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Electrochemical and electrochemiluminescence study of $Ru(bpy)_3^{2+}$-doped silica nanoparticles with covalently grafted biomacromolecules

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Abstract

Spherical $Ru(bpy)_3^{2+}$-doped silica (RuSi) nanoparticles were prepared via a water-in-oil microemulsion approach. The electrochemical and electrochemiluminescent properties of the RuSi nanoparticles immobilized on an indium tin oxide (ITO) electrode were investigated. Further, electrochemiluminescence (ECL) of the RuSi nanoparticles with covalently coated biomacromolecules was studied. By covalent cross-linking with glutaraldehyde, γ-(aminopropyl) triethoxysilane (APTES)-pretreated RuSi nanoparticles were coupled with different concentrations of bovine serum albumin (BSA), hemoglobin, and myoglobin, respectively. ECL from these biomacromolecule-coated RuSi nanoparticles decreased with the increase of the loading of biomacromolecules. Moreover, the ECL of coreactants with different sizes was studied. The ECL decrease could be assigned to the steric hindrance and limited diffusion of coreactant molecules into the RuSi nanoparticles after biomacromolecule conjugation. Since tens of thousands of $Ru(bpy)_3^{2+}$ molecules are contained in the silica particles and the RuSi nanoparticle surface modification could improve their biocompatibility, the biomacromolecule-coated RuSi nanoparticles could be readily used as efficient and stable ECL tag materials in the future.

Keywords: Electrochemiluminescence; Silica nanoparticles; Biomacromolecules; ECL tag

1. Introduction

Small molecules, such as transition metal complexes and organic dyes, can be encapsulated into sol–gel to form doped polymer materials, and this technique provides an attractive alternative to conventional organic polymers [1–6]. Among all the doped sol–gel systems, the tris(2,2′-bipyridyl)ruthenium(II) [$Ru(bpy)_3^{2+}$]-doped sol–gel system has been extensively studied due to its application in optical sensors [7], photosensitizers in solar energy conversion schemes [8], quantification of surface binding of molecules to metallic nanoparticles [9], fluorescence sensors [10–12], and electrochemiluminescence (ECL) [13–21].

ECL is luminescence from excited molecules generated by electrochemical redox reactions [22–25]. Among many organic and inorganic ECL systems, ECL based on $Ru(bpy)_3^{2+}$ has proven to be the most valuable since its discovery [26] due to its strong luminescence and its inherent sensitivity, selectivity, and wide linear range in utility in different analytical areas [22–25, 27–40].

Silica-based materials, especially silica nanomaterials, show great promise for application in many research areas, such as bionanomaterials and bioassays because of their biocompatibility, chemical stability, and easy surface modification [41–43]. Dye-doped silica nanomaterials as functional materials have been intensively investigated and used in biosensors [10–12]. Spherical $Ru(bpy)_3^{2+}$-doped silica (RuSi) nanoparticles have been prepared and used for multiplexed signaling in bioanalysis [11]. Recently stable and sensitive ECL biosensors based on RuSi nanoparticles have been prepared via a water-in-oil microemulsion method [39,40]. Fang's group has realized ECL detection of DNA using RuSi nanoparticles [44]. Very recently,

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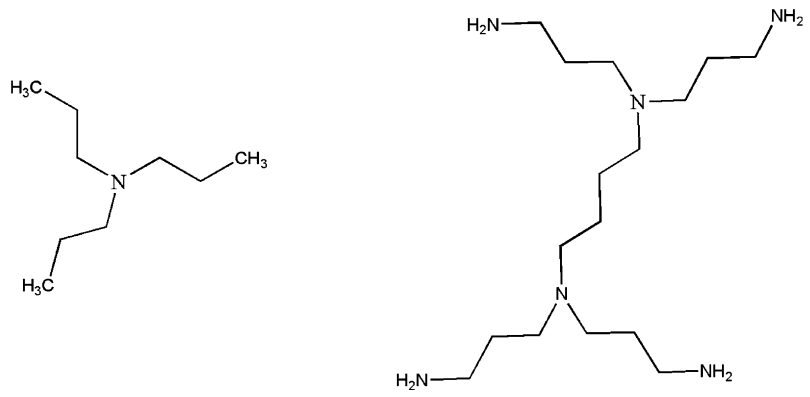

tripropylamine (TPA) **polypropylenimine tetraamine dendrimer (PPI)**

Fig. 1. Molecular structures of ECL coreactants used in this study.

we have studied the RuSi nanoparticles with layer-by-layer biomolecular surface modification [45].

