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Short communication

A new strategy for photoelectrochemical DNA biosensor using chemiluminescence reaction as light source

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ABSTRACT

Physical light source is absolutely necessary for usual photoelectrochemical measurement. In this work, chemiluminescence reaction rather than physical light source was used for the development of a novel photoelectrochemical DNA biosensor. CIPO (bis(2,4,5-trichlro-6-*n*-pentoxycarbonylphenyl)oxalate)–H_2O_2–9,10-diphenylanthrancene was selected as a CL system, which can produce appropriate exciting light and excite photoelectro active materials $Ru(bpy)_2dppz^{2+}$ intercalated into the double-stranded DNA. Using such simple intercalation method, a detection limit of 4.5×10^{-9} M target DNA was achieved without any amplification process. In addition, the selected CL system could be used to excite AuNPs–$Ru(bpy)_2dppz^{2+}$ complex as well as CdSe QD multilayer, which indicated a good applicability for the established method.

1. Introduction

Optical and electrical properties are important phenomena around us. These phenomena alone, as well as their combination have now been widely used in the field of biosensors, for example, fluorescence (Peng et al., 2010; Zhang et al., 2010), electrochemistry (Xiao et al., 2006; Zhang et al., 2008; Hu et al., 2008; Patolsky et al., 2002), electrogenerated chemiluminescence (ECL) (Yin et al., 2009; Hu et al., 2009) and photoelectrochemistry (Ran et al., 2008; Gill et al., 2005; Haddour et al., 2006). Among them, photoelectrochemical biosensors are very promising. Firstly, due to the different forms of energy for excitation (light) and detection (current), this method is very sensitive with low background signals. Secondly, compared to optical detection methods such as fluorescence, chemiluminescence (CL) and ECL which use complex and expensive optical imaging devices, the instrument of photoelectrochemstry is much simpler and low cost (Liang et al., 2008; Liang and Guo, 2007).

The photoelectrochemical method was widely used in the detection of biological affinity reaction (Dong et al., 2004), DNA (Liu et al., 2006; Gao and Tansil, 2005; Willner et al., 2001), DNA damage (Liang et al., 2008; Liang and Guo, 2007), enzyme (Yildiz et al., 2008), antigen (Haddour et al., 2006; Wang et al., 2009) and small molecule (Cooper et al., 1998; Pardo-Yissar et al., 2003). Photoelectrochemically active species usually used are rutheniumbipyridine derivatives (Liang et al., 2006, 2008; Wang et al., 2008), CdSe/CdS nanoparticles (Willner et al., 2001; Sheeney-Haj-Ichia et al., 2005; Hojeij et al., 2008) and dyes (Cooper et al., 1998; Okamoto et al., 2004; Yamada et al., 2008). Strategies for the photoelectrochemical analysis can be classified into two categories: label-free (Haddour et al., 2006; Wang et al., 2009) and labeled methods (Willner et al., 2001). All these methods embody the merits of relatively high sensitivity, simplicity and low instrument cost.

In all these work, in order to produce photocurrents, photoexcitation of light source is required. However, the appendant light source makes the instrument complicated. In addition, the exciting wavelengths of various photoelectro active materials are different. Hence, monochromator is needed to bring appropriate exciting light, which makes the volume of the instrument bigger and departures from the portable trend for biosensor. Consequently, a strategy for substitution of physical light source is highly deserved. Chemiluminescence is defined as a process in which excited molecules or atoms generated from chemical reactions release the excess of energy in light form (Pinheiro et al., 1999). Different CL systems can bring emission light of various wavelengths. At the same time, reaction conditions such as type of fluorescence reagent, reaction solution can also affect the emission wavelength (Barni et al., 2007). Thus, by adjusting the conditions of CL reaction, various photoelectrochemically active species can be excited theoretically, which can realize the photoelectrochemical detection free from physical light source.

Herein, we utilized chemiluminescence reaction instead of light source establishing CL-photoelectrochemstry (CLE) method for the first time. CIPO–H_2O_2–9,10-diphenylanthrancene was used as a CL system to generate appropriate exciting light, which could excite photoelectro active materials $Ru(bpy)_2dppz^{2+}$ and engender pho-

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tocurrent. We reasoned that this strategy processed the inherited advantages of photoelectrochemstry, while it obviated the use of physical light source so that a simple electrochemical analyzer could work. The practicability of this method was investigated in DNA sensor using $Ru(bpy)_2dppz^{2+}$ as an intercalator.

