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Distance-dependent quenching and enhancing of electrochemiluminescence from tris(2, 2′-bipyridine) ruthenium (II)/tripropylamine system by gold nanoparticles and its sensing applications

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ABSTRACT

Understanding the role of gold nanoparticles (AuNPs) in electrochemiluminescence (ECL) processes of the Ru$(bpy)_3^{2+}$ (bpy = 2, 2′-bipyridine)/tripropylamine (TPA) system would be beneficial to develop novel ECL sensors for a variety of applications. In this work, we found that the AuNPs on the surface of indium tin oxide (ITO) electrode could catalyze the electrochemical oxidation of TPA, greatly enhancing the ECL signal of $Ru(bpy)_3^{2+}$/TPA, present in the solution. If physical separation of AuNPs away from electrode surface after hybridization with target ssDNA, ECL signal decreased dramatically due to the loss of electrochemical activity of AuNPs, based on which a sensitive and specific DNA sensor in a “switch-off” mode was constructed with a detection limit of 0.2 pM. In addition, a suppressing effect of the AuNPs on the ECL of $Ru(bpy)_3^{2+}$ was experimentally confirmed by decreasing the electrocatalytic effect to overall ECL emission, including selection of oxalate as a coreactant instead of TPA, or introduction of gold electrode as substrate. Furthermore, when $Ru(bpy)_3^{2+}$ and AuNPs were both immobilized on the ITO electrode at close proximity, the ECL quenching induced by energy/electron transfer was predominant. ECL emission of the $Ru(bpy)_3^{2+}$/TPA system resulted from a competition between electrocatalytic enhancement and quenching effect. However, the quenched ECL signal would return in case of the AuNPs moving far away from ECL emitters after a hybridization reaction as before, and a separation distance dependent surface enhancement was observed as well. Based on the role change for AuNPs from quenching to enhancing ECL intensity of $Ru(bpy)_3^{2+}$/TPA system, a novel ECL DNA sensing strategy in a “turn-on” mode was developed, enabling quantitative analysis of target ssDNA over the range of 0.05 pM to 0.5 nM with a detection limit of 12 fM. Overall, we demonstrated the existence of three effects of AuNPs on the ECL of $Ru(bpy)_3^{2+}$/TPA system, and which played the leading role was dependent on the placement of AuNPs, $Ru(bpy)_3^{2+}$, and their separation distance. The ECL sensors based on the role change for AuNPs showed both high sensitivity and excellent selectivity.

1. Introduction

Electrochemiluminescence (ECL) based on metallic complex, Ru$(bpy)_3^{2+}$ (bpy = 2, 2′-bipyridyl), has been widely used in scientific research and clinical diagnosis because of its excellent photochemical stability, high luminescence quantum yield in aqueous solutions, good water solubility and the regeneration ability in electrochemical reaction (Tokel and Bard, 1972; Van Houten and Watts, 1976). In most cases, the ECL emission results from electrochemical reactions between Ru$(bpy)_3^{2+}$ and its coreactants (Miao and Choi, 2004), such as oxalate (Rubinstein and Bard, 1981), peroxydisulfate (White and Bard, 1982), amine-related compounds including alkylamides (Leland and Powell, 1990; Noffsinger et al., 1987), amino acids (Li et al., 1990), nicotinamide adenine dinucleotide (Jameison et al., 1996). Until now, the use of $Ru(bpy)_3^{2+}$ or one of its derivatives as the signal reporter and tripropylamine (TPA) as an efficient co-reactant is still the most successful ECL system. In fact, the clinical immunoassay analyzers based on $Ru(bpy)_3^{2+}$/TPA ECL system have been successfully commercialized (Debad et al., 2004).

