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Short communication

Highly sensitive electrochemical detection of human telomerase activity based on bio-barcode method

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ABSTRACT

In the present study, an electrochemical method for highly sensitive detection of human telomerase activity was developed based on bio-barcode amplification assay. Telomerase was extracted from HeLa cells, then the extract was mixed with telomerase substrate (TS) primer to perform extension reaction. The extension product was hybridized with the capture DNA immobilized on the Au electrode and then reacted with the signal DNA on Au nanoparticles to form a sandwich hybridization mode. Electrochemical signals were generated by chronocoulometric interrogation of $[Ru(NH_3)_6]^{3+}$ that quantitatively binds to the DNA on Au nanoparticles *via* electrostatic interaction. This method can detect the telomerase activity from as little as 10 cultured cancer cells without the polymerase chain reaction (PCR) amplification of telomerase extension product.

1. Introduction

Human telomerase is a ribonucleoprotein reverse transcriptase, it is responsible for maintaining telomere length by adding the repeated sequence TTAGGG to the ends of telomeres (Hammond and Cech, 1997; Nakamura et al., 1997). In normal human somatic cells, the repetitive DNA sequences reduce in length with each round of cell division and DNA replication. This process is believed to be one of the factors that limits the times of normal cell dividing. In contrast, cancer cells are able to divide indefinitely and maintain their telomere lengths by expressing telomerase (Sachin et al., 2004; Hahn et al., 1999). So telomerase has been regarded as a biomarker for cancer diagnosis as well as a therapeutic target and the assay of telomerase activity is very useful (Shay and Bacchetti, 1997; Savoysky et al., 1996).

The most popular assay for telomerase is the polymerase chain reaction (PCR)-based telomeric repeat amplification protocol (TRAP) (Kim et al., 1994; Savoysky et al., 1996; Herbert et al., 2006). The major limitations of the TRAP assay are related to PCR-derived artifacts because of the susceptibility to polymerase inhibition by clinical extracts. In recent years, a variety of techniques without PCR have been developed to analyze telomerase activity. Willner reported several methods for telomerase assay by using the catalytic DNAzyme, which consists of hemin and single-stranded guanine-rich nucleic acids and possesses peroxidase-like activities (Xiao et al., 2004; Niazov et al., 2004; Pavlov et al., 2004). Several electrochemical methods were developed using ferrocenylnaphthalene diimide as a tetraplex DNA-specific binder (Sato et al., 2005) or the electrochemical oxidation signal of guanine (Shao et al., 2008). Xing reported magnetic bead and nanoparticle based electrochemiluminescence amplification assay of telomerase activity (Zhou et al., 2009). Mirkin introduced a new approach to amplified telomerase detection by incorporating polyvalent oligonucleotide nanoparticle conjugates with silver staining technique (Zheng et al., 2008). These assays for telomerase activity are all based on PCR-free method, however, some of them suffer from drawbacks: low sensitivity, complicated manipulation or immobilization of telomerase substrate (TS) primer on solid supports, which will reduce the efficiency of telomerase extension reaction and limit the practical application in the real samples.

To further improve the sensitivity for the detection of telomerase activity and develop a more simple method, bio-barcode amplification coupled with $[Ru(NH_3)_6]^{3+}$ as indicator was applied to telomerase assay. Nanoparticle-based bio-barcode approach is the only biodetection method that has the PCR-like sensitivity without a need for enzymatic amplification (Chang et al., 2007; Nam et al., 2003). In a conventional bio-barcode amplification assay (Oh et al., 2006), the magnetic microparticle (MMP) probes react with target and then the nanoparticle probes with oligonucleotides (barcode DNA) and a second group that has an affinity for the same target are added to form a sandwich structure. A magnetic field is used to separate such sandwich complexes and the dehybridization

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of the barcode DNA followed by scanometric microarray detection allows one to identify the barcode sequences and quantify the amount of target. However, the conventional bio-barcode assay has some disadvantages including the release of barcode DNA probes and immobilization on a chip. In recent years, some novel bio-barcode detection methods have been reported (Nam et al., 2005; Zhu et al., 2008).

In this strategy, DNA–Au NP conjugates were fabricated by Au NPs and two kinds of oligonucleotides. One can specifically hybridize with the telomerase extension product, while the other (barcode DNA) cannot, reducing the cross-reaction of targets with DNA loaded on the same one Au NP. $[Ru(NH_3)_6]^{3+}$, a electroactive complex, which can bind to the anionic phosphate of DNA strands through electrostatic interaction (Yu et al., 2003; Zhang et al., 2006), serves as a signaling transducer. With this strategy, the assay can detect telomerase activity from cell extracts equivalent down to 10 HeLa cells. It shows a great potential in the application for the clinical diagnosis of cancer based on telomerase activity detection.

2. Experimental

2.1. Materials and apparatus

2.1.1. Materials

All of synthetic oligonucleotides were purchased from SBS Genetech. Co. Ltd. (China) with the following sequences: TS primer: 5′-AAT CCG TCG AGC AGA GTT-3′; capture DNA (S1): 5′-GCT CGA CGG ATT TTT-SH-3′; signal DNA (S3): 5′-SH-TTT TTT TTT TTT TTT CTA ACC CTA ACC CTA ACC-3′; barcode DNA (S4): 5′-SH-TTT TTT TGT ATC GCT CAT ATG GAC-3′.

