Talanta 178 (2018) 458–463

Contents lists available at ScienceDirect

Talanta

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

Convertible DNA ends-based silver nanoprobes for colorimetric detection human telomerase activity

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ARTICLE INFO

Keywords:
Silver nanoprobes
Convertible DNA ends
Telomerase activity
Colorimetric detection

ABSTRACT

Human telomerase is an endogenous ribonucleoprotein that is over-expressed in most types of malignant cancer cells. Sensitive and specific detection of telomerase activity is crucial for better understanding its role in cancer cells and further exploring its function in cancer diagnosis. Here, we develop convertible DNA ends-based silver nanoprobes for sensitive and specific colorimetric detection telomerase activity. Silver nanoprobes are constructed by modifying telomerase binding substrates (TS) that are pre-hybridized with complementary sequences onto silver nanoparticles (AgNPs), via the coordination between consecutive cytosines in TS strand and AgNPs. This forms blunt-end terminated, double-stranded DNA on the surface of AgNPs. Under the action of telomerase, TS on the silver nanoprobes are elongated with telomeric repeats, converting DNA stiff blunt ends to flexible single-stranded dangling ends. The dangling ends enhance the stability of nanoprobes and relieve their salt-induced aggregation, and the solution shows a yellow color. When telomerase is inactive, the blunt end-terminated nanoprobes cannot resist salt-induced aggregation, resulting in a gray color of solution. Based on telomerase-regulated DNA "blunt-dangling" ends conversion-induced AgNPs' dispersity and color change, colorimetric detection of the endogenous telomerase with AgNPs is realized. The detection limit is equivalent to 1 cell/μL of telomerase activity, and extracts from cancer cells and normal cells are visually distinguished through color difference. The proposed strategy will offer a new approach for reliable, convenient quantification of telomerase activity in biochemical research and clinical diagnosis.

1. Introduction

Human telomerase is an endogenous ribonucleoprotein comprised of telomerase RNA and telomerase reverse transcriptase and can catalyze the addition of telomeric repeats $(TTAGGG)_n$ onto the 3′-end of telomere inside cells [1]. This process compensates the shortening of telomere during cell division so as to avoid reaching critical telomere length that triggers cellular senescence and apoptosis, extending cellular lifespan [2]. Telomerase is found to be over-expressed in most types of malignant cancer cells, enabling cancer cells to circumvent telomere-dependent pathway of cell mortality and divide infinitely [3]. Telomerase has thus been regarded as a promising biomarker and a potential therapeutic target for cancer [4]. Sensitive and specific detection of telomerase activity is crucial for better understanding its role in cancer cells and further exploring its function in cancer diagnosis.

Telomerase activity detection is mainly based on the elongation of telomerase binding substrates (TS) under the action of telomerase [5]. Conventional detection methods include primer extension assay [6] and telomeric repeat amplification protocol (TRAP) [7]. To avoid polymerase-derived artifacts and radioactive/mutagenic hazards, emerging techniques including fluorescence [8–11], electrochemistry [12–15], field effect transistor [16], microarray [17] and scanometric chip [18] have been developed. Compared with these methods, colorimetry enables visual determination and minimizes the reliance on complicated instruments, which would facilitate a portable, on-site and more maneuverable assay of telomerase. Noble metal nanoparticles' surface plasmon resonance (SPR) frequency is sensitive to the proximity of individual colloids, rendering their optical properties strongly dependent on interparticle distance [19], thus can serve as ideal optical reporters in colorimetry. Gold nanoparticle (AuNP)-based colorimetric detection of telomerase activity has been developed [20–23], in which elongated TS would fold secondary structures or serve as cross-linkers to change AuNPs' dispersity.

