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A facile label-free electrochemiluminescence biosensor for target protein specific recognition based on the controlled-release delivery system

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ABSTRACT

This paper described a novel label-free electrochemiluminescence assay for target protein based on a controlled delivery system. Iron oxide magnetic mesoporous silica nanocontainers were prepared by using a general procedure. The prepared magnetic mesoporous silica nanocontainers were applied to load the guest molecules $[Ru(bpy)_3]^{2+}$. Aptamers were used as gatekeepers on the pore outlets of the nanocontainers. In the presence of target proteins, the specific aptamer–protein interactions were employed as triggers for uncapping the pores and releasing the guest molecules from the nanocontainers. The amount of the guest molecule $[Ru(bpy)_3]^{2+}$ released from the magnetic mesoporous silica nanocontainers was monitored by the electrochemiluminescence assay. The results show that the releasing amount of $[Ru(bpy)_3]^{2+}$ is proportional to the thrombin concentration in the range of 0.6 pM–0.8 nM with a detection limit of 0.5 pM ($S/N=3$). The present work demonstrates that the fabricated nanocontainer using aptamer as the cap is a highly sensitive and selective key-in lock gating system for the label-free ECL biosensor.

1. Introduction

Electrochemiluminescence (ECL) has been a very powerful analytical technique which is widely used in the areas of clinical tests and biomolecule detection [1]. $[Ru(bpy)_3]^{2+}$ is a well-known ECL luminophore with high ECL efficiency, which is often used to label DNA, antibody or antigen in ECL biosensor [2–5]. In order to tag biomolecule, it is necessary to functionalize $[Ru(bpy)_3]^{2+}$ with active group, such as –COOH, $–NH_2$, –SH, etc, resulting in the complicated and time-consuming labeling process. Compared with the conventional label-based methods, label-free ECL biosensors have attracted much attention in recent years owing to avoiding the need for expensive and complex molecular labels [6–12]. However, most of label-free ECL biosensors were "signal-off" architectures, in which the presence of targets could reduce ECL intensity and sensitivity. Therefore, developing a signal-on, low-cost and efficient approach is the key issue to improve the ECL label-free biosensor.

Magnetic mesoporous silica nanocontainers (MMSNs) have gained increasing attention for their interesting magnetic property, large surface area, accessible pore volume, and well-defined surface property. MMSNs have provided an alternative for site-specific drug targeting delivery because they could be concentrated and held in position by means of an external field [13]. Furthermore, they could be used in bioseparation of protein or cell, and diagnosis as potential contrast agents in magnetic resonance imaging [14]. There have been some reports on the preparation of magnetic iron nanoparticles coated with mesoporous shells [15–19]. And there have been several reports on the controlled-release of the cargos based on the magnetic mesoporous silica nanoparticles for drug delivery, simultaneous magnetic resonance and fluorescence imaging [15–20]. However, to the best of our knowledge, the label-free ECL biosensors with signal-on architecture based on a controlled-release MMSNs system has not been reported yet.

Herein, we described a general procedure for the fabrication of Fe_3O_4 magnetic nanoparticles embedded within mesoporous silica nanocontainers. Furthermore, these monodisperse MMSNs were applied to the uptake and controlled release of guest molecules by using aptamers as gatekeepers. Aptamers are the short single-stranded nucleic acids which bind to their respective target molecules with high affinity and specificity [21–24]. As excellent alternatives to antibodies, aptamers have been used in molecule recognition and detection as well as targeted drug-delivery systems [25–28]. In this work, the specific aptamer–protein interactions were employed as triggers for uncapping the pores and releasing the guest molecules from MMSNs. Thrombin, a multifunctional serine protease involves in the coagulation cascade and converts fibrinogen to insoluble fibrin that forms the fibrin gel, was chosen as the target protein model [29]. Accordingly, the aptamer of thrombin was chosen as the gatekeeper. $[Ru(bpy)_3]^{2+}$,

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a typical ECL substrate, was employed as the guest molecule to be encapsulated within the MMSNs. This flexible ECL biosensor exhibited not only high sensitivity and specificity but also excellent performance in real human plasma samples.

2. Experimental

2.1. Reagents and apparatus

Tetraethylorthosilicate (TEOS), n-cetyltrimethylammonium bromide (CTAB) and oleic acid were purchased from Bodi Chemical Co., Ltd. (Tianjin, China). Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), iron (II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$) and cerium(III) nitrate hexahydrate ($Ce(NO_3)_3 \cdot 6H_2O$) were obtained from DaMao Chemical Reagent Factory (Tianjin, China). 3-Aminopropyltriethoxysilane (APTS), and Tris(2,2′-bipyridyl)ruthenium(II) chloride hexahydrate ($[Ru(bpy)_3]Cl_2 \cdot 6H_2O$) were purchased from J&K Chemical LTD. (Beijing, China). Purified thrombin (1000 U/mg, lyophilized powder) was purchased from Dingguo biological Technology Corporation (Beijing, China). Lysozyme, trypsin and bovine serum albumin (BSA) were purchased from Sigma (USA). Analytical reagent grade chemicals and deionized, doubly distilled water (18.2 MΩ cm) were used throughout.

The compositions of the buffer solutions used for the experiments were as follows: buffer solution for washing and for the immobilization of the aptamer onto NH_2–MSN was 20 mM Tris–HCl solution, for the interaction between aptamer and thrombin physiological buffer was PBS solution (0.1 M NaH_2PO_4–Na_2HPO_4, pH=7.0, containing 10 mM KCl, 2 mM $MgCl_2$), and the solution for ECL detection consisted of Britton–Robinson buffer (B–R buffer, 0.04 M H_3BO_3, 0.04 M H_3PO_4, and 0.04 M CH_3COOH, pH=8.69), 0.1 M potassium persulfate, and 2.0 M NaCl.