2. Experiment performance

2.1. Materials and apparatus

2.1.1. Materials

All the reagents were analytical grade and used without further purification. Doubly distilled water (DDW) was used through out this work. 3-Aminopropyltriethoxysilane (APS), glutaraldehyde, poly(dimethyldiallylammonium chloride (PDDA), 3-mercaptopropyl- triethoxysilane (MPTES), mercaptoundecanoic acid (MUA), mercaptoacetic acid (MAA) were purchased from Acros. Fifteen percent tin (IV) oxide, as a colloidal dispersion of 15-nm particles in water, was obtained from Alfa Aesar. Synthesis of $Ru(bpy)_2(dppz)^{2+}$ was described in Supplementary material. The oligonucleotides used in this study were purchased from SBS Genetech Co., Ltd. (China) and sequences of all oligonucleotides are listed as follows. Capture DNA: 5′-H_2N-AGG AGT TGG ATG GCG GCG GAG GCA GAT-3′; target DNA: 5′-ATC TGC CTC CGC CGC CAT CCA ACT-3′; two-base mismatched sequences: 5′-ATC TGC CGC CGC CGC CAT TCA ACT-3′.

2.1.2. Apparatus

Photocurrent was measured on a CHI832B electrochemical analyzer (Shanghai CH Instrument Company, China). A three-electrode system was employed with Ag/AgCl as reference electrode, platinum wire as auxiliary electrode and ITO conductive glass supplied by Weiguang Corp. (Shenzhen, People's Republic of China, ITO coating 180 ± 25 nm, sheet resistance $\leq 10\,\Omega$/square) as working electrode. CL spectra were measured on a model FL 4500 spectrofluorometer (HITACHI) with the excitation light source being turned off.

2.2. Assembly processes on electrode

The SnO_2 modified ITO electrode (see Supplementary material for preparation process) was silanized in an ethanol solvent containing 1 mM APS through sonication for 2 h at room temperature. After that, the samples were rinsed with DDW, dried under N_2 atmosphere and then cured at 110 °C for 15 min in an oven.

DNA probes were assembled on electrode using cross-linking reagent glutaraldehyde. Firstly, silanized ITO electrode was immersed into a tube containing 5 mL of 5% glutaraldehyde solution for 2 h in a water bath at 37 °C. Secondly, the modified electrode was immersed in 0.1 M PBS (pH 7.4) containing 2.0×10^{-6} M 5′-amino group capped capture DNA for 2 h in a water bath at 37 °C. Then, the capture DNA-modified ITO electrodes were incubated with appropriate concentrations target DNA solutions in 0.1 M PBS buffer for 1 h at 37 °C. Finally, the assembled electrode was immersed into 0.1 M PBS solution (pH 7.4) containing 5.0×10^{-5} mol/L $Ru(bpy)_2dppz^{2+}$ for 45 min. The electrode surface was washed three times with 0.1 M PBS (pH 7.4) after each step of fabrication process in order to remove nonspecifically adsorption.

2.3. Detection process

600 μL sodium oxalate solution was added into a 10 mL beaker containing 3.4 mL of 0.1 M PBS (pH 5.8). A Three-electrode system was introduced. The small beaker was then placed inside another 25 mL bigger one, which contained 1.7 g CIPO, 0.8 g 9,10-diphenylanthrancene, 0.1 g sodium salicylate, 1.5 mL *tert*-butyl alcohol and 7.5 mL diethyl phthalate. The whole device was placed into a lightproof container. Then, the electrochemical analyzer began to record. After 40 s, the CL reaction was initiated by injecting H_2O_2 (5 mL, 50% in *tert*-butyl alcohol) into the bigger beaker with a syringe. The experiment was carried out at 25 °C. Potential bias was optimized for bigger photocurrents, and a bias voltage of +0.3 V was selected according to the result of Fig. S1 (see supplementary material).

2.4. Preparation of Ru–AuNPs modified ITO electrode

Ru–AuNPs modified planar gold electrode was prepared according to the literature (Sun et al., 2005). Briefly, 300 μL of 0.05 M $Ru(bpy)_2dppz^{2+}$ aqueous solution was added into 20 mL of AuNPs (diameter of 13 nm) solution under vigorous stirring at room temperature. Several minutes later, a large amount of black precipitate was formed. Keeping still for 1 h, the resulting precipitate was collected by centrifugation, washed several times with water, and then suspended in 3 mL of water. At the same time, SnO_2 modified ITO electrode was immersed in 2 mM MPTES ethanol solution for 1 day. The so prepared electrode was air-dried at room temperature. 10 μL of the suspension of Ru–AuNPs obtained above was then dipped on the sulfhydryl-derivated ITO electrode surface. The prepared electrode was air-dried at room temperature in the dark.

