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Electrochemiluminescence resonance energy transfer system between GNRs and $Ru(bpy)_3^{2+}$: Application in magnetic aptasensor for β-amyloid

Hong Ke, Haifeng Sha, Yinfang Wang, Weiwei Guo, Xin Zhang, Zhiming Wang, Chusen Huang, Nengqin Jia*

The Education Ministry Key Laboratory of Resource Chemistry, Shanghai Key Laboratory of Rare Earth Functional Materials, Department of Chemistry, Shanghai Normal University, Shanghai 200234, People's Republic of China

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ABSTRACT

Electrochemiluminescent (ECL) assay has gradually drawn increasing interest in the biomedical analysis. This paper proposed a new methodology for ultrasensitive and facile detection of Alzheimer's disease marker β-amyloid (Aβ) by fabricating a sandwich-type ECL sensing platform. Herein, electrochemiluminescence resonance energy transfer (ECL-RET) was employed to determine Aβ concentration, which can be attributed to the quenching effect from RET between $Ru(bpy)_3^{2+}$ and gold nanorods (GNRs) acting as ECL-RET electron donor and acceptor, respectively. In this protocol, mesoporous carbon nanospheres were adopted to immobilize ECL reactant $Ru(bpy)_3^{2+}$ and antibody via nafion to acquire the RET donor nanocomposites (MOCs/nafion/$Ru(bpy)_3^{2+}$/antibody), which were tightly interconnected with epoxy group functionalized Fe_3O_4 nanoparticles. It is of vital importance that GNRs with exquisite rod shape were synthesized and exhibited a typical absorption peak at 650 nm to quench ECL signal of $Ru(bpy)_3^{2+}$ effectively. In addition, the ECL emission decreased linearly with the logarithm of Aβ concentration in a wide linear range from 1.0×10^{-5} to 100 ng/mL with a detection limit of 4.2×10^{-6} ng/mL. Furthermore, distinctive and desirable properties were verified to declare the promise for being applicable to analyze the Aβ content in real Alzheimer's cerebrospinal fluid samples with satisfactory results.

1. Introduction

Alzheimer's disease (AD) (Schulte et al., 2015) is a major worldwide public health problem and accounts for the vast majority of dementia cases worldwide, which is characterized by pathological symptoms such as gradual loss of memory, abnormal formation of brain nerves (Schütz et al., 2015) and cognitive decline (Wang et al., 2016c). Neuropathologically, the disease involves the formation and accumulation of amyloid-β (Aβ) species (Chen et al., 2016; Diomede et al., 2016) in the cerebrospinal fluid and it has been closely linked to senile plaque (Hernandez-Rapp et al., 2016) in the brain. Traditionally, Aβ has been subscribed to the belief that Aβ can be used as one of the reliable molecular biomarkers (Wang et al., 2016a) for the detection, early screening and diagnosis of AD. Furthermore, the last decade has witnessed increasingly rapid advances in Aβ assay and the strategies employed thus far are listed as electrical impedimetric (EI) (Rushworth et al., 2014), electrochemical (EC) (Liu et al., 2015a), fluorescence (Pi et al., 2016), as well as localized surface plasmon resonance (LSPR) (Kang et al., 2015) and electrochemiluminescence (ECL) and so forth.

Specifically, tremendous attention has been gradually rising about ECL assay owing to the perfect coalition of unique advantages for both electrochemical methods and chemiluminescent spectroscopy in recent years. Electrochemiluminescence (ECL) (He et al., 2016; Li et al., 2017; Zhongyuan Liu and Guobao, 2015; Zhou et al., 2015) was defined as an electrogenerated chemiluminescence (CL) phenomenon during the conversion from electrochemical energy to luminous energy, which possess overwhelming popularity for fascinating advantages, such as low background signal, high and stable luminescent efficiency, extraordinary broad dynamic detection range and low determination limit among various assay systems. Electrochemiluminescence resonance energy transfer (ECL-RET) (Lei et al., 2015; Qi et al., 2013) was based on the basic principle of resonance energy transfer (RET) phenomenon between a perfectly matched acceptor and donor electron pair, which is an effective strategy that could be employed to fabricate stable and sensitive ECL sensing platforms. Central to the entire discipline of efficient ECL-RET is the critical matching of energy overlapped donor–acceptor pair. In light of recent advances in ECL-RET, it is of vital significance to search for a well-matched donor–acceptor pair, which

* Corresponding author.
E-mail address: nqjia@shnu.edu.cn (N. Jia).