From an optical sensing point of view, silver nanoparticles (AgNPs) are also important optical reporters besides AuNPs [24]. As plasmonic materials, silver supports surface plasmons from the visible till to near-

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http://dx.doi.org/10.1016/j.talanta.2017.09.057
Received 20 July 2017; Received in revised form 15 September 2017; Accepted 17 September 2017
Available online 19 September 2017
0039-9140/

infrared regions, thus can serve as plasmonic candidates at the desired resonance wavelength [25]. Moreover, AgNPs own several to hundreds times higher of molar extinction coefficient than its nanoparticles made from silver congeners [26], and may endow colorimetric systems more quantitative alternatives besides absorption [27]. Additionally, silver is relatively cheap among the metals that support plasmons [28], enabling the assay more cost-effective and easily-extended. Despite of AgNPs' excellent optical properties, the analytes in AgNPs-based colorimetric methods are typically synthetic/exogenous targets spiked in buffer and biofluids [29–32]. Their further application in real sample analysis still needs to be studied, as detection of endogenous target originating from organism is a prerequisite for risk assessment and disease diagnosis [33].

Herein we explore AgNPs for colorimetric detection of endogenous target, by taking human telomerase extracted from HeLa cancer cells as a model. Convertible DNA ends-based silver nanoprobes are constructed by modifying TS that are pre-hybridized with complementary sequences onto silver nanoparticles (AgNPs), via the coordination between consecutive cytosines in TS strand and AgNPs [34]. This forms blunt-end terminated, double-stranded DNA on the surface of AgNPs. Under the action of telomerase, TS on the silver nanoprobes are elongated with telomeric repeats, converting DNA stiff blunt ends to flexible single-stranded dangling ends. Due to the increased flexibility of DNA on colloid surface [35], the nanoprobes get stabilized to relieve salt-induced aggregation, showing a yellow color. When telomerase is inactive, the single-stranded dangling ends cannot be generated. As a result of DNA stiff ends and base stacking interaction [36], the nanoprobes cannot resist salt-induced aggregation, resulting in a gray color of solution. Based on telomerase-regulated DNA “blunt-dangling” ends conversion-induced AgNPs' dispersity and color change, colorimetric detection of the endogenous telomerase with AgNPs is realized. The detection limit is equivalent to 1 cell/μL of telomerase activity, and extracts from cancer cells and normal cells are visually distinguished through color difference. The proposed strategy will offer a new approach for reliable, convenient quantification of telomerase activity in biochemical research and clinical diagnosis.

2. Experimental section

2.1. Reagents and apparatus

Silver nitrate, sodium citrate, sodium borohydride, gold(III) chloride trihydrate ($HAuCl_4{\cdot}3H_2O$), bovine serum albumin (BSA) and 3′-azido-3′-deoxythymidine (AZT) were purchased from Sigma-Alrich (St. Louis, MO, USA). CHAPS lysis buffer was ordered from Millipore (Bedford, MA, USA). Tris base, boric acid, ethylenediaminetetraacetic acid tetrasodium (EDTA), egtazic acid-glycol ether diamine tetraacetic acid (EGTA), deoxynucleotide triphosphates (dNTPs), agarose and 40% (w/v) acrylamide/bis-acrylamide solution (19:1) were obtained from Sangon Biotech Co., Ltd. (Shanghai, China). Diethyl pyrocarbonate (DEPC) and dithiothreitol (DTT) were purchased from BBI Life Sciences (Markham, Ontario, Canada). Tween-20 was obtained from Salarbio (Beijing, China). SYBR gold nucleic acid gel stain was bought from Invitrogen. All other reagents were of analytical grade and used as received. Ultrapure water (18.25 MΩ cm) obtained from a UP water purification system was used throughout the experiment. DNA oligonucleotides were synthesized in Sangon Biotech Co., Ltd. (Shanghai, China), with sequences listed in Table S1.

UV–vis absorption spectra were recorded on a U-2910 spectrophotometer equipped with a temperature controller (Hitachi, Japan). Fluorescence spectra were measured on an F-7000 fluorescence spectrophotometer (Hitachi, Japan). The excitation wavelength was set at 498 nm to record the fluorescence within the range from 510 nm to 750 nm, and the excitation and emission slits were both set as 5 nm. Transmission electron microscopy (TEM) measurements were made on a JSM-6700F transmission electron microscope operated at an accelerating voltage of 200 kV (JEOL, Japan). Dynamic light scattering (DLS) measurements were performed on a Zetasizer Nano ZS (Malvern, UK). The samples for DLS characterization were adjusted to an AgNP concentration of 4.2 nM, and were transferred to 50-μL disposable cuvettes (Sarstedt, Germany) for measurements. The polyacrylamide gel was imaged by GelDocTM XR^+ imaging system (Bio-RAD Laboratories Inc. USA). Polymerase chain reaction (PCR) reaction was performed on a T-gradient thermoblock (Biometra, Germany).

