Analytica Chimica Acta 850 (2014) 33–40

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

A label-free and high sensitive aptamer biosensor based on hyperbranched polyester microspheres for thrombin detection

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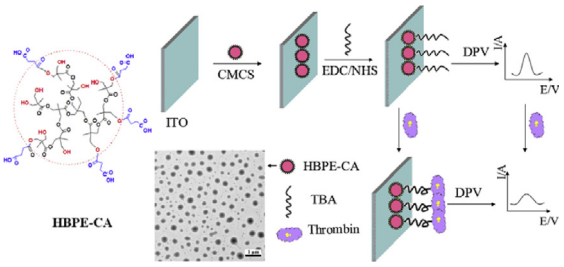
HIGHLIGHTS

- A label-free thrombin aptamer biosensor applied in whole blood has been developed.
- The aptamer biosensor showed a wide detection range and a low detection limit.
- The antibiofouling idea utilized for biosensor is significant for diagnostics.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 July 2013
Received in revised form 6 August 2014
Accepted 7 August 2014
Available online 11 August 2014

Keywords:
Hyperbranched polyester microspheres
Whole blood
Antibiofouling
Aptamer biosensor
Thrombin

ABSTRACT

In this paper, we have synthesized hyperbranched polyester microspheres with carboxylic acid functional groups (HBPE-CA) and developed a label-free electrochemical aptamer biosensor using thrombin-binding aptamer (TBA) as receptor for the measurement of thrombin in whole blood. The indium tin oxide (ITO) electrode surface modified with HBPE-CA microspheres was grafted with TBA, which has excellent binding affinity and selectivity for thrombin. Binding of the thrombin at the modified ITO electrode surface greatly restrained access of electrons for a redox probe of $[Fe(CN)_6]^{3-/4-}$. Moreover, the aptamer biosensor could be used for detection of thrombin in whole blood, a wide detection range (10 fM–100 nM) and a detection limit on the order of 0.90 fM were demonstrated. Control experiments were also carried out by using bull serum albumin (BSA) and lysozyme in the absence of thrombin. The good stability and repeatability of this aptamer biosensor were also proved. We expect that this demonstration will lead to the development of highly sensitive label-free sensors based on aptamer with lower cost than current technology. The integration of the technologies, which include anticoagulant, sensor and nanoscience, will bring significant input to high-performance biosensors relevant to diagnostics and therapy of interest for human health.

1. Introduction

Thrombin, a kind of serine protease, plays an essential role in some physiological and pathological processes, such as blood solidification, wound cicatrisation and inflammation [1]. The concentration of thrombin in blood varies considerably and can be virtually absent in healthy subjects. However, in the coagulation

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http://dx.doi.org/10.1016/j.aca.2014.08.010
0003-2670/

process, the concentration of thrombin in blood ranges from nM to low mM levels [2,3]. Therefore, the specific recognition and quantitative detection of thrombin is extremely crucial to fundamental research as well as to clinical practice. Electrochemical [4,5] and optical techniques [6,7] were widely applied for thrombin detection. Aptamers are artificial oligonucleic acids to bind specific target molecules, which are in vitro selected by SELEX (systematic evolution of ligands by exponential enrichment) technology [8,9]. Theoretically, it is possible to obtain all kinds of aptamers to recognize virtually different target molecules with high affinity and specificity. Aptamers used for specific protein binding studies have drawn much interest recently [10–15], especially for thrombin [4,5,16,17]. However, as we know, the detection methods of thrombin concentration are mainly performed in serum which is isolated from whole blood [18,19]. When the electrode surface is contact with blood directly, the foreign materials are prone to initiate the formation of clots, as platelets and other components of the blood coagulation system are activated. At present, it is very difficult to design and prepare an electrochemical biosensor that can be used in whole blood just because the biofouling of electrode surface can be developed by platelet, fibrin and blood cell adhesion in the complex environment of whole blood media. The focus of this paper is the development and investigation of antibiofouling properties of new nanostructured architecture for electrochemical aptamer biosensors that can be applied in whole blood directly.

Hyperbranched polymers have attracted significant attention for their unique architecture and novel properties including good solubility, special viscosity behavior, and high density of their functional groups [20,21]. Owing to the multifunctionality in hyperbranched polymers, the physical properties can be adjusted to a large extent by the chemical modification of the terminal-groups [22,23]. The use of hyperbranched polymers by the chemical modification has attracted increasing attention in recent years [24–27]. At present, the original material of hyperbranched polyester (HBPE) can only be dissolved in organic solvent (e.g., dimethyl sulphoxide (DMSO), tetrahydrofuran (THF)) and not in water which is not suitable for meeting bioapplications. In this paper, we synthesized water-soluble microspheres by the chemical modification of aliphatic HBPE with carboxylic acid functional groups (HBPE-CA). The preparation and blood compatibility of the HBPE-CA microspheres were investigated. Moreover, a label-free and sensitive aptamer biosensor based on the HBPE-CA microspheres was prepared due to the coherence between blood compatibility and antifouling property which in our knowledge has never been reported. Meanwhile, the aptamer biosensor showed a wide detection range and a low detection limit. More details were presented.