2.5. Preparation of CdSe QDs multilayer film modified planar gold electrode

The CdSe QDs multilayer film was prepared according to the literature (Wang et al., 2009). Briefly, the gold layer was initially modified with a monolayer of MUA by immersion into an ethanolic solution of 1 mM MUA for 12 h. Loosely bound MUA molecules were removed from the surface by rinsing successively with ethanol and water before drying the electrode under a nitrogen stream. The CdSe multilayer film was grown by alternately dipping of modified planar gold electrode into a solution of 2% PDDA containing 0.5 M NaCl and the MAA-stabilized CdSe QDs solution (Rogach et al., 2000) for 10 min, respectively. Between each dip, the surface was rinsed with DDW and blown dry under a nitrogen stream. This process was repeated five times to obtain $(PDDA/CdSe)_5$ multilayer film assembled planar gold electrode.

3. Results and discussion

3.1. The equipment and fabrication of the biosensor

The equipment for our CLE method was very simple (see Fig. 1A). A samll beaker was placed into a bigger one. A three-electrode system was placed into the former, while CL reactants were placed into the latter. After injection of peroxide into the bigger beaker, CL reaction was initiated. Subsequently, the emitted light excited the photoelectro active materials immobilized on the working electrode. The so produced current was measured by CHI 832 electrochemical analyzer.

The process for the fabrication of the DNA biosensor was shown in Fig. 1B. The attachment involved the formation of a monolayer of APS on SnO_2 nanoparticles modified ITO electrode. The surface was modified further with a cross-linker, glutaraldehyde, by means of a Schiff base formation. The remaining aldehyde group could be used to covalent immobilization of amine-capped capture DNA. After hybridization with target DNA and intercalation with $Ru(bpy)_2dppz^{2+}$, the target DNA could be quantitatively detected when the photoelectrochemically active species rutheniumbipyridine derivatives were excited by CL reaction.

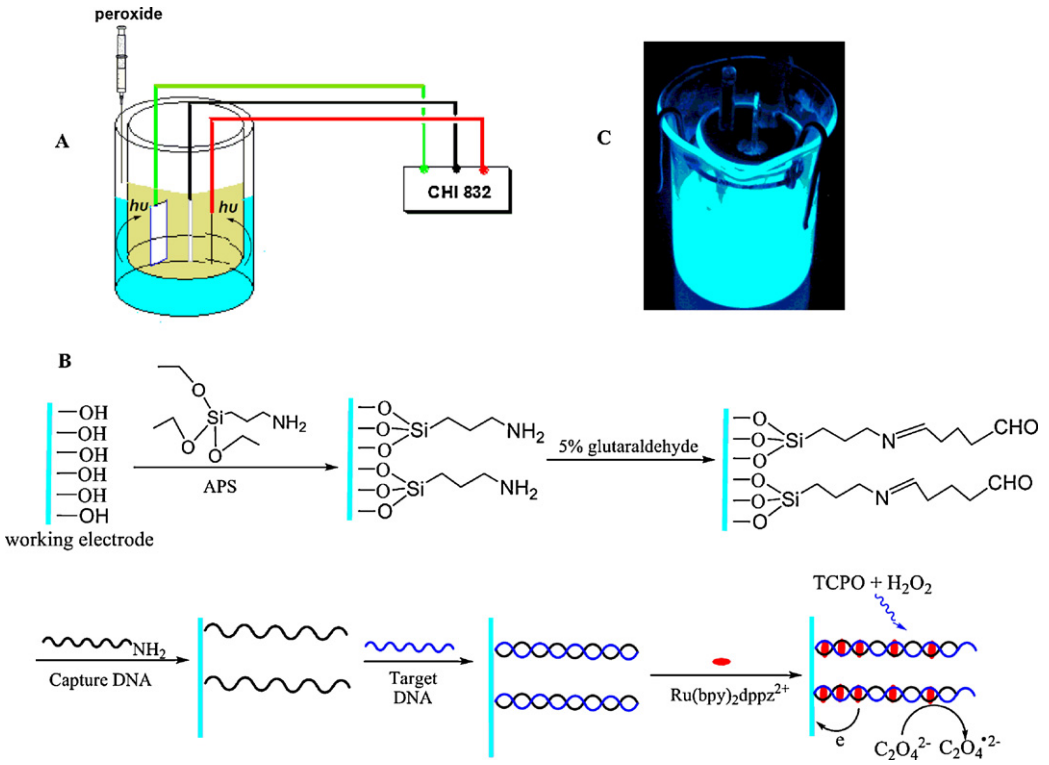

Fig. 1. Schematic diagram for (A) the equipment of CLE method; (B) the biosensor fabrication; (C) CL image of CIPO–9,10-diphenylanthrancene–H_2O_2 in our experiment.

