Electrochemiluminescence-based DNA Detection Using Guanine Oxidation at Electrostatic Self-assembly of $Ru(bpy)_3^{2+}$-doped Silica Nanoparticles on Indium Tin Oxide Electrode

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A $Ru(bpy)_3^{2+}$-doped silica nanoparticle- [Ru@Silica] modified indium tin oxide electrode was prepared by simple electrostatic self-assembly technique, and one-electron catalytic oxidation of guanine bases in double-strand and denatured DNA was realized using the electrochemiluminescence detection means.

DNA detection is of great scientific and technological importance to, for example, clinical tests and pathogen detection.[1] Electrochemistry has advantages of simplicity, sensitivity, selectivity, low cost for the detection of DNA hybridization and damage.[2] One of the most important approaches based on Thorp group's pioneer work[3] is the catalytic oxidation of guanine bases in DNA using tris(2,2′-bipyridine)ruthenium(II) [$Ru(bpy)_3^{2+}$] and its analogues. And much of research has been devoted to this field.[4]

By employing luminophore-active species as labels on DNA, electrochemiluminescence (ECL)[5] provides a sensitive method of detection.[6,7] And self-assembly provides an effective and versatile approach to construct ordered and well-defined architectures at a molecular level.[8,9] Nanometer-scale materials offer promise for application in many research areas, such as biomolecular materials and biomolecule detection.[10] Silica nanoparticles are extensively studied and widely used in bioassay owing to their excellent biocompatibility and in versatile methods for synthesis such as acid catalytic approach, base catalytic approach and the Stöber method.[11,12] In this work, 60-nm $Ru(bpy)_3^{2+}$-doped silica nanoparticles (Ru@Silica) were prepared following the Stöber method according to a previous literature (Figure 1).[12] Then, the Ru@Silica assembled with a poly(diallyldimethylammonium chloride) (PDDA) premodified indium tin oxide (ITO) electrode through electrostatic self-assembly interaction. And the one-electron oxidation of guanine bases in calf thymus DNA was realized using the ECL detection.

The ITO substrate used for modification was pretreated using a literature method to obtain a negatively charged surface.[13] Then, the negatively charged ITO electrode was treated by a 2.5% aqueous PDDA solution for 30 min to form positive surface. After the PDDA electrostatic assembly, the ITO electrode was washed with distilled water and dried in a nitrogen stream. Finally, the negatively charged Ru@Silica nanoparticles (2 mg/mL dispersed in 100 mM phosphate buffer solution, pH 7.4) was immobilized on the electrode through electrostatic interaction (Figure 1B).

The modified ITO electrode was washed with copious water and dried. The size of Ru@Silica nanoparticles immobilized on the ITO substrate was characterized using an XL30 ESEM FEG scanning electron microscope at an accelerating voltage of 25 kV (Figure 2). The result indicates that the nanoparticles dispersed well on the electrode have an average diameter of 60 nm. For further characterization of the modified ITO electrode, the UV–vis absorption spectroscopy was employed (see Supporting Information). The spectrum exhibits a broad absorption band in the visible region (ca. 450 nm) due to spin-allowed $d\pi(Ru)$–$\pi(ligand)^*$ metal-to-ligand charge-transfer (MLCT) transitions.[14]

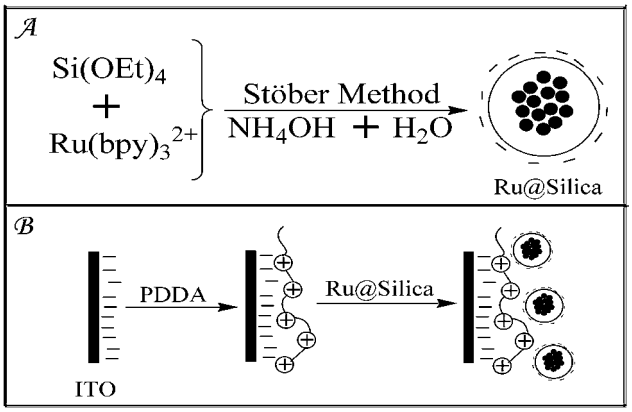

Figure 1. Scheme illustrating (A) the preparation of $Ru(bpy)_3^{2+}$-doped nano-silica (Ru@Silica) and (B) the immobilization of Ru@Silica on PDDA-modified ITO electrode surface.

For electrochemical experiment, a CHI 832 workstation (CH Instruments Co., Austin, TX) was employed using a three-electrode system (the modified ITO electrode with an area

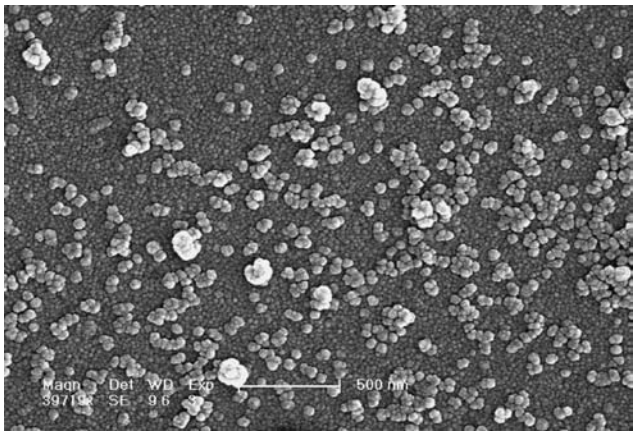

Figure 2. SEM image of Ru@Silica nanoparticles with an average diameter of 60 nm immobilized on the ITO electrode.