Oligonucleotides were synthesized by SBS-bio Genetech. Co. Ltd. (Shanghai, China). Their base sequences were as follows:

Aptamer : 5′–TTT TTT GGT TGG TGT GGT TGG–3′.

Transmission electron microscopy (TEM), Zeta Potential, Dynamic Light Scattering (DLS), XRD microscopy, Vibrating Sample Magnetometer (VSM) and N_2 adsorption–desorption were employed to characterize the synthesized materials. TEM image was taken with a JEOL JEM-2100 instrument (HITACHI). Zeta Potential and DLS were performed on a Malvern Zetasizer-Nano instrument equipped with a 4 mW He–Ne laser (633 nm) and avalanche photodiode detector. X-ray measurements were performed on a D/max2500PC diffractometer (RIGAKU) using Cu-Kα radiation. N_2 adsorption–desorption isotherms were recorded on a Micromeritics Tristar 3000 analyzer. The samples were degassed at 120 °C in vacuum overnight. The specific surface areas were calculated from the adsorption data in the low pressures range using the Barrett–Emmett–Teller (BET) model. Pore volume and pore size distributions for the adsorption branch of the isotherm were determined following the Barrett–Joyner–Halanda (BJH) method. UV–vis spectra were carried out on a Cary 50 UV–vis–NIR spectrophotometer (Varian).

2.2. Preparation of Fe_3O_4 nanoparticles

The magnetic nanoparticles were prepared via a convenient chemical coprecipitation process described previously with a slight modification [17]. Briefly, 4.80 g $FeCl_3 \cdot 6H_2O$ and 2.00 g $FeCl_2 \cdot 4H_2O$ were added to 120 mL of deionized water under nitrogen atmosphere with vigorous stirring. 20 mL of ammonium hydroxide (14 wt%) was then added to the mixture solution at 70 °C and kept for 30 min. 0.85 mL oleic acid was added and the reaction was kept for another 2.5 h at 70 °C. The black precipitate was collected by magnetic separation at room temperature, washed with ethanol 3 times by ultrasonication for 5 min, collected by centrifugation at 10,000 r/min, and kept wet at 4 °C for use.

2.3. Synthesis of magnetic mesoporous silica nanocontainers (MMSNs)

The MMSNs were prepared according to the method reported previously with a slight modification [17–19]. 0.15 g as-prepared Fe_3O_4 nanoparticles were dispersed in 20 mL of aqueous solution containing 0.40 g of CTAB with vigorous stirring. Then 20 mL of the as-synthesized aqueous phase monodisperse Fe_3O_4 nanoparticles was added into the 172 mL of aqueous solution containing 0.10 g of CTAB and 1.4 mL of NaOH (2.0 M) with vigorous stirring, and the mixed solution was heated to 75 °C and 2.5 mL of TEOS was introduced under vigorous stirring. After 15 min of stirring, 100 μL of APTS was added into the mixtures, and the solution was stirred for 2 h. The light brown product was collected by filtration and dried at room temperature. The CTAB surfactants were removed from the mesopores by dispersing the as-synthesized materials in a solution of 0.160 g of ammonium nitrate and 60 mL of 95% ethanol and heating the mixture at 60 °C for 15 min to fabricate the NH_2-group functionalized MMSNs (NH_2–MMSNs).

2.4. Preparation of dye encapsulated NH_2–MMSNs

0.1 g the above NH_2–MMSNs solid and the dye tris (2,2′-bipyridyl) ruthenium chloride (0.06 g, 0.08 mmol) were suspended in 2 mL of anhydrous acetonitrile under N_2 atmosphere. The suspension was stirred for 24 h at room temperature with the aim of encapsulating dye into the pores of the MMSN scaffolding. The solution was dried in vacuum to remove acetonitrile, and then the obtained orange solid was suspended in 2 mL Tris–HCl (20 mM) buffer.

2.5. Fabrication of the aptamer as the cap of the controlled-release system

20 μL aptamer solutions were added into 5 μL NH_2–MMSNs loaded with guest dyes suspension, incubated for 30 min at 37 °C with frequent shaking. The resulting solid was isolated by magnetic separation and washed 3 times with Tris–HCl buffer to remove the residual dye and the free aptamer.

2.6. Release of the guest molecules

20 μL thrombin solutions were added in the above aptamer-capped NH_2–MMSNs solid loaded with guest molecules, incubated at 37 °C for 2 h. Supernatant containing the guest molecules released from MMSNs was obtained after magnetic separation. The release efficiency of the guest molecules was determined by UV–vis spectra at a wavelength of 286 nm, using an extinction coefficient of 7.95×10^4 L mol^{-1} cm^{-1}.

2.7. ECL detection

The ECL emission was detected by the use of a model MPI-E ECL analyzer (Xi'an Remax Electronic Science & Technology Co. Ltd., Xi'an, China) at room temperature. A commercial cylindroid glass cell with three-electrode, a 2.5-mm diameter Au as the working electrode, a Pt wire as the counter electrode, and an Ag/AgCl (sat.) as the reference electrode, was used. 20 μL supernatant containing the guest molecules released from MMSNs, 800 μL detection solution (B–R buffer solution, 0.1 M potassium persulfate, and 2.0 M NaCl), and 180 μL 1.0 mM cerium nitrate solution were added in a quartz cell for ECL detection. Cyclic voltammetry mode with continuously potential scanning from −1.9 to 0.0 V and with 350 mV s^{-1} scanning rate