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has an as large as possible overlapping area between the ECL emission spectrum of donor and absorption spectrum of acceptor. Besides, RET has often occurred to establish various sensors for fluorescence (FRET) (He et al., 2017; Lin et al., 2017), bioluminescence (BRET) (Alam et al., 2016; Yoshida et al., 2016) and chemiluminesence (CRET) (Li et al., 2016; Wang et al., 2015) in analysis of ion (Coskun and Akkaya, 2005), protein (Willard et al., 2001), and DNA (Freeman et al., 2011), etc.

Recently, there has been renewed interest in gold-related nanomaterials due to their distinctive physical properties (for example, optical and electronic characteristics) and controllable growth into various shapes and sizes. Moreover, there is a growing body of literature that recognizes the unique shape- and size-dependent optical absorption property of gold nanorods (GNRs) due to the creation of surface plasmon resonances. So far, however, there has been little discussion about the promising role of GNRs in ECL-RET despite the above-mentioned very fascinating superiorities to fuel further applications. Additionally, a number of advanced synthetic methods have been taken consideration to achieve successful synthesis of appropriate GNRs size, aiming at obtaining a characteristic absorption peak at about 650 nm to subsequently make up half of the fabricated ECL-RET donor and acceptor pair. Many previous reported studies are suitable candidates for GNRs synthesis, for instance, aqueous wet-chemical CTAB-mediated method (Babak Nikoobakht, 2003; Jana et al., 2001), template-aided reduction of gold (Martin, 1996, 1998) and seed-mediated procedure (Chang et al., 1999; Yu et al., 1997) and so forth.

On the basis of the above consideration, a novel sandwich-type ECL-RET sensing platform involving energy transfer from classical ECL reactant $Ru(bpy)_3^{2+}$ to gold nanorods was designed for accurate Alzheimer's disease biomarker amyloid-β (Aβ) analysis. Accordingly, the fabricated ECL aptasensor was comprised of RET donor nanohybrids (MOCs@nafion/$Ru(bpy)_3^{2+}$/antibody) and RET acceptor nanocomposites (GNRs/Aptamer). After being incubated with target Aβ protein and GNRs based aptamer, an ultrasensitive ECL signal decrease was clearly observed owing to the ECL-RET quenching effect between $Ru(bpy)_3^{2+}$ and GNRs acting as ECL-RET donor and acceptor, respectively. As a result, this quenching ECL aptasensor is prone to realize favorable quantitative analytical performance for Aβ antigen, paving an innovative approach in assay for Aβ in actual samples.

2. Experimental section

2.1. Reagents and materials

Tris(2,2-bipyridyl) diclo-roruthenium(II) hexahydrate ($Ru(bpy)_3Cl_2 \cdot 6H_2O$) was purchased from Tokyo Chemical Industry Co., Ltd. (TCI). Bovine serum albumin (BSA, > 99.8%), tripropylamine (TPA, ≥ 98.0%) and nafion (5 wt%) were bought from Sigma-Aldrich (USA). Cetyltrimethyl Ammonium Bromide (CTAB) was purchased from Sinopharm Chemical Reagent Co., Ltd. The epoxy-functionalized Fe_3O_4 nanoparticles were bought from Aladdin Biochemical Technology Co., Ltd (Shanghai, China). β-amyloid (Aβ) protein and its antibody were both obtained from Shanghai Xin Yu Biotech Co., Ltd. (Shanghai, China). The aptamer sequence of Aβ oligomers (5′-HS-GCCTGTGTTGGGGCGGGTGCG), which targeted specifically at Aβ-40, was screened out by Tsukakoshi et al. (2012), and was synthesized by Sangon Biotech Co., Ltd (Shanghai, China). Tween 20, potassium ferricyanide, sodium dihydrogen phosphate and disodiumhydrogen phosphate dodecahydrate were all collected from Sinopharm Chemical Reagent Co., Ltd. Human chorionic gonadotropin (HCG), α-1-Fetoprotein (AFP), carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9) and their relative antibodies were all purchased from Shanghai Linc-Bio Science Co. Ltd. Phosphate buffered solutions (PBS) with different pH were prepared by mixing Na_2HPO_4 and NaH_2PO_4 according to different proportions. All other reagents were of analytical reagent grade and the ultrapure water (18.2 MΩ) was employed throughout this work.

