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Label free electrochemiluminescence protocol for sensitive DNA detection with a *tris*(2,2′-bipyridyl)ruthenium(II) modified electrode based on nucleic acid oxidation

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Abstract

Label free electrochemiluminescence (ECL) DNA detection based on catalytic guanine and adenine bases oxidation using *tris*(2,2′-bipyridyl)ruthenium(II) [$Ru(bpy)_3^{2+}$] modified glassy carbon (GC) electrode was demonstrated in this work. The modified GC electrode was prepared by casting carbon nanotubes (CNT)/Nafion/$Ru(bpy)_3^{2+}$ composite film on the electrode surface. ECL signals of double-stranded DNA and their thermally denatured counterparts can be distinctly discriminated using cyclic voltammetry (CV) with a low concentration (3.04×10^{-8} mol/L for Salmon Testes-DNA). Most importantly, sensitive single-base mismatch detection of p53 gene sequence segment was realized with 3.93×10^{-10} mol/L employing CV stimulation (ECL signal of C/A mismatched DNA oligonucleotides was 1.5-fold higher than that of fully base-paired DNA oligonucleotides). Label free, high sensitivity and simplicity for single-base mismatch discrimination were the main advantages of the present ECL technique for DNA detection over the traditional DNA sensors.
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Keywords: Electrochemiluminescence; DNA sensors; Label free; Single base mismatch

1. Introduction

DNA detection is of great importance to, for example, DNA sequencing, profiling, clinical test and mutated genes diagnosis associated with human diseases [1–3]. In order to meet all these objectives, a number of analysis methods have been developed over past decades, including classical fluorescence techniques [4], mass spectrum [5], electrochemical means [3,6,7] and recently emerged DNA sensors based on nanotechnology [8,9].

Among all these methods, electrochemical protocols can provide quite significant advantages such as simplicity, sensitivity, selectivity and low cost for the detection of DNA hybridization and damage. Since Paleček's pioneer work about half a century ago [10], a lot of efforts have been devoted to the research of electrochemical DNA biosensors [11,12]. One of the most interesting electrochemical approaches for DNA detection based on Thorp group's pioneer work [13] is the catalytic oxidation of guanine bases using *tris*(2,2′-bipyridyl)ruthenium (II) [$Ru(bpy)_3^{2+}$] and its analogues [14].

Recently, considerable advances have been made in DNA biosensors using $Ru(bpy)_3^{2+}$ electrochemiluminescence (ECL) [15] detection protocol, owing to its inherent sensitivity, selectivity, and wide linear range in the utility in different analytical areas such as clinical tests and biomolecules detection [16–20].

Rusling et al. developed direct ECL detection of DNA in poly(vinylpyridine)[PVP] ultra-thin film using cationic polymer $[Ru(bpy)_2(PVP)_{10}]^{2+}$ or $[Os(bpy)_2(PVP)_{10}]^{2+}$, and

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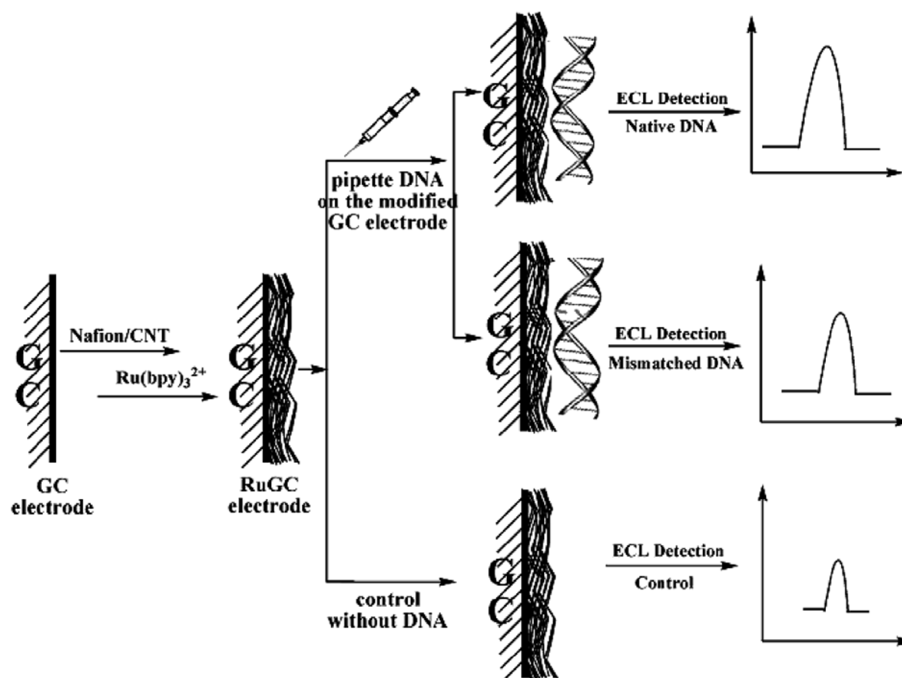