3.2. Luminescence emission spectrum of CIPO–9,10-diphenylanthrancene–H_2O_2 CL system

The photocurrent response of $Ru(bpy)_2dppz^{2+}$ as a function of excitation wavelength was studied by Liu et al. (2006). The results showed that in a solution of $Ru(bpy)_2dppz^{2+}$, the photocurrents had good responses in the range of 400–500 nm, and reached their peak at 470 nm. For comparison, the emitting light of CIPO–H_2O_2–9,10-diphenylanthrancene CL system was measured in this work. As shown in Fig. S2, the produced CL emission wavelength was around 440 nm, which indicated that the selected CL system could be used to excite $Ru(bpy)_2dppz^{2+}$. CIPO–9,10-diphenylanthrancene–H_2O_2 system could emit bright blue light in a dark room. The CL image was shown in Fig. 1C. Because the intensity of CL is important for the accuracy of the established biosensor, the reaction conditions such as concentration, solvent and temperature should be controlled strictly to insure the same light intensity of the CL system in each experiment.

3.3. Sensitivity and selectivity of the DNA biosensor

The sensitivity of the developed biosensor was investigated by varying the concentrations of target DNA. With the increasing concentrations of target DNA, the amount of intercalator $Ru(bpy)_2dppz^{2+}$ was increased accordingly. Thus, the bigger photocurrent was obtained. As shown in Fig. 2A, the peak current had a good linear relation versus the concentration of target DNA in the range of 2.0×10^{-8} to 4.0×10^{-7} M, with a correlation coefficient of 0.9976. The regression equation was $y = -2.4366 - 2.1530x$ ($r = -0.9976$). A detection limit of 4.5×10^{-9} M of the target DNA

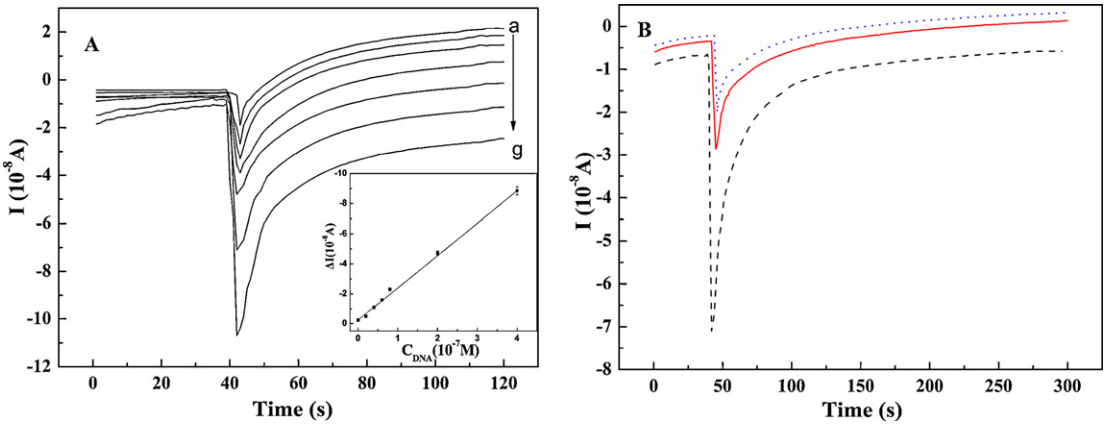

Fig. 2. Sensitivity and selectivity. (A) Photocurrent responses for increasing levels (a–g) of the target DNA (a) 0, (b) 2.0, (c) 4.0, (d) 6.0, (e) 8.0, (f) 20, and (g) 40 ($\times 10^{-8}$ M); inset is the calibration plot of peak current versus target DNA concentration in the range of 2.0×10^{-8} up to 4.0×10^{-7} M in CLE assay. (B) Comparison of photocurrent when capture DNA hybridized with 2.0×10^{-7} M noncomplementary sequences (dot line), two-base mismatched sequences (solid line) and complementary sequences (dash line).