According to the accepted ECL reaction mechanism for $Ru(bpy)_3^{2+}$/TPA system, electro-oxidation of TPA plays critical roles in ECL signal generation, especially when detecting low concentration of $Ru(bpy)_3^{2+}$ ($\leq$ 1 μM) (Chen and Zu, 2008; Miao et al., 2002). This relies on the surface property of the electrode, halide ion, and surfactant (Chen and Zu, 2009; Cole et al., 2003; Factor et al., 2001; Li and Zu, 2004; Zu and Bard, 2000, 2001). As a typical of metallic nanoparticle, the AuNPs are

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widely used in ECL system due to their excellent electrocatalytic activity, electrical conductivity, ease of synthesis, and well-established surface chemistry (Huang and Guo, 2013). The functions of AuNPs in ECL system of $Ru(bpy)_3^{2+}$/TPA are mainly divided into three major categories: (1) Catalytic oxidation of coreactant, TPA. Zheng et al. found that the AuNPs-modified ITO electrodes exhibited similar behavior as a bulk gold electrode in producing ECL of the $Ru(bpy)_3^{2+}$/TPA system (Chen and Zu, 2007). The main reason was the much more facile TPA oxidation in the presence of AuNPs. (2) Working as the carriers of coreactants or conventional ECL emitting molecules to enhance signal (Yin et al., 2005). Zhang's group also introduced AuNPs as an ECL emitters carrier to fabricate ECL biosensors for the detection of DNA hybridization. Due to multiple signal reporters per hybridization event, a detection limit of target ssDNA as low as 5 pM was achieved (Wang et al., 2006). (3) Enhancing ECL intensity by localized surface plasmon resonance (LSPR). Lakowicz firstly reported the excited $Ru(bpy)_3^{2+}$ generated by electrochemical reaction could excite the surface plasmon on a thin gold film coated a glass substrate, which was the basis of the SPR-field enhanced ECL by metal nanoparticle (Zhang et al., 2004). Guo's group experimentally demonstrated that the ECL response of the $Ru(bpy)_3^{2+}$/TPA system could be significantly enhanced in the presence of AuNP-SiO_2 core-shell nanocomposites (Wang et al., 2015a). As much as 10 times ECL enhancement compared with that in the absence of AuNPs was observed under the optimal conditions. Later, they presented a new approach for ultrasensitive determination of carcinoembryonic antigen in human serum based on LSPR enhanced ECL of $Ru(bpy)_3^{2+}$, in which $Ru(bpy)_3^{2+}$-doped SiO_2 nanoparticles acted as ECL luminophores (Wang et al., 2015b).

Previous studies showed experimentally and theoretically the existence of the interactions with surface plasmons of metallic surfaces could enhance or diminish photoluminescence due to the competition between SPR-field enhancement and Förster resonance energy transfer (FRET). The FRET quenching is a short-range effect, and it would be weakened with the distance much faster than the enhanced SPR field possibly responsible for the enhancement in luminescence. The distance dependence of energy transfer plays an important role in a variety of sensing applications, which has recently been applied for sensitive determination of proteins adsorbed on AuNPs using the gated resonance energy transfer technique (Stobiecka, 2014; Stobiecka and Chalupa, 2015). Xu's group studied the effects of separation distance, spectral overlapping and magnetic field on the SPR induced ECL enhancement, which was further applied for ultrasensitive thrombin detection (Wang et al., 2011). Zhou et al. reported a novel ECL resonance energy transfer (RET) system using CdS:Eu NCs as an ECL donor and Au nanorods as an ECL acceptor (Zhou et al., 2012). After investigation of the distance, spectrum and shape effects on the efficiency of ECL-RET were also studied, an ultra-sensitive and specific ECL method was proposed to detect target ssDNA. Based on the function conversion from ECL quenching to SPR-field enhancement for AuNPs, some novel ECL sensing strategies have been developed for the sensitive detection of nucleic acid or methylation level (Cheng et al., 2014; Shan et al., 2009; Zhang et al., 2014).

