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A sensitive, label-free electrochemical detection of telomerase activity without modification or immobilization

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ABSTRACT

Telomerase has become one of the most typical tumor marker because it is closely related to cancers. In this paper, a simple label-free electrochemical detection of telomerase activity by using methylene blue (MB) as a G-quadruplex binding probe was proposed, avoiding commonly used complex label procedures, nano-probe synthesis, complicated electrode modification, probe immobilization or signal amplification. In the presence of telomerase substrate (TS) primer, the binding of MB on primer was weak. When repeats of (TTAGGG) were extended on the TS primer under the action of telomerase, they formed multiple G-quadruplexes with the help of K^+. As a result, a large amount of MB bounded to multiple G-quadruplexes because they have more strong interaction with G-quadruplexes than TS primer. As a result, the diffusion current of MB decreased sharply, which was strongly dependent on the telomerase activity. The DPV current change has a linear correlation with the logarithm of HeLa cell number in the range of 10–10,000 cells, with the detection limit of 3 cells. The high sensitivity was due to the formed multiple G-quadruplexes. Using indium tin oxide (ITO) as working electrode without modification ensured the good reproducibility of the method. The method was also simple, rapid, and has been successfully applied in the telomerase activity detection in urine with good selectivity and reproducibility, which is significant for cancer diagnosis, anticancer drugs screening, and cancer therapy evaluation.

1. Introduction

Telomeres is essential to ensure the accuracy of genetic information (Moyzis et al., 1998), which located at the end of the eukaryotic chromosome. Telomerase add the telomeric repeats $(TTAGGG)_x$ to telomeric DNA to protect telomeres from erosion during cell division (Feng et al., 1995). Its activity were highly activated in most human cancers cells, leading to overgrowth of cancer cells (Zheng et al., 2005). Thus, It is urgent to develop simple and sensitive method for telomerase activity detection (Wu and Qu, 2015).

Telomeric repeat amplification protocol, the traditional method for detection of telomerase activity, has been extensively used in various methods (Kim et al., 1994; De Cian et al., 2007; Xiao et al., 2010). Fluorescence methods combined with signal amplification strategy have attracted more and more attentions (Ding et al., 2016; Gao et al., 2016a, 2016b; Li et al., 2016; Lou et al., 2015; Zhang et al., 2016b; Zhuang et al., 2015). Xia reported several novel fluorescence method to detect telomerase based on aggregation induced emission (AIE) of TPE-Z and Silole-R for quantification and monitoring of the telomerase activity (Jia, 2016; Lou et al., 2015; Zhuang et al., 2015, 2016b). Qu developed an upconversion nanoparticle modified cellulose paper to detect telomerase activity. This method is convenient and sensitive. The low level of telomerase can be discerned by naked eye (Wang et al., 2015a). Colorimetric methods based on gold nanoparticle (Duan et al., 2014; Wang et al., 2012, 2014; Zhang et al., 2016a), chemiluminescence method based on luminol (Li et al., 2011; Wang et al., 2013; Zhang et al., 2014b), surface enhanced Raman scattering (SERS) (Shi et al., 2016; Zong et al., 2013, 2014), photostable imaging (Zhuang et al., 2016a) and lectrochemical methods (Alizadeh-Ghodsi et al., 2016; Li et al., 2015; Ling et al., 2016; Liu et al., 2015, 2016a, 201b; Wang et al., 2015b) have been developed in recent years. Most of them suffers from nano-probe synthesis, complex label procedures and complicated probe immobilization.

Methylene blue (MB) is a positively charged electroactive indicator molecule. It interact with single-stranded DNA (ssDNA) via weak electrostatic binding and intercalate into double-stranded DNA (dsDNA) through π-π stacking interactions (Gill et al., 2005). The binding of MB to dsDNA often decreases its diffusion current in

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solution greatly due to a much lower diffusion rate of the MB/dsDNA compared with MB/ssDNA. All these properties of MB allowed the extensive application of it in electrochemical biosensors detection (Dou et al., 2016; Miao et al., 2016; Wang et al., 2016; Xuan, Luo and Hsing, 2013). Zhang et al. fabricated a simple electrochemical biosensor for cocaine detection based on systematically investigated the interaction of MB with ssDNA, dsDNA and G-quadruplex. The binding between MB and G-quadruplex was even stronger than that between MB and dsDNA, as a result, the decrease of diffusion current was more obvious than that for MB/dsDNA (Zhang et al., 2014a). Li et al. developed electrochemical methods for sensitive detection of ATP, alkaline phosphatase activity, and miRNA. The release of intercalated MB from dsDNA and G-quadrulexes led to sharp signal decrease (Hou et al., 2015; Liu et al., 2013; Zhang et al., 2015).

Inspired by the aforementioned works, a very simple label-free electrochemical detection of telomerase activity by using MB as a G-quadruplex binding probe was proposed. In the presence of TS primer, a portion of MB interact with TS primer, while most MB was free in the solution and produced a high DPV signal, which come from the diffusion current of free MB. Under the action of telomerase, telomerase extension reaction generated and G-rich sequence formed G-quadruplexes in the presence of K^+. In this case, plenty of MB interacted with G-quadruplexes and a very small portion of MB were free in solution. While used the indium tin oxide (ITO) as the working electrode, the repulsion between DNA and the negatively charged ITO working electrode surface and the large probe size prevented the intercalated MB from reaching to the ITO working electrode surface. As a result, the diffusion current of MB reduced greatly, which was dependent on the formed G-quadruplexes that closely related to telomerase activity. The proposed method avoided the expensive labelled procedures, complex nano-probe synthesis, electrode modification, probe immobilization and signal amplification.

