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Electrogenerated chemiluminescence energy transfer and its application in label-free sensing long DNA

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ABSTRACT

An easily operated label-free DNA sensor for sensing long DNA molecule based on electrogenerated chemiluminescence (ECL) energy transfer from luminol to $[Ru(bpy)_3]^{2+}$ was proposed in this work. Excited luminol was used as energy donor and $[Ru(bpy)_3]^{2+}$ combined with DNA as energy acceptor. ECL reaction of luminol was triggered by step pulse signal and excited luminol molecules transferred their energy to $[Ru(bpy)_3]^{2+}$ molecules. Excited $[Ru(bpy)_3]^{2+}$ molecules released energy in photon form in the process of going back to the ground state. Compared with single-stranded DNA (ssDNA), double-stranded DNA (dsDNA) binds more $[Ru(bpy)_3]^{2+}$ molecules mainly be electrostatic attraction, which results in stronger ECL intensity from $[Ru(bpy)_3]^{2+}$–dsDNA. Most important, the proposed method can detect DNA with length as long as the thickness of diffusion layer theoretically. In this work, 15 bps DNA was used as a model to investigate the ECL energy transfer behaviors and the analytical performance of DNA detection. Under optimal conditions, the label-free DNA ECL biosensor showed a good linear range over 1.0×10^{-14} mol/L to 1.0×10^{-10} mol/L with detection limit of 2.0×10^{-15} mol/L for 15 bps DNA. What is more, this proposed method could distinguish ssDNA and dsDNA with 35 bps and 100 bps, which exceeded the upper limit in fluorescence resonance energy transfer and electrochemical methods.

1. Introduction

In recent years, the detection of specific DNA sequences has become more important due to its widely applications in clinical diagnostics, early screening of human genetic diseases, biological warfare agents, and forensic testing [1–5]. Consequently, numerous analytical methods such as fluorescence [6,7], chemiluminescence [8–10], surface plasmon resonance [11,12], electrochemical techniques [13–15], and electrogenerated chemiluminescence (ECL) [16–21] have been developed for the detection of DNA sequences. Unfortunately, those methods are usually fit for DNA with less than ~30 base pairs. For example, in fluorescence resonance energy transfer (FRET), dye molecules act as the energy donor and acceptor respectively [22,23]. For the high donor quantum yield, the distance between donors and acceptors (R_0) is generally in the 1–10 nm [24,25]. Because of the upper limit on R_0, it is difficult to use FRET in DNA detection when the donors and acceptors may be more than 10 nm apart, which is about 30 base pairs.

In addition, electrochemical methods are particularly attractive for DNA detection due to their unique advantages of high sensitivity, low cost, good selectivity, rapid response, and easy miniaturization [26–29]. Of the current, the electrochemical biosensors based on label and intercalator were developed to detect DNA [30–32]. Heeger and co-worker [33] reported electrochemical-DNA hairpin sensor by using methylene blue or ferrocene as labels. When there was no target DNA, the probe DNA resulted in a hairpin configuration where electron transfer was unhindered between the electroactive species and electrode. Upon hybridization, the electroactive species were moved farther away from electrode surface, which made signal decrease. Although this sensor exhibited high selectivity and sensitivity, some inherent issues still cannot be avoided, such as high cost, low yield, time-consuming, labor-intensive. Thus, the fabrication of a label-free, sensitive, simple, and low cost biosensor system has become highly focusing. Millan and Mikkelsen [34] developed a prototype sensor to electrochemically discriminate hybridized double-stranded (ds) DNA from single-stranded (ss) by using redox-active hybridization indicators. The presence of the immobilized dsDNA caused preconcentration of $Co(bpy)_3^{3+}$ in the DNA layer at the electrode surface, and it resulted in much larger voltammetric peak currents than immobilized ssDNA. Ju and co-workers [35] designed a

