Talanta 178 (2018) 594–599

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

A PCR-free colorimetric strategy for visualized assay of telomerase activity

Tao Yu, Wei Zhao*, Jing-Juan Xu*, Hong-Yuan Chen

State Key Laboratory of Analytical Chemistry for Life Science and Collaborative Innovation Center of Chemistry for Life Sciences, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, China

ARTICLE INFO

Keywords:
Telomerase
PCR-free
Au NPs
Visual detection
Colorimetry

ABSTRACT

A simple yet powerful polymerase chain reaction (PCR)-free strategy for visualized assay of human telomerase activity was reported in this work. Gold nanoparticles (AuNPs) based colorimetric strategy was applied with well-designed enzyme-aided cyclic amplification. Briefly, the detection relies on the elongated primers of telomerase substrate (TS) induced by telomerase, which open the hairpin DNA and hybridize with linker DNA, the trigger of AuNPs aggregation. Nicking endonuclease was added in the sensing system, which cleaved linker DNA after hybridization and released complimentary strand for cyclic hybridization with linker DNA, resulted in high sensitivity for the detection of telomerase. Down to 25 HeLa cells with high expression of telomerase could be recognized. The proposed strategy provides a good platform for the determination of telomerase activity, differentiation of cancer cell lines from normal cell line and screening of telomerase-targeted anticancer drugs.

1. Introduction

Telomere, a unique nucleic acid that locates at the end of eukaryotic chromosome, is a critical element which protects chromosome from deterioration, recombination, or fusion with neighbouring chromosomes during cell mitosis process at the cost of shortening themselves (about 50–200 nt per cycle), resulting in cellular senescence and apotosis [1–3]. Human telomerase is a ribonucleoprotein with reverse transcriptase activity, which adds repeated segments of DNA $(TTAGGG)_n$ to the 3′ ends of telomere and maintains its length, hence avoids cell death [4]. In most normal somatic cells, telomerase activity is highly depressed, while in approximately 90% of known human tumors telomerase is overexpressed. The overexpression of telomerase can prevent telomeres from shortening during cell mitosis, making cancer cells mitose indefinitely [5]. The inherent relationship between telomerase and cancers makes the enzyme a valuable biomarker for early diagnosis of cancer and anticancer-drug discovery.

Among the strategies used to detect telomerase activity, the most conventional method is the polymerase chain reaction (PCR)-based telomeric repeat amplification protocol (TRAP), which is extraordinary sensitive, but time-consuming and easily contaminated [6,7]. PCR-free strategies based on electrochemistry [8–10], fluorescence [11–15], chemical luminescence [16], electrogenerated chemiluminescence [17], surface plasmon resonance [18] and so on [19] have been developed for telomerase sensing. For example, recently, our group reported a method based on Ag-Ag plasmon rulers (PRs) for in-situ monitoring the extension process of telomerase primer (TSP) activated by single telomerase [18]. This method allowed us to uncover telomerase activity at single-molecule level. However, as other instrument based analytical methods, the operation process of such strategy was relatively complicated and the performance was heavily depending on the status of the instrument [20]. In recent years, gold nanoparticle (AuNP)-based colorimetric assays have been demonstrated as a highly competitive biosensing technology for the detection of metal ions [21], DNA [22] and microRNA [23] due to the specialties of low cost, simplicity, and practicality. One major drawback of the colorimetry compared with other methods is the relative lower sensitivity [24].

This work presents an oligonucleotide-modified AuNPs based colorimetric sensor for the analysis of telomerase. The detection principle is based on the elongated primers of telomerase substrate (TS) induced by telomerase that open the hairpin DNA and hybridize with linker DNAs, which prevent the aggregation of AuNPs triggered by linker DNA. With enzyme-aided cyclic amplification using nicking endonuclease, linker DNAs were largely consumed, which greatly enhanced the sensitivity of the method. Down to 25 HeLa cells could be detected, and cancer cells could be easily differentiated from normal human cell lines. The proposed PCR-free strategy doesn't require complicated thermal-cycling procedure, which could be extended to a high-throughput clinical detection.