In this work, we further extended the work to study the interactions between the RuSi nanoparticles and some biomacromolecules through covalent attachment. ECL from these biomacromolecule-coated RuSi nanoparticles decreased with the increase of the loading of biomacromolecules, which is similar to our previous results [45]. Moreover, the ECL of coreactants with different sizes was studied.

2. Materials and methods

2.1. Materials

Tris(2,2′-bipyridyl)ruthenium(II) chloride hexahydrate [Ru(bpy)$_3$Cl$_2$·6H$_2$O], tetraethyloxysilane (TEOS), γ-(aminopropyl) triethoxysilane (APTES), polypropylenimine tetraamine dendrimer (PPI) (Fig. 1), and polystyrene (M_w = 280,000) were obtained from Aldrich (WI, USA). Myoglobin was obtained from Sigma (Milwaukee, WI). Bovine serum albumin (BSA) was from Randox (Antrim, UK). Hemoglobin was from Xinjiang Institute of Chemistry (Urumqi, China). Glutaraldehyde (50% aqueous solution) was from Tianjin Tiantai Fine Chemicals Company (Tianjin, China). Tripropylamine (TPA) was obtained from Acros (Morris Plains, NJ) (Fig. 1). Other reagents and chemicals were at least analytical reagent grade. All aqueous solutions were prepared with water purified by a Milli-Q system (Millipore, Bedford, MA) and stored at 4 °C in a refrigerator.

2.2. Preparation of RuSi nanoparticle-modified electrode

The RuSi nanoparticles used for preparation of modified electrode were prepared according to the previous method [45]. The amounts 5.31 mL of Triton X-100, 22.5 mL of cyclohexane, 5.4 mL of *n*-hexanol, and 1020 μL of water were mixed together to form the water-in-oil microemulsion. Concentrated $Ru(bpy)_3^{2+}$ solution was then added into the microemulsion system to a final concentration of 1.2 mM. After addition of 300 μL of TEOS and 180 μL of NH_4OH, the hydrolysis reaction was allowed to continue for 24 h. Acetone was then added to destroy the emulsion and to isolate the deep orange-colored nanoparticles, followed by centrifuging and washing with ethanol and water and by ultrasonication, removing any surfactant molecules. Finally, the orange-colored RuSi nanoparticles were obtained and stored in a refrigerator (4 °C) until use. The size of this prepared RuSi nanoparticles was about 39 nm according to transmission electron microscopy.

The immobilization of RuSi nanoparticles on an ITO electrode was similar to the procedure adopted by Villemure's method [46]. The amount of 50 μL of a suspended solution of polystyrene in tetrahydrofuran (1 mg/mL) containing 1 mg of RuSi nanoparticles was spread on a 0.5 × 2.5 cm^2 piece of ITO-coated glass substrate, and the solvent was allowed to evaporate. The final film was left to be 0.5 × 1.5 cm^2.

2.3. Covalent binding of proteins to RuSi nanoparticles

For covalent binding of protein to silica particles, BSA, hemoglobin, and myoglobin were dissolved in 0.15 mol/L, pH 7.4, phosphate-buffered saline (PBS) with the concentration range from 10^{-9} to 10^{-3} mol/L. About 2 mg RuSi nanoparticles was added into 1.5-mL tube and treated with 0.5 mL of 10% APTES alcoholic solution, centrifuged, rinsed with PBS solution, reacted with glutaraldehyde (25% aqueous solution), centrifuged, and washed with PBS thoroughly. Different concentrations of BSA, hemoglobin, and myoglobin PBS were immediately added into the tubes for coupling, followed by centrifuging and washing of the RuSi nanoparticles with PBS, and finally dispersed into PBS.

2.4. Instrumentation

The electrochemical and ECL measurements were performed with an MPI-A ECL detector (Xi'an Remax Electron-