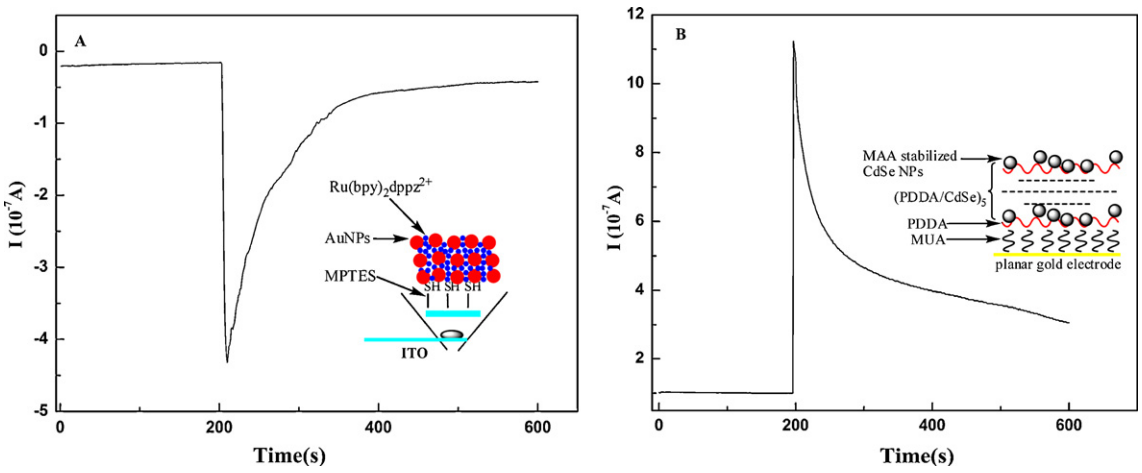

Fig. 3. The applicability of selected CL system. (A) Photocurrent responses of Ru–AuNPs modified ITO electrode excited by ClPO–H_2O_2–9,10-diphenylanthrancene CL system. 30 mM sodium oxalate was used as electron donor and potential bias is +0.3 V. Inset is the representation of Ru–AuNPs modified ITO electrode. (B) Photocurrent responses of $(PDDA/CdSe)_5$ multilayer modified planar gold electrode excited by ClPO–H_2O_2–9,10-diphenylanthrancene CL system. 0.1 M ascorbic acid was used as electron donor and potential bias is −0.09 V. Inset is the representation of $(PDDA/CdSe)_5$ multilayer modified planar gold electrode.

could be estimated using 3σ (where σ was the standard deviation of the blank solution, $n = 7$). Although the sensitivity was not very high in this experiment, the biosensor was aimed at testing the feasibility of the developed CLE method. After further amplification process, such as making use of Au nanoparticles, the sensitivity could be enhanced accordingly.

The selectivity of the present biosensor in discriminating completely complementary target DNA from two-base mismatched and noncomplementary sequences was investigated under the concentration of 2.0×10^{-7} M target DNA. As shown in Fig. 2B, a well-defined photocurrent was obtained for the complementary sequences. The signal intensity of two-base mismatched sequences was much weaker than that of the complementary target sequences, and the noncomplementary sequences showed even lower response. The results showed that a good selectivity with this CLE DNA biosensor was achieved.

3.4. The applicability of selected CL system

Two kinds of other photoelectrochemistry systems were further investigated for verifying the applicability of our CLE method. Insert of Fig. 3A was the complex of $Ru(bpy)_2dppz^{2+}$ and AuNPs assemmbled on MPTES modified ITO electrode and its CLE response was shown in Fig. 3A. Insert of Fig. 3B was the layer-by-layer assembling of CdSe QDs and PDDA multilayer film on planar gold electrode. Fig. 3B was the CLE response of $(PDDA/CdSe)_5$ multilayers modified planar gold electrode. The results showed that $Ru(bpy)_2dppz^{2+}$–AuNPs complex and $(PDDA/CdSe)_5$ multilayers could also be excited by CL of ClPO–H_2O_2–9,10-diphenylanthrancene and generated preferable photocurrent signals. Therefore, such electrodes could be used in label-free CLE detection. Preliminary exploration on the label-free CLE detection of thrombin using $(PDDA/CdSe)_5$ multilayers modified planar gold electrode was showed in Supplementary material.

4. Conclusions

In summary, a new method combing CL with photoelectrochemistry was developed here. In this way, a simple electrochemical analyzer could realize the photoelectrochemical detection without the usage of physical light source involved in conventional photoelectrochemistry assays. This strategy has been successfully employed in DNA detection and can possibly find general use in other bio-active molecules. Furthermore, the selected CL system can also be used to excied Ru–AuNPs complex as well as $(PDDA/CdSe)_5$ multilayers and produce preferable photocurrent. Although the sensitivity in this experiment is not high enough, the proposed method opens the window for light source free photoelectrochemistry detection. Further design aimed at improving the sensitivity is currently under investigation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.09.051.

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