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A electrochemiluminescence aptasensor for detection of thrombin incorporating the capture aptamer labeled with gold nanoparticles immobilized onto the thio-silanized ITO electrode

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ABSTRACT

A novel electrochemiluminescence (ECL) aptasensor was proposed for sensitive and cost-effective detection of the target thrombin adopted an aptamer-based sandwich format. To detect thrombin, capture aptamers labeled with gold nanoparticles (AuNPs) were first immobilized onto the thio-silanized ITO electrode surface through strong Au–S bonds. After catching the target thrombin, signal aptamers tagged with ECL labels were attached to the assembled electrode surface. As a result, an AuNPs-capture-aptamer/thrombin/ECL-tagged-signal-aptamer sandwich type was formed. Treating the resulting electrode surface with tri-*n*-propylamine (TPA) and applying a swept potential to the electrode, ECL response was generated which realized the detection of target protein. Spectroscopy and electrochemical impedance techniques were used to characterize and confirm the fabrication of the ECL aptasensor. AuNPs amplification and smart sensor fabrication art were implemented for the sensitive and cost-effective detection purpose. Signal-to-dose curve excellently followed a sandwich format equation and could be used to quantify the protein, and the detection limit was estimated to be 10 nM. Other forms of thrombin such as β- and γ-thrombins had negligible response, which indicated a high specificity of α-thrombin detection. The aptasensor opened up new fields of aptamer applications in ECL domain, a highly sensitive technique, and had a promising perspective to be applied in microarray analysis.

1. Introduction

A rapid, effective and highly specific detection and quantification of biological substances are increasingly needed [1,2]. These biochemical materials can be determined by conventional binding methods with high degree of specificity such as antigen–antibody system, nucleic acid hybridization, avidin–biotin interaction and protein–ligand systems. In recent years, aptamers emerged as an attractive element in diverse analytical applications and bioassays [3–6].

Aptamers are short single-stranded nucleic acids that fold into well-defined three-dimensional structures in the conditions of their in vitro selection process called SELEX (systematic evolution of ligands by exponential enrichment)

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[7]. Because of the high diversity of possible nucleotide sequence, aptamers can bind to their respective targets with high affinity, selectivity and specificity ranging from small molecules to proteins even whole cells [8–14]. Aptamer used as biorecognition element in analytical devices was extensively investigated for reasons of simple synthesis, good stability, easily chemical modification and wide applicability to extreme conditions [7,15]. A variety of detection methods were coupled to aptamer-based analysis including optical transduction [16], circular dichroism [17], electrochemistry [18–20], fluorescence [13,21], colorimetry [22], AFM [23], SPR [24] and quartz crystal microbalance [15].

ECL is inherently a highly sensitive and selective detection means which attracts considerable attention in pharmaceutical analysis, clinical diagnosis, environmental and food analysis and immunoassay as well as DNA detections, which were systematically reviewed by Richter [25] and Guilbault [26]. But tris(2,2′-bipyridine) ruthenium(II) [$Ru(bpy)_3^{2+}$]-based ECL immunoassay and DNA probe assays are not so popular, which used functional ruthenium complex as label since proteins and DNA cannot be directly detected by tris(2,2′-bipyridine) ruthenium(II). Previous work used commercial ECL labels, usually activated ruthenium NHS esters to modify biomacromolecules such as DNA and proteins [27–30]. The labels are very expensive, inconvenient and do not be sold without buying corresponding equipments (e.g. IGEN). Notice that there are some intrinsic distinct advantages of ruthenium labeling such as high stability, wide dynamic range, wide pH tolerance, easy multiple labeling without affecting bioactivities [27]. Extending ECL scope and exerting ECL superiority into bioanalysis are very helpful, valuable and of consequence. To our best knowledge, ECL or EIS aptasensor has been reported based on capture aptamers [31–33], but ECL approach for aptamer-based thrombin detection was seldom reported until now [34,35]. They used $Ru(bpy)_3^{2+}$-doped silica nanoparticle as DNA tags to get ECL signal enhancement. But, preparation of those tags was complicated and introduction of functional groups onto the surface of silica nanoparticles was tedious but necessary for labeling [36]. Still, the linkage of $Ru(bpy)_3^{2+}$ from silica nanoparticle was troublesome which would spoil the detection stability. In our work, we incorporate aptamer, the novel and efficient biorecognition element, and TBR ECL, the highly sensitive and favorable detection approach bridged by our self-synthesized activated $Ru(bpy)_3^{2+}$ NHS ester for protein detection. It is a simple, cost-effective and selective method. We just utilized an ITO-coated glass slide as assembly substrate, reversibly bound with a hollow PDMS layer on the ITO electrode surface to confine the area of ITO electrode, combined with self-assembly and AuNPs amplification techniques to fabricate a novel, smart, low-cost and sensitive ECL aptasensor for the detection of model protein, thrombin.