Hexaammineruthenium(III) chloride (98%), ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid (CHAPS), phenylmethylsulfonyl fluoride (PMSF) and tris(2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Beijing BioDee biotechnology Corporation Ltd. (China). RNase A was acquired from Sigma. Other chemicals employed were all of analytical reagent grade and were used as received. All the water used in the work was RNase-free.

2.1.2. Apparatus

Cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and chronocoulometry (CC) were carried out on a CHI 660C electrochemical analyzer (Shanghai CH Instrument Company, China) using a three-electrode system consisting of a platinum wire as an auxiliary electrode, a Ag/AgCl electrode as reference electrode, and Au electrode (2 mm in diameter) as working electrode. UV–vis absorption spectra were carried out on a Cary 50 UV–vis–NIR spectrophotometer (Varian). Transmission electron microscopy (TEM) image was taken with JEOL JSM-6700F instrument (Hitachi).

2.2. Preparation of DNA–Au NP conjugates

The process of signal and barcode DNA labeling was performed as follows (Taton et al., 2000): The mixture of 1.0×10^{-10} mol of signal DNA (S3) and 7.0×10^{-10} mol of barcode DNA (S4) was activated with 10 μL of 30 mM TCEP for 1 h, then added to 1 mL of freshly prepared gold nanoparticles, and shaken gently for 16 h. After that, the solution was aged in salts (0.1 M NaCl, 10 mM acetate buffer) for another 24 h. Excess reagents were removed by centrifuging at 10 000 rpm for 30 min. The red precipitate was washed and centrifuged repeatedly for three times. The resulting nanoparticles were dispersed into a buffer solution (0.01 mM PBS, pH 8.2, 0.3 M NaCl) and stored at 4 °C.

2.3. Telomerase extract preparation and extension reaction

HeLa cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, and the cells were maintained at 37 °C in a humidified atmosphere (95% air and 5% CO_2). Cells were collected in the exponential phase of growth, and 5×10^6 cells were dispensed in a 1.5 mL EP tube, washed twice with ice-cold PBS (0.1 M, pH 7.4), and resuspended in 200 μL of ice-cold CHAPS lysis buffer (10 mM Tris–HCl, pH 7.5, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM PMSF, 0.5% CHAPS, 10% glycerol). The lysate was incubated for 30 min on ice and centrifuged 20 min at 16 000 rpm, 4 °C, and the supernatant was collected carefully. The lysate was used immediately for telomerase assay or frozen at −80 °C.

Telomerase extracts were diluted in lysis buffer with respective number of cells and added to 200 μL of extension solution (20 mM Tris–HCl, pH 8.3, 4 mM $MgCl_2$, 1 mM EGTA, 63 mM KCl, 0.05% Tween 20) containing 0.5 mM dATP, dTTP, dGTP and 1 nM TS primer. Then the solution was incubated at 37 °C for 1 h and stopped by heat denaturing of telomerase. For control experiments, telomerase extracts were pretreated with RNase for 15 min at 37 °C or heat-treated (95 °C for 10 min).

2.4. Fabrication of the biosensor for telomerase activity detection

A gold electrode was polished carefully with alumina slurries (1, 0.3, 0.05 μm) and washed ultrasonically with deionized water. Subsequently, the electrode was electrochemically cleaned in 0.5 M H_2SO_4 solution by cyclic potential scanning between −0.2 and 1.5 V until a standard cyclic voltammogram (CV) was obtained. Then it was rinsed with deionized and doubly distilled water and absolute ethanol in turn.

The capture DNA (S1) self-assembly process was performed by pipetting 5 μL of 0.01 μM S1 onto the surface of the electrode. Then the modified electrode was immersed in 100 mM PBS (pH 7.4) containing 1.0 mM MCH for 1 h to block the uncovered gold surface. For the hybridization reaction, the S1 modified electrode was incubated for 1.5 h with various telomerase extension products at 37 °C, and secondly hybridized with the signal DNA loaded on Au NPs for 2 h at 37 °C. The electrode surface was rinsed with 0.01 M PBS containing 1‰ SDS (pH 7.4) after each step of fabrication process in order to remove nonspecifically adsorbed thrombin and DNA sequences.

2.5. Electrochemical measurements

All electrochemical measurements were carried out at room temperature in a single-compartment, three-electrode glass cell using an electrochemical analyzer. EIS and CV were carried out in 10 mM PBS (pH 7.4) containing 2.5 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ and 0.1 M KCl. CC was performed in 2 mL of 10 mM Tris–HCl solution (pH 7.0) containing 50 μM $[Ru(NH_3)_6]^{3+}$, with a pulse period of 250 ms. The chronocoulometric response curves were converted to Anson plots by plotting charge versus $t^{1/2}$. The linear part of the plot was then extrapolated back to 0 $s^{1/2}$ and the intercept was taken as readout.

3. Results and discussion

3.1. Sensing system based on bio-barcode method

Scheme 1 shows the method used for the detection of telomerase activity. For telomerase analysis, TS primer was incubated with telomerase extracts from the cancer cells in the presence of the nucleotide mixture dNTPs. TTAGGG repeat units were continuously added to the 3′ end of the primer by telomerase to form a longer single strand DNA (*S2*). Subsequently, the 12-base segment close to the 5′ end of S2 was hybridized with S1 immobilized on