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one-target-multitriggered hybridization chain reaction (MHCR) strategy using $[Ru(NH_3)_6]^{3+}$ as an electrochemically active indicator to interact with the MHCR product for electrochemical detection of DNA. While these intercalator-based DNA sensors take advantage of design simplicity and operation convenience, they often suffer from high background signals that are associated with nonspecific binding of intercalators to unhybridized ssDNA. These methods lie in the alteration of the efficiency of electron transfer between the electrode and the redox species based on the structure changes of DNA before and after hybridization, so electron transfer distance plays an important role in these biosensors. Therefore, electrochemical DNA sensor in generally can only be used for the detection of short DNA due to the limit to the distance of electron transfer. The response mechanisms of direct ECL sensors based on label and intercalator are similar to electrochemical DNA sensor, except for detection signal, so they are also limited by DNA strand length.

However, normal tissue cells death through apoptosis can release uniform DNA fragment within 185–300 bps. Tumor necrosis results in the release of abnormal DNA segment, which produces different length DNA fragments in a certain range due to free and incomplete digestion of DNA, and the DNA strands related to diseases existing in the organisms are usually lengthy DNA. Therefore, it is urgently needed to develop a simple and sensitive method for the detection of lengthy DNA.

In order to increase the distance between the donors and acceptors for FRET, researchers added a third chromophore as a bridge of energy transfer to extend distance between the donors and receptors for amplifying energy transfer [36]. For instance, Elke Haustein et al. [37] designed two coupled FRET pairs by utilizing three different dyes including Rhodamine Green, TAMRA, and Cy5 labeled on the different sites of the DNA, TAMRA as donor/bridging dye, which transferred the energy to the Cy5. With this method, the distance between the donors and acceptors can be expanded by about 50%. Following this work, Medintz et al. [38] developed a two-step FRET maltose sensors, QDs were coupled with Cy3-labeled *Escherichia coli* maltose binding protein (MBP) following binding with β-cyclodextrin-Cy3.5, Cy3 acted as a donor/bridging, which maximally transferred the energy to β-cyclodextrin-Cy3.5. In this case, this designed sensor overcome inherent QD donor–acceptor distance limitation. Lakowicz et al. [39] found that the effects of nanostructured silver island films on resonance energy transfer (RET) between the donor 4,6-diamidino-2-phenylindole (DAPI) and the acceptor propidium iodide (PI), resulted in the RET distance from 3.5 nm to 16.6 nm. Although these methods can extend energy transfer distance of donors and acceptors, but they need complicated labeling. In addition, the silver nanoparticles (AgNPs) for in vivo study also have its drawbacks, because the fluorescent protein labeled with AgNPs is difficult to be introduced into cells, and they also have toxicity to cells. More importantly, these methods for extending the distance between donors and acceptors are limited.

Herein, we reported a label-free DNA sensor for sensing lengthy DNA molecules based on ECL energy transfer from luminol to $[Ru(bpy)_3]^{2+}$. Luminophor luminol were excited by electrochemically oxidized at the electrode surface and then excited luminol acted as energy donor. $[Ru(bpy)_3]^{2+}$ combined with DNA acted as indictor and energy acceptor. Excited luminol molecules transfer their energy to $[Ru(bpy)_3]^{2+}$ combined with DNA and make the $[Ru(bpy)_3]^{2+}$ be in their excited state. Then excited indicator $[Ru(bpy)_3]^{2+}$ released energy in photon form in the process of going back to the ground state. Because $Ru(bpy)_3{}^{2+}$ bound electrostatically in the minor groove of dsDNA [16,34,40], the microenvironment and amount of hybridization indictor $[Ru(bpy)_3]^{2+}$ associated with dsDNA was different from that bound to ssDNA, which resulted in different ECL intensity between ssDNA and dsDNA.