2. Experimental

2.1. Chemicals and materials

The oligonucleotides used in this study were purchased from Takara Biotechnology (Dalian) Co., Ltd. with the following sequences: 15-mer 5′SH-$(CH_2)_6$-GGTTGGTGTGGTTGG as capture aptamer and 29-mer 5′NH_2-$(CH_2)_6$-AGTCCGTGGTAGGGCAGGTTGGGGTGACT as signal aptamer. 3-Mercaptopropyltrimethoxysilane (MPTMS), tetrachloroauric acid ($HAuCl_4$), *N*-hydroxysuccinimide (NHS), thrombin, glutaraldehyde, bovine serum albumin (BSA), *N*,*N*′-dicyclohexylcarbodiimide (DCC), tri-*n*-propylamine (TPA), and anhydrous *N*,*N*′-dimethylformide (DMF) were purchased from Sigma–Aldrich. Human β-thrombin and γ-thrombin were purchased from Haematologic Technologies, Inc., (Essex Junction, VT, USA). Sodium citrate, methanol, ethanol, and acetone were bought from Beijing Chemical Corp. and were at least analytical reagent graded. All reagents were used as-received without further purification. ITO-coated glass (150-nM thick and $<15\,\Omega^{-2}$-resistant) was purchased from HIVAC Technology Co., Ltd. (Shenzhen, China). Sylgard 184 silicone elastomer and curing agent were obtained from Dow Corning (Midland, MI).

2.2. Apparatus and equipments

ECL is described as chemiluminescence produced directly or indirectly as a result of electrochemical reactions. It comprises electrochemical reactions on the electrode and light emitting process in the vicinity of the electrode. So, electrochemical reactions were carried out with a conventional three-electrode setup and monitored on a CH Instruments 832 Voltammetric Analyzer (CH Instrument Inc., USA). The ECL response was recorded on a Model BPCL Ultra-weak Luminescent Analyzer (Institute of Biophysics, Chinese Academy of Sciences) in which the photomultiplier tube was biased at −950 V. Working electrodes were ITO electrodes self-assembled layer-by-layer with different biomolecules. Ag/AgCl (saturated KCl) reference electrode and platinum counter electrode were used for all the electrochemical and ECL measurements.

The electrochemical impedance experiments (EIS) of the self-assemblies of biomolecules were performed in the presence of 1 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) as the redox probe and the binding buffers (20 mM Tris–HCl, 140 mM NaCl, 5 mM KCl, and 1 mM $MgCl_2$) as supporting electrolyte. The spectra were measured by Autolab with PGSTAT 30 (Eco Chemie B.V., Utrecht, Netherlands) and with the aid of a frequency response analysis (FRA) system software under an oscillation potential of 5 mV over a frequency range of 10 kHz to 0.1 Hz. The impedance *Z* is expressed in term of a real (Z_{re}) and an imaginary (Z_{im}) component. UV spectra were recorded on a Cary 500 scan UV–vis–NIR spectrophotometer (Varian, Harbor City, CA). All measurements were performed at room temperature.

2.3. Preparation of oligonucleotide–AuNPs conjugate

AuNPs with a diameter of approximately 13 nM were prepared by the citrate reduction of $HAuCl_4$ in aqueous solution according to a well-known method [37]. In brief, 50 mL of aqueous solution containing 0.0167 g of $HAuCl_4$ was brought to a boiling reflux with stirring, and then 1.94 mL of 0.1 M sodium citrate solution was introduced quickly. After a fast color change, the solution was kept boiling for additional 20 min and left to cool to room temperature.

The synthesis of oligonucleotide–AuNPs conjugate was consulted to previous work [38] with necessary modification