in whole blood were presented in detail.

2. Experimental section

2.1. Synthesis of HBPE-SO_3 NPs

Second-generation aliphatic hyperbranched polyester (HBPE-OH) was prepared using quasi-one-step method with abundant of terminal hydroxyl groups and then modified by sulfonic acid groups to get HBPE-SO_3 NPs, which were presented in a previous paper by our group (Han et al., 2013). Briefly, in the presence of *p*-toluenesulfonic acid as catalyst, the esterification reaction between trimethylol propane and 2,2-bis (hydroxymethyl)propionic acid was carried out at 140 °C to form HBPE-OH. And the final product was obtained by precipitating crude polymer from acetone in n-hexane. Second, to a 150 mL three-necked flask with a magnetic stir bar, the mixtures of HBPE-OH and excess NaH in anhydrous tetrahydrofuran were added to react under reflux overnight before adding 1,3-propane sultone, and then allowed to react for another 12 h under reflux. The resulting solution was filtered, then, the crude product was exhaustively dialyzed by pure water, and the purified product of HBPE-SO_3 was obtained. In addition, the antithrombogenicity of HBPE-SO_3 NPs was further evaluated by coagulative time tests, including activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin time (TT). The detailed experimental procedures were described in Supporting information.

2.2. Characterization of TBA/HBPE-SO_3

When binding to TB, thrombin-binding aptamer (TBA), a 15-mer G-rich DNA oligonucleotide (5′-GGT TGG TGT GGT TGG-3′) (Xi et al., 2015), could change its secondary structure from random coil to chair-like antiparallel G-quadruplex (Heydari-Bafrooei et al., 2016; Aaldering et al., 2017). Thus, the ultraviolet visible (UV–vis) spectrophotometer was employed to measure the configuration transition of TBA when incubating with HBPE-SO_3 NPs for further investigating the biocompatibility of HBPE-SO_3.

2.3. Construction of TBA/HBPE-SO_3/Au/MPTMS/GCE and measurements

Prior to modification, the glassy carbon electrode (GCE) was typically polished by alumina slurry in succession, and then sonicated in pure ethanol and distilled water, finally allowed to air-dry at room temperature for further to use (Arab Chamjangali et al., 2015). The construction process of proposed aptasensor was presented in Scheme 1 with layer-by-layer self-assembly method. Firstly, 8.0 μL of 0.1% 3-mercaptopropyltrimethoxysilane (MPTMS) solution (anhydrous alcohol as solvent) was successfully grafted onto the pretreated electrochemical electrode surface for increasing the amount of hydroxyl groups on the electrode surface (Hao et al., 2007), and dried in air. The obtained MPTMS/GCE was modified by 8.0 μL of positively charged Au NPs (see Supporting information) aqueous with the terminal thiol groups of MPTMS molecule, subsequently. Then, 8.0 μL of HBPE-SO_3 NPs (0.1 mg/mL) was immobilized by electrostatic absorption, and the obtained HBPE-SO_3/Au/MPTMS/GCE was stored at 4 °C before use.

Finally, the HBPE-SO_3 modified GCE was immersed into 1.0 mL of a mixing solution containing 1.0 μM TBA (5′-amino-modified), 0.1 M NaCl and 4 mM EDTA for 24 h, which was adjusted pH to 7.4 with 20 mM Na_2HPO_4, followed by rinsing with phosphate buffered saline (PBS, 0.1 M, pH 7.4) to remove unreacted TBA. Here, TBA was grafted onto the surface of modified GCE *via* weak electrostatic interactions. The TBA/HBPE-SO_3/Au/MPTMS/GCE was then incubated with 2% bovine serum albumin (BSA) to block possible active sites against nonspecific adsorption. The other different modified electrodes that used as contrast groups were prepared by the same modified method. Since the target molecule of TB was appeared, a complex of G-quadruplex-TB was formed and such a complex increased the steric hindrance that greatly restrained the electrons transfer occurring on the electrode surface. When not in use, the electrodes were stored at 4 °C.

2.4. Hemocompatibility tests of HBPE-SO_3 NPs modified electrodes

2.4.1. Whole blood and platelet adhesion test

In detail, for evaluation of the anti-biofouling effect of HBPE-SO_3 NPs modified the electrode surface, the whole blood and platelet adhesion tests were performed. However, the GCE was very difficult to be tailored into pieces for test. So, here, the indium tin oxide (ITO) electrodes with the same modified method instead of GCE were placed in the 24-well microplates and immersed in PBS for 24 h. After removing the PBS, the fresh rabbit whole blood with sodium citrate were dropped on each well with an incubation time of 1 h at 37 °C, followed by removing the blood with an aspirator and rinsing three times with 1 mL PBS. Then, 1 mL of 2.5 vol% glutaraldehyde in PBS was used to immobilize the bounded blood cells for 30 min at room temperature. The electrodes were washed with PBS again and then subsequently dehydrated by a series of ethanol-water mixing solutions (50%, 60%, 70%, 80%, 90%, 95% and 100% (v/v)) for 30 min each and allowed to air-dry. The electrodes were coated with gold, and then the treated ITO surface was observed by scanning electron microscopy (SEM). Instead of whole blood, platelet rich plasma, which was prepared by centrifuging anticoagulated whole blood at 800 rpm for 15 min, was applied to clarify the adhesion of platelet using the same procedures as described above (Wang et al., 2013a, 2013b).

And other hemocompatible experimental procedures including hemolysis assay and morphological changes of red blood cells (RBCs) were also presented in Supporting information.

3. Result and discussion

3.1. Characterization of the HBPE-SO_3 NPs and Au NPs

Learned from the previous literature, the HBPE-SO_3 NPs were incomplete sulfonated-terminal modified with retaining part of hydroxyl groups, and well-distributed in aqueous with nanoscale fake-spherical morphology (Han et al., 2013). Furthermore, the organic compound, 4-dimethylaminopyridine was adopted to realize the phase transfer of Au NPs from organic to aqueous solutions. And the Au NPs with positively charged was obtained ($+14.5 \pm 2.3$ mV, Fig. S2). As shown in Fig. S3, the Au NPs had an ultraviolet absorption peak at 528 nm, and their hydration radius were 60.3 ± 0.5 nm by dynamic light scattering (DLS), which was consistent with the results observed by transmission electron microscopy (TEM).