2.2. Assay apparatus

A model MPI-E ECL analyzer (Xi'An Remax Electronic Science & Technology Co. Ltd., China) was employed to perform ECL detections. EIS and CV experiments were taken by CHI 660B electrochemistry workstation (Shanghai Chenhua Apparatus Inc., China) in 0.1 M PBS (pH 7.4) containing 10 mM $K_3Fe(CN)_6$/ $K_4Fe(CN)_6$ (1:1) and 0.1 M KCl as the supporting electrolyte. A conventional three-electrode system was made up of an Ag/AgCl (3 M KCl) and a platinum wire as reference electrode and auxiliary electrode, respectively. Moreover, a differently decorated magnetic glass carbon electrode (MGCE, 3.0 mm in diameter) was adopted as working electrode. Additionally, the morphologies of gold nanorods (GNRs) and mesoporous carbon nanospheres (MOCs) were characterized by Transmission electron microscopy (TEM) (Hitachi model H-800 TEM) and Scanning electron microscopy (SEM) of using a LEO1530 field emission SEM system (Germany).

2.3. Preparation of MOCs based ECL-RET donor antibody nanocomposites

In this protocol, we provide a successful approach for preparing the MOCs with much higher yield, which was proposed according to a strategy employed by previously published literature methods (Hu et al., 2014; Li et al., 2011; Liang and Dai, 2006) with appropriate modifications, which was demonstrated in Supplemental information. Typically, the resultant dark powder should be ultrasonically dispersed in PBS to receive a homogeneous solution. Firstly, 2 mL 1 mg/mL acquired MOCs solution was mixed with 5 mL 5 wt% nafion and stirred constantly with the assistance of magnetic force. Then, the MOCs@nafion hybrids were collected by centrifugation and got rid of unreacted nafion carefully, followed by redispersed in 2 mL PBS after washed by PBS for several times. As a classical ECL reagent, $Ru(bpy)_3^{2+}$ was employed as ECL resonance energy transfer donor on the basis of many distinctive and desirable electrochemiluminescence properties at about 650 nm. In brief, 1 mL 20 mM $Ru(bpy)_3^{2+}$ was added rapidly with stirring in ice-water bath. The target composites (MOCs@nafion/$Ru(bpy)_3^{2+}$) could be obtained after separating through ultracentrifugation and further washing for 3 times with PBS. Subsequently, 550 μL 2.5 μg/mL Aβ antibody was taken to be further attached with 500 μL the above-obtained MOCs@nafion/$Ru(bpy)_3^{2+}$ solution with shaking for 2 h in ice- water bath. In this case, antibody could be conjugated onto the surface of MOCs@nafion/$Ru(bpy)_3^{2+}$. Briefly, the ultimate products were collected by centrifugation and redispersed in 2 mL PBS after washing thoroughly for several times. Finally, the RET donor nanocomposites (MOCs@nafion/$Ru(bpy)_3^{2+}$/antibody) should be stored against exposure to light in the refrigerator at 4 °C until use.

2.4. Synthesis of GNRs and β-amyloid aptamer based ECL-RET acceptor

The GNRs selected as ECL resonance energy transfer acceptor were synthesized by previously reported method with minor modification. Herein, seed-induced growth method was primarily adopted to produce GNRs with uniform size and shape. Initially, 0.25 mL 15 mM chloroauric acid ($HAuCl_4 \cdot 6H_2O$) solution was diluted to 5 mL in deionized water and mixed with 8 mL 0.1 M CTAB with vigorous stirring for 5 min. After adding 0.5 mL 0.01 M sodium borohydride ($NaBH_4$) to the above-obtained mixture with stirring for another 3 min, the seed solution was kept for 3 h at 28 °C. Then, 100 μL resulting gold seed solution was added rapidly into a homogeneous growth solution containing 25 mL 0.1 M CTAB, 1.0 mL 6 mM silver nitrate, 25 mL 15 mM $HAuCl_4 \cdot 6H_2O$ and 0.50 mL 0.0788 M ascorbic acid with stirring at 28 °C for 2 h and left overnight. Next, 1 mL as-prepared GNRs was taken to 1 mL PBS and reacted with 1.5 mL 8 μg/mL Aβ aptamer to realize complete interaction as much as possible.