Fig. 1. Schematic diagram of GC electrode modification and DNA ECL detection procedures.

also chemically induced damage by styrene oxide of DNA [21,22]. Single-base mismatch detection, however, has not been reported through catalytic oxidation of DNA with an ECL detection on a modified electrode [23,24]. Landers' group recently reported an interesting quenching ECL method for quantitive and sequence-specific DNA detection [25]. By employing luminophore-active species as labels on biomolecules such as DNA and proteins, ECL opens a rapid, selective, and sensitive avenue for bioassay and detection. Bard's group employed $Ru(bpy)_3^{2+}$ as ECL label combined with tri-*n*-propylamine [TPrA] as a coreactant to determine DNA [19].

Here we presented a label free ECL protocol for DNA detection using carbon nanotubes (CNT)/Nafion/ $Ru(bpy)_3^{2+}$ composite film modified glassy carbon electrode (abbreviated as RuGC) (Fig. 1). ECL signals can distinctly discriminate double-stranded Salmon Testes-DNA and their thermally denatured counterparts using cyclic voltammetry (CV) with a low concentration (3.04×10^{-8} mol/L). Moreover, sensitive single-base mismatch detection of p53 gene sequence segment was realized with 3.93×10^{-10} mol/L employing CV stimulation (ECL signal of C/A mismatched DNA oligonucleotides was 1.5-fold higher than that of fully base-paired DNA oligonucleotides).

2. Experimental

2.1. Chemicals and materials

Tris(2,2′-bipyridyl)ruthenium(II) chloride hexahydrate and Nafion (perfluorinated ion-exchange powder, 5 wt% solution in a mixture of lower aliphatic alcohols and water) were obtained from Aldrich (Milwaukee, WI, USA). The multi-wall carbon nanotubes (CNT) were purchased from Shenzhen Nanotech. Port. Co. Ltd. (Shenzhen, China) and were purified according to a literature method [26]. 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, USA) and stored at 4 °C in a refrigerator.

Salmon Testes (ST) double-stranded (ds) DNA were purchased from Sigma. Poly(guanadylic acid) (5′) poly[G], poly(adenylic acid) (5′) poly[A] and oligonucleotides containing specific sequences (see Table 1) were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China).

The concentrations of oligonucleotides were determined using the 260 nm UV absorbance and the corresponding extinction coefficient. The extinction coefficient of Salmon Testes DNA was taken as 6600 M^{-1} cm^{-1}. The extinction coefficients of single strands were calculated by the sum of the extinction coefficients of the individual bases: $\varepsilon(dA) =$ 15,400 M^{-1} cm^{-1}, $\varepsilon(dG) = 11{,}500$ M^{-1} cm^{-1}, $\varepsilon(dC) =$ 7400 M^{-1} cm^{-1}, $\varepsilon(dT) = 8700)$ M^{-1} cm^{-1}.

Table 1
Oligonucleotides synthesized in this experiment

Sequence 1 (**Seq 1**)	5′ GCA GGG GC***C*** GCC GGT 3′ (primary sequence)
Sequence 2 (**Seq 2**)	5′ ACC GGC ***G***GC CCC TGC 3′ (complete complementary sequence)
Sequence 3 (**Seq 3**)	5′ ACC GGC ***A***GC CCC TGC 3′ (single-base mismatch complementary sequence)

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