2.2. Preparation of silver nanoprobes

AgNPs were synthesized by reduction of $AgNO_3$ by $NaBH_4$ in the presence of sodium citrate [37]. Silver nanoprobes were prepared through consecutive cytosine-mediated DNA conjugation [34], under the low pH condition to accelerate the DNA conjugation [38,39]. For preparation of C10-dTS-AgNP nanoprobe, stoichiometric moles of C10-TS and partially complementary cDNA were annealed at 95 °C for 10 min and then slowly cooled down to room temperature. A small volume of hybridized duplex was then mixed with a large volume of synthesized AgNP with a molar ratio of 3000:1. The mixture was kept under stirring at room temperature for 2 h. Subsequently, a small volume of citrate buffer (500 mM, pH 3.0) was added to reach a final citrate concentration of 4 mM, followed by an incubation of 30 min. Another small volume of citrate buffer (500 mM, pH 3.0) was added to reach a final citrate concentration of 8 mM, followed by another incubation of 30 min. Then a small volume of phosphate buffer (200 mM, pH 7.4) was added to reach a final PB concentration of 21 mM, followed by an incubation of 1 h. The nanoprobe was washed with Tris-HCl buffer (10 mM, pH 8.0) for three times and finally dispersed in the same buffer with proper concentration, storing in dark in a refrigerator at 4 °C before use. For the preparation of C20-dTS-AgNP and C30-dTS-AgNP nanoprobes, the procedure was the same as mentioned above except for the adoption of C20-dTS and C30-dTS oligonucleotides. DNA surface density was quantified by DTT-displacement method [40].

2.3. Cell culture and telomerase extraction

HeLa cells and normal liver cells were cultured in a flask in RPMI medium 1640 (GIBCO) supplemented with 10% fetal calf serum at 37 °C in a humidified atmosphere containing 5% CO_2. Cell numbers were determined with a Qiujing cell counter (Shanghai, China). Telomerase was extracted by the CHAPS method [41]. Briefly, 1×10^6 cells were dispensed in a 1.5-mL EP tube and washed twice with ice-cold PBS buffer. Then cell pellets were re-suspended in 200 μL of CHAPS lysis buffer and incubated on ice for 30 min. The mixture was centrifuged at 12,000 rpm for 20 min at 4 °C. The clear cellular lysates in the supernatant were transferred, aliquoted and stored at −80 °C for further use.

2.4. Colorimetric detection of telomerase

For colorimetric detection of telomerase with silver nanoprobes, 10 μL of 5 × TRAP buffer (100 mM Tris-HCl, 7.5 mM $MgCl_2$, 315 mM KCl, 0.025% Tween-20, 5 mM EGTA, pH 8.3), 5 μL of 10 mM dNTPs, 5 μL of 1 mg/mL BSA, 5 μL of DEPC-treated, deionized water, 20 μL of 3.5 nM consecutive cytosine-dTS-AgNP and 5 μL of HeLa cell lysates were mixed. The mixture was kept at 30 °C, followed by the measurement of UV–vis absorption spectra in a 50-μL cuvette with a time interval of 2 min or the visual observation. To visually distinguish active and inactive telomerase, telomerase extracted from 200 cells/μL HeLa was deactivated by heating at 90 °C for 10 min or by incubating with AZT (200 μM) for 6 h. The above treated telomerase and untreated telomerase were complexed with C20-dTS-AgNP to perform visual observation. To visually distinguish telomerase activity extracted from cancer cell (with HeLa cell as a model) and normal somatic cell (with normal liver cell as a model), lysates from the two model cells of 50