* Corresponding authors.
E-mail addresses: weizhao@nju.edu.cn (W. Zhao), xujj@nju.edu.cn (J.-J. Xu).

http://dx.doi.org/10.1016/j.talanta.2017.09.070
Received 3 August 2017; Received in revised form 17 September 2017; Accepted 26 September 2017
Available online 28 September 2017
0039-9140/

Table 1
DNA sequences employed in this work.[a]

Name	DNA sequence (5′-3′)
TS primer	AAT CCG TCG AGC AGA GTT
DNA1	SH-$(CH_2)_6$-CGT TTC GCC TCA
DNA2	GCT ATT GTT TGC-$(CH_2)_6$-SH
Linker DNA	GCA AAC AAT AGC↓TGA GGC GAA ACG
Artificial elongated product	TTAGGGTTAGGGTTAGGG
Hairpin DNA	**GGGTTAGGGCCT**CAGCTAAAAAAA**AGGCCCTAACCC***TAACCCTA*

[a] Bold letters are stem sequences of hairpin DNA probes, italic type in Hairpin DNA shows sticky ends. Underlined letters are the NEase recognition sequence. The arrow indicates the nicking position.

2. Experimental section

2.1. Materials and apparatus

All of the oligonucleotides used in this work were synthesized by Sangon Biotech. Co., Ltd. (Shanghai, China), which were purified by HPLC and confirmed by mass spectrometry (Table 1). Fetal bovine serum (FBS), new bovine calf serum (NBCS), Dulbecco's modified eagle medium (DMEM), and penicillin/streptomycin were purchased from Gibco (USA). DNase I endonuclease was purchased from KeyGen Biotech. NEase (Nb. BbvCI) and 10 × NEB buffer (500 mM NaCl, 100 mM Tris-HCl, 100 mM $MgCl_2$ and 10 mM dithiothreitol, pH 7.9) were purchased from the New England Biolabs, Inc. Chloroauric acid ($HAuCl_4$), trisodium citrate were purchased from Aldrich. All other reagents and chemicals were of at least analytical reagent grade. Sequences of DNAs are shown in Table 1.

Transmission electron microscopy (TEM) was performed with a JEOL model 2000 instrument. The UV–vis absorption spectra were recorded on a Shimadzu UV-3600 UV–vis–NIR photospectrometer (Shimadzu Co., Japan) at room temperature. Dynamic light scattering (DLS) and Zeta potential analysis of Au NPs were performed on a Zeta Sizer Nano ZS (Brookhaven Instruments Ltd.). The images of gel electrophoresis were scanned by the Gel Image Analysis System (Bio-Rad, U.S.A.). The water (18.2 MΩ cm) used throughout the experiments was pretreated with Milli-Q (Millipore, Inc., U.S.A.).

2.2. Preparation of Au NPs and DNA-modified Au NPs

Au NPs with an average diameter of 13 nm were prepared according to a previously described protocol [25]. In a typical experiment, 100 mL aqueous solution of $HAuCl_4$ (1 mM) was heated to reflux under stirring, then 10 mL of trisodium citrate (38.8 mM) was added quickly, resulting in a change in solution color from pale yellow to deep red. The solution was heated under reflux for 20 min and then cooled to room temperature. Then it was filtered through a 0.22 μm Millipore membrane filter. The prepared Au NPs were stored in brown glass bottles at 4 °C.