2. Experiments

2.1. Materials

Thrombin-binding aptamer (TBA) was purchased from Sangon Biotechnology Co., Ltd. (China) with HPLC purification. The 5′-terminus of TBA contained 15 bases with its sequence as follows: 5′-NH_2-GGT TGG TGT GGT TGG-3′. Thrombin (human α-Thrombin), *N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide (EDC) and *N*-hydroxysuccinimidobiotin (NHS) were obtained from Sigma–Aldrich (USA). Carboxymethyl chitosan (CMCS), bull serum albumin (BSA) and lysozyme were purchased from Aladdin Chemistry Co., Ltd. (China). Butanedioic anhydride was purchased from Energy Chemical Co., Ltd. (China). Triethylamine, THF and crystal violet were purchased from Sinopharm Chemical reagent Co., Ltd. (China). Meanwhile, triethylamine and THF were refluxed with CaH_2 and sodium respectively, then distilled prior to use. Other reagents were used without further purification. All solutions were prepared with double-distilled water and high purity N_2 was applied for deaeration. Phosphate buffer solution (PBS) was prepared with 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 solutions. The ITO electrodes were purchased from Zhongjingkeyi Technology Co., Ltd. (China).

2.2. Instrumentation

Proton nuclear magnetic resonance spectroscopy (^{1}H NMR) experiment was performed on a Bruker Avance 400 spectrometers (Bruker, Germany) with D_2O as solvent at ambient temperature. The chemical shifts were referenced to tetramethylsilane (TMS) standard. The electro-spray ionization mass spectrometry (ESI-MS) was obtained from mass spectrometer (LCQ/M/Z = 150–2000, Finnigan, USA). Transmission electron microscopy (TEM) image was obtained using an interface high-resolution transmission electron microscopy (HITACHI H-7650, Japan).

2.3. Procedures

2.3.1. Synthesis of HBPE-CA microspheres

The HBPE-CA microspheres were prepared by a procedure described in the previous literatures [28]. In brief, the hyperbranched polymer HBPE (1.02 g with 0.87 mM —OH groups) was dissolved in 60 mL THF. Then 2.07 g butanedioic anhydride and 1.0 mL triethylamine were dissolved in 40 mL THF and added into the polymer solution. The reaction mixtures were stirred at room temperature for 20 h. Upon completion, white viscous products were filtrated on the bottom of the flask and the solvent was removed. After the viscous products were dissolved in ethanol and precipitated with THF for three times, the filtrate was washed by THF for several times, then the hyperbranched polyester HBPE-CA microspheres were obtained. Yield 80%.

2.3.2. Hemocompatibility evalution of HBPE-CA microspheres

The coagulation assays were performed and measured by using a Semi automated Coagulometer (RT-2204C, Rayto, USA). Expression of the fluorescently labeled platelet activation marker anti-CD62P and the platelet pan-marker anti-CD42a was detected using a BD FACSCalibur (BD Biosciences, USA). All the platelet activation experiments were done in triplicates.

2.3.3. Preparation of the HBPE-CA microspheres modified GCE

Prior to each experiment, the indium tin oxide (ITO) electrodes with dimension of 2 cm × 0.5 cm were sonicated alternately with chemical detergent solution, deionized water, acetone and ethanol, each for 10 min to get clean ITO surface. After rinsing with ethanol and drying with N_2 stream, 1/3 part of the ITO electrode was immersed in the mixture of CMCS and HBPE-CA (V/V = 1/4). The modification process of the electrode was shown as Scheme 1. CMCS was chosen as an adhesive molecule to immobilize HBPE-CA on the clear surface of the ITO electrode, and dried in the air freely. The HBPE-CA modified ITO electrode was immersed in 0.10 M PBS (pH 7.4) which contained EDC and NHS as a coupling agent for about 16 h to activate the carboxyl-terminated surface of the HBPE-CA [29], followed by rinsing with PBS and dried with N_2. Then the ITO electrode was immersed in PBS (pH 7.4) containing 1 μM TBA at 4 °C for 24 h, followed by rinsing with PBS and dried with N_2 again. Here, TBA was molecularly grafted onto the surface of modified GCE via covalent binding. After blocking the nonspecific binding sites by 1% BSA, such prepared ITO electrode was incubated with thrombin. The control experiment employed to detect thrombin in human serum was followed according to the literature [30]. First, the fluorescence spectra of crystal violet were recorded on a fluorometer (Cary Eclipse, Varian, USA) with