Although the SPR-field and electrocatalysis based enhancement by AuNPs are widely reported, the quenching effect of the AuNPs on the ECL of $Ru(bpy)_3^{2+}$/TPA system have not been studied and applied in ECL sensing system until now. Continuous investigation of enhancing or quenching of $Ru(bpy)_3^{2+}$/TPA ECL system is critically important to extending its applications. Additionally, we found that most of research works usually focused on one role of the AuNPs and ignored their other functions, when the AuNPs were introduced to develop novel ECL sensing systems. Therefore, it is necessary to present experimental evidence to demonstrate all the roles of AuNPs and to study the influencing factors. In this work, enhancing and quenching of ECL from $Ru(bpy)_3^{2+}$/TPA system by AuNPs are studied in detail, in which $Ru(bpy)_3^{2+}$ is either present in the solution or immobilized on working electrode. The rigid dsDNA with varied length formed through a DNA hybridization reaction are introduced to adjust the separation distance between working electrode surface or ECL emitters with AuNPs. According to the change of ECL intensity, the effects of AuNPs on the ECL system of $Ru(bpy)_3^{2+}$/TPA are concluded and well-explained, which are further applied to design novel ECL sensors to detect target ssDNA in hybridization reactions.

2. Experimental section

2.1. Reagents and materials

Indium-doped tin oxide (ITO, film thickness: 900 ± 100 Å, conductivity: 19 ± 2.1 Ω/□) conductive glass was purchased from Weiguang Corp. (Shenzhen, China). Tripropylamine (TPA), ethanolamine, $Ru(bpy)_3Cl_2$, poly (diallyldimethylammonium chloride) (PDDA), and Bis(2, 2′-bipyridine)-4′-methyl-4-carboxybipyridine -ruthenium N-succinimidyl ester bis(hexafluorophosphate) (abbreviation: Ru-NHS) were obtained from Sigma-Aldrich (St. Louis, MO, USA). (3-glycidoxypropyl) -trimethoxysilane (GPTMS), $HAuCl_4 \cdot 3H_2O$ were purchased from J&K Scientific Ltd. (Beijing, China). All the DNA molecules reported in this article were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China), as shown in Table S1. The other chemicals were from Kelong Reagent Corporation of Chengdu (Chengdu, China). All the reagents were of analytical-reagent grade without further purification. A concentration of 10 mM phosphate buffer saline (PBS, pH 7.00, 0.10 M KCl + 10 mM KH_2PO_4/K_2HPO_4) was used to dissolve DNA.

2.2. Apparatus

Electrochemical measurements were performed on a CHI660E electrochemistry analyzer (CH Instruments, Austin, TX, USA) with a Pt counter electrode and an Ag/AgCl (3 M KCl, also from CH Instruments) reference electrode. Potential scan rate was 100 mV/s. The ECL emission was transformed into an electrical signal by an R456 photomultiplier (PMT) (Xi'an Remax Electronic Science Tech. Co. Ltd., Xi'an, China), which was operated at -800 V if without notification. Scanning electron microscope (SEM) images were taken on a Hitachi model S4800 (Tokya, Japan).

2.3. Preparation of hairpin-shaped DNA (hpDNA)-AuNP conjugates

According to Frens' method (Frens, 1973), the AuNPs with an average diameter of 10 nm were prepared by reducing $HAuCl_4$ with citrate (Fig. S1). Assuming the spherical shape and a density equivalent to bulk gold (19.30 g/cm^3), the mole concentration of AuNPs in solution was calculated to be 9.5 nM. The thiolated hpDNA (3′-end) was attached to the surface of AuNPs through the sulfur-gold linkage. Then 500 μL of AuNPs solution was added to the solution and stored at 4 °C for 3 h to gain the Au-S bonding. After centrifuge at 12,000 rpm for 30 min, the suspension was discarded and the precipitate was re-dispersed in 10 mM PBS containing 1% PEG.

2.4. Preparation of AuNPs-modified electrode

Gold electrode (2 mm in diameter) was polished to a mirror finish with 0.1 μm and 0.05 μm of alumina aqueous slurry in turn. After washed with high-purity water (18.3 MΩ cm) under sonication for 3 min, gold electrode was electrochemically scanned with cyclic voltammetry (CV) in a potential range from 0.1 V to 1.6 V in 0.05 M H_2SO_4 until steady CV curves were obtained. The preparation of AuNPs-modified electrodes was achieved by casting 5 μL of AuNPs solution on gold electrode surface, which was denoted as Au/AuNPs. The bare ITO electrodes were prepared and cleaned as described before (Xu et al., 2001). The AuNPs-modified ITO electrodes (ITO/AuNPs) were assembled by layer-by-layer electrostatic adsorption. Cleaned ITO