The DNA-modified AuNPs were prepared as reported previously [26]. The thiol-modified DNA 1 and DNA 2 were first activated by 2 μL of 10 mM TCEP for 1 h, respectively. Then, TCEP-activated DNA 1 and DNA 2 were incubated with Au NPs and stirred at room temperature overnight, and then PBS solution containing 2 M NaCl was added to the mixture and brought to a final concentration of 0.15 M NaCl through a stepwise process for stabilizing the probe. This mixed solution was further incubated for 24 h at room temperature. To remove excess reagents, the solution was centrifuged for 25 min at 14,000 rpm. The precipitate was washed three times with a PBS buffer (20 mM Tris-acetate, pH 7.9), and finally dispersed in the Tris-HCl buffer and stored at 4 °C until use.

2.3. Cell culture

To investigate the selectivity of the biosensor for the detection of different cells, four kinds of different cell lines (HeLa, A549, HepG2 and QSG7701) were cultured in a humidified atmosphere (37 °C, 95% air and 5% CO_2). HeLa and HepG2 cells were cultured in DMEM supplemented with 10% fetal bovine serum (Gibco), penicillin (80 U/mL) and streptomycin (0.08 mg/mL). A549 cells were cultured in RPMI-1640 supplemented with 10% fetal bovine serum (Gibco), penicillin (80 U/mL) and streptomycin (0.08 mg/mL). QSG7701 cells were cultured in RPMI 1640 supplemented with 10% calf serum (Gibco) and penicillin/streptomycin. Cell density was determined using a hemocytometer prior to each experiment.

2.4. Detection of telomerase activity

All kinds of cells were collected in the exponential phase of growth and 1 × 10^6 cells were dispensed in a 1.5 mL EP tube, washed twice with ice-cold PBS (0.1 M, pH 7.4), then resuspended in 200 μL of ice-cold CHAPS lysis buffer containing 10 mM Tris-HCl (pH 7.5), 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM (w/v) PMSF, 0.5% CHAPS and 10% (v/v) glycerol. The mixture was incubated for 30 min on ice and centrifuged at 12,000 rpm and 4 °C for 20 min. After centrifugation, the supernatant was carefully transferred into a fresh RNase-free tube, flash frozen and stored at −80 °C before use [27]. For negative control experiment, CHAPS lysis buffer was used instead of telomerase extracts. For the thermal inactivation control experiment, the telomerase extracts were first heated at 95 °C for 15 min [28].

2.5. Telomerase extension reaction

HeLa extracts were serially diluted in lysis buffer with respective number of cells (25, 50, 100, 250, 500, 1000, 1500, 2000) and used as a source for telomerase. 20 μL of telomerase extract was added to 30 μL of extension buffer containing TRAP buffer (20 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 63 mM KCl, 0.005% (V/V) Tween 20, 1 mM EGTA), 1 mM dNTPs and 20 nM TS probe. The mixture was incubated at 30 °C for 1 h.

Then, hairpin DNA was added in, the reaction mixture was incubated at 37 °C for 15 min. Then, based on our previous work, the above mixture was incubated with 15 u/mL Nt.BbvCI at 37 °C for 30 min [29]. Third, the mixtures were introduced to the solution containing DNA1-AuNPs and DNA2-AuNPs to allow assembly of the Au NPs. The resulting samples were photographed and tested with a UV–vis spectrometer.

2.6. Conventional TRAP assays

5 μL of telomerization products were added into 45 μL of solution which contains 1 × PCR buffer, 200 μM dNTPs, 3U of Taq DNA polymerase, 400 nM of TS primer, and 400 nM of ACX primer. PCR was carried out using thermal cycler with the following program: 94 °C for 4 min; 30 cycles at 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s; 72 °C for 5 min; 4 °C hold. The 15% polyacrylamide gels were prepared using a 1 × TBE buffer and a volume of 10 μL of different PCR products with loading buffer was added to each well. Gels were run at room temperature for 1 h at 120 V and then scanned using the gel image analysis system.

3. Results and discussion

3.1. Principle of the assay

The principle of this PCR-free colorimetric assay for detection of telomerase activity is illustrated in Scheme 1. Two kinds of DNA modified AuNPs with 13 nm in diameter (DNA1-Au NPs and DNA2-Au