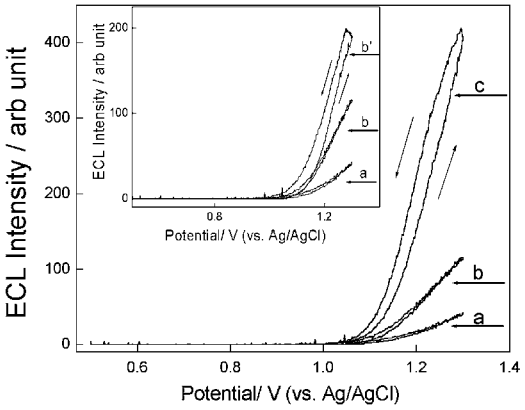

Figure 3. ECL intensity–potential curve of (a) buffer, (b) 1.26 μg/mL DNA and (c) 12.6 μg/mL DNA using CV scanning from 0.5 to 1.3 V (scanning rate = 50 mV/s). Insert is the ECL intensity–potential curve of (a) buffer, (b) 1.26 μg/mL DNA and (b′) 1.26 μg/mL DNA (denatured) using CV scanning from 0.5 to 1.3 V (scanning rate = 50 mV/s).

about 0.64 cm^2 as the working electrode, a KCl-saturated Ag/AgCl electrode as the reference electrode and a platinum wire as the counter electrode). ECL signals were collected using a MCFL-A multifunctional chemiluminescent and bioluminescent analytical system (Xi'an Remax Electronic Science-Tech Co., Ltd, Xi'an, China) with the biased voltage of the photomultiplier tube set at 800 V. Concentrations of DNA in phosphate buffer were measured assuming 1 $OD_{\lambda=260\,nm} = 33\,\mu g$ DNA. For DNA catalytic oxidation detection, the modified ITO electrode was dipped into DNA phosphate buffer solution for 30 min just before each measurement. Different concentrations of double-strand calf thymus DNA at 1.26 and 12.6 μg/mL for the catalytic oxidation were investigated. Figure 3 shows the ECL intensity–potential curve using cyclic voltammetry (CV) scanning from 0.5 to 1.3 V (vs. Ag/AgCl) at the scanning rate of 50 mV/s. As shown in Figure 3, the onset of luminescence occurred about 1.0–1.1 V, at which $Ru(bpy)_3^{2+}$ was oxidized to $Ru(bpy)_3^{3+}$, activating the catalytic cycle of the ruthenium–guanine system. For a higher concentration (12.6 μg/mL), the ECL signal arose more sharply. Compared to the immobilization method using polymer ultrathin films,[4] even low DNA concentration (1.26 μg/mL) in our experiment gave a significant ECL signal response (Figure 3).

For the further study, thermally denatured calf thymus DNA was employed. And the denaturation was accomplished by heating a native double-strand DNA in a boiling water bath for about 5 min and then cooling in an ice bath. Compared to double-strand calf thymus DNA, denatured calf thymus DNA gave a significant ECL signal increase (about twice the ECL signal, Figure 3 insert).

The calibration curve of native DNA was linear from 0.63 to 12.6 μg/mL with a detection limit of 0.252 μg/mL while the calibration curve of denatured DNA was linear from 0.63 to 12.6 μg/mL with a detection limit of 0.126 μg/mL (see Supporting Information).

On the basis of previous proposals for the mechanism of the one-electron catalytic oxidation of guanine bases using $Ru(bpy)_3^{2+}$, here a proper reaction pathway in this work could be presented as in Scheme 1.[3,4]

$$Ru^{2+}@Silica \longleftrightarrow Ru^{3+}@Silica + e^- \quad (1)$$

$$Ru^{3+}@Silica + G \longrightarrow Ru^{2+}@Silica + G^{\bullet} \quad (2)$$

$$G^{\bullet} + Ru^{3+}@Silica \longrightarrow G_{2ox} + Ru^{2+*}@Silica \quad (3)$$

$$Ru^{2+*}@Silica \longrightarrow Ru^{2+}@Silica + h\nu \quad (4)$$

Scheme 1.

Initial oxidation by electron transfer from Ru^{2+}@Silica to the electrode at sufficiently positive potentials (i.e., potentials exceeding 1.0 V vs. AgCl/Ag) gives the Ru^{3+}@Silica oxidant (eq 1), which reacts with guanine bases in calf thymus DNA to give guanine radical $G^{\bullet}$ (eq 2). The radical may reduce Ru^{3+}@Silica directly to produce Ru^{2+*}@Silica (eq 3) and the ECL signal is obtained when the excited state complex Ru^{2+*}@Silica decayed back to the ground state (eq 4).

In conclusion, a PDDA/Ru@Silica-modified ITO electrode was prepared, and the catalytic oxidation of guanine bases in double-strand and denatured calf thymus DNA was realized on the electrode using an ECL detection means.

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15 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

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