2. Experiment

2.1. Reagents and materials

ITO was purchased from Zhuhai Kaivo Optoelectronic Technology Co., Ltd. (Zhuhai, China). TS primer (AATCCGTCGAGCAGAGTT) was synthesized by Sangon Biotech Co. Ltd. (Shanghai, China). Methylene Blue hydrate was purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Dulbecco's modified eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from Life Technologies (California, USA). Phenylmethanesulfonyl fluoride (PMSF), 3-[(3-Cholamidopropyl)dimethylammonio]propanesulfonate (CHAPS), and ethylene glycol tetraacetic acid (EGTA) were purchased from Sigma-Aldrich Co. Ltd. (St. Louis, MO). Ultrapure water (18.2 MΩ cm, Barnstead, Thermo Scientific, USA) was used throughout the study. Real urine samples were received from Nanjing General Hospital of Nanjing Military Command.

The buffer solutions employed in this study including pH 7.4 tris buffer solution (10 mM Tris-HCl), pH 7.4 phosphate buffered saline (PBS) solution containing 136.89 mM NaCl and 2.67 mM KCl, CHAPS lysis buffer and telomerase extension reaction buffer.

2.2. Cell culture and telomerase extraction and extension procedures

Various cell lines used in these experiments including HeLa, A549, MCF-7 and MDA-MB-231 cells were incubated according to our previously reported methods (Liu et al., 2016a, 2016b). Extraction of telomerase from HeLa cells and urine samples were also operated according to these literature reported procedures by using pH 7.5 CHAPS lysis buffer that containing 0.5% (w/v) CHAPS, 10 mM Tris-HCl, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM PMSF and 10% (v/v) glycerol. *Telomerase extension reaction* were also operated according to traditions method (Liu et al., 2016a, 2016b). 5 μL above telomerase extraction solution was mixed with 1 μM TS primer, 2 μM MB in pH 8.3 telomerase extension reaction buffer that containing 20 mM Tris-HCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 63 mM KCl, 0.005% (v/v) Tween 20, and 1 mM dNTPs. BIBR 1532 and curcumin was used to inhibit the telomerase extension by adding them to above solutions. Heated telomerase extracts were used as negative control samples.

2.3. Electrode pretreatment

ITO slice was first being sonicated in acetone, ethanol and ultrapure water for 15 min sequentially. Then, the prepared electrode was immersed into 1 mM of NaOH solution for 5 h at room temperature, followed by being sonicated further in ultrapure water. The chip is similar to the DNA microchip which previously reported by Hsing for the immobilization-free sequence-specific electrochemical detection of DNA (Luo et al., 2008; Xuan et al., 2012).

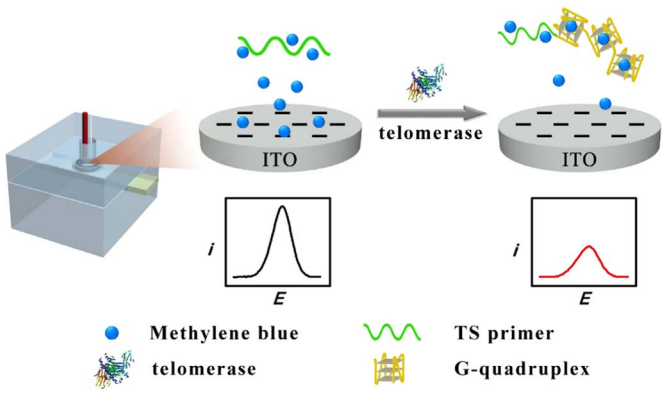
2.4. Electrochemical measurements

All electrochemical measurements were performed with a home-made device as shown in Sheme 1. Electrochemical measurements were performed on a CHI 660C electrochemical analyzer (Chenhua Instrument, Shanghai, China), with a conventional three-electrode system by using Ag/AgCl and Pt as reference and counter electrode, respectively. Negatively charged ITO was used as the working electrode. 100 μL mixture solution was pitted into the hole of the device for each detection. Then differential pulse voltammetry (DPV) measurements was performed with the potential sweeping from −0.5 to 0 V. The pulse height, the pulse width, the pulse increment and the pulse period were set to 0.05 V, 0.05 s, 0.004 V, and 0.5 s, respectively.

3. Results and discussion

3.1. Principle of the assay

The principle of electrochemical detection of telomerase activity was illustrated in Scheme 1. Firstly, we designed the device that containing ITO, Ag/AgCl and Pt electrode as working electrode, reference electrode, and counter electrode, respectively. ITO electrode was placed into the groove of the bottom polymethyl methacrylate (PMMA), which was covered by the upper PMMA with a 200 μL volume of cavity. The seal ring was used to prevent the solution leaking out. Electrolyte solution that containing TS primer (AATCCGTCGAGCAGAGTT) and MB, as indicated probe, were added into the container. A small number of MB adsorbed on TS primer, while most MB molecules are free in solution. These free MB showed a strong diffusion toward the negatively charged ITO electrode surface due to electrostatic interaction, which resulting in a large DPV signal. In the presence of telomerase and dNTPs, repetitive sequence



Scheme 1. Schematic illustration of the telomerase activity detection by using methylene blue as a G-quadruplex binding probe.