In this proposed method, the oxidation potential of luminol was low, so thiolated DNA film on Au surface which had a narrow electrochemical window cannot be damaged. Moreover, because the diffusion layer thickness of electrochemical reaction was between 10 μm and 100 μm, that is to say, luminophor luminol in their excited state can be produced in the range of 10–100 μm apart from electrode surface. So, the proposed method can detect DNA with length as long as the thickness of diffusion layer theoretically. In our work, ssDNA and dsDNA with 15 bps were used as a model to investigate the ECL energy transfer behaviors. To further confirm the proposed method, ssDNA and dsDNA with 35 bps and 100 bps also can be distinguished, which exceeded the upper limit in fluorescence resonance energy transfer and electrochemical methods. To the best of our knowledge, this ECL energy transfer biosensor holds a great promise for simple and sensitive detection of the more longer DNA.

2. Experimental

2.1. Materials and chemicals

Tris (hydroxymethyl) aminomethane was purchased from Xi'an worldbio biotechnology Ltd. (Xi'an, China). 6-Mercapto-1-hexanol (MCH), Tris (2, 2-bipyridyl) dichlororuthenium (II) hexahydrate $[Ru(bpy)_3]Cl_2{\cdot}6H_2O$, 3-Aminophthalhydrazide (Luminol), and Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) were bought from Sigma–Aldrich. All solutions were prepared with ultrapure water (specific resistance of 18 ΩM cm) obtained from a Milli-Q water purification system.

All oligonucleotides were synthesized by Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China). The thiolated capture probes were listed below, ADNA: 5′-SH–$(CH_2)_6$–GTT CAT GCC GCC CAT-3′; CDNA: 5′-SH–$(CH_2)_6$–GTT CAT GCC GCC CAT TGG ATC GGC GTT TTA TTC TT-3′; EDNA: 5′-SH–$(CH_2)_6$–GTT CAT GCC GCC CAT TGG ATC GGC GTT TTA TTC TTG TTC AGA TAT CAC GAC GTT GCA TGT CGT TGT AGA CTG ACG GCT AGG AGC GGC GCA ACA TTC AGG T-3′; The complementary sequences were listed below, BDNA: 5′-ATG GGC GGC ATG AAC-3′; DDNA: 5′-AAG AAT AAA ACG CCG ATC CAA TGG GCG GCA TGA AC-3′; FDNA: 5′-ACC TGA ATG TTG CGC CGC TCC TAG CCG TCA GTC TAC AAC GAC ATG CAA CGT CGT GAT ATC TGA ACA AGA ATA AAA CGC CGA TCC AAT GGG CGG CAT GAA C-3′; single-base mismatch DNA (GDNA): 5′-ATG GGC GG*T* ATG AAC-3′.

The buffers involved in this work were as follows: DNA immobilization buffer (I-buffer), 10 mM Tris–HCl, 1 mM EDTA, 1.0 M NaCl, and 10 mM TCEP (pH 7.4). Note that TCEP was employed to cleave disulfides. DNA hybridization buffer (H-buffer), 10 mM Tris–HCl, 1 mM EDTA, and 50 mM NaCl (pH 7.4).

Luminol stocking solution (1.0×10^{-2} mol/L) was prepared by dissolving 0.1771 g luminol (Sigma) into 100 mL 0.1 mol/L sodium hydroxide. $[Ru(bpy)_3]^{2+}$ stocking solution (1.0×10^{-3} mol/L) was prepared by ultrapure water.

2.2. Apparatus

The electrochemical measurements were performed using a REC-100 workstation (Xi'An Remax Science & Technology Co. Ltd., Xi'An, China). A traditional three-electrode system contained a DNA modified 1 mm diameter Au disk electrode as the working electrode, platinum wire and an Ag/AgCl as the counter electrode and the reference electrode, respectively. The ECL emission was detected by a model RFL-1 Chemiluminescence Analyzer Systems (Xi'An Remax Science & Technology Co. Ltd., Xi'An, China) at room temperature, and the voltage of PMT was set at −900 V in the detection process.