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Article

Chemistry

A label-free colorimetric assay for detection of *c-Myc* mRNA based on peptide nucleic acid and silver nanoparticles

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Abstract A label-free colorimetric protocol based on peptide nucleic acid/silver nanoparticles (PNA/AgNPs) has been initially proposed for specific recognition of mRNA. Making use of the controlled silver nanoparticles aggregation/dispersion by PNA/PNA–RNA complex, proto-oncogene *c-Myc* mRNA detection can be achieved. Moreover, the PNA/AgNPs platform can undergo color change in response to target *c-Myc* mRNA with single-base-mismatch sensitivity, which could further help in visually identify single nucleotide differences in target genes.

Keywords Colorimetric · *c-Myc* mRNA · Peptide nucleic acid · Silver nanoparticles

1 Introduction

Given the important role in functioning as oncogenes or tumor suppressors, mRNAs have emerged as potential biomarkers for cancer detection. In particular, abnormal expression of mRNAs is commonly observed at early stages of cancer development [1]. Therefore, sensitive detection of specific ribonucleic acid (RNA) transcripts has become a promising approach to achieving early clinical diagnosis of genetic diseases, such as tumor or cancer. Current methods for detecting specific mRNA include various types of biosensors with fluorescence [2], electrochemical [3] and electrochemiluminescence readouts [4] and amplified by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) [5]. Despite the fact that these admirably sensitive means are widely used, the tedious assay processes and costly detection systems have hindered their widespread use in practical biomedical use. Therefore, the development of a simple and low-cost method for identifying specific RNA sequences without any sophisticated instruments is a desirable clinical goal.

In this regard, gold nanoparticle (AuNP)-based visual detection assays have attracted fast-growing interest. Since the use of DNA oligonucleotide–AuNP conjugates was pioneered by Mirkin and co-workers [6], this platform has been applied for the detection of large variety of targets including nucleic acids, small molecules and cells [7–9]. From an optical sensing point of view, silver nanoparticles (AgNPs) are also good candidates besides AuNPs, which likewise exhibit a distance-dependent color and a high extinction coefficient. Recently, DNA-functionalized AgNPs have attracted more attention in the development of ultrasensitive biomolecules probes [10–14]. However, traditional methods for DNA-functionalized AgNPs were time-consuming with low efficiency. To solve this

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problem, Liu group [10] further developed a rapid method for DNA-functionalized AgNPs by tuning the pH of silver solution, resulting in a significant reduction in the total operation time. Xu group [11] have demonstrated a very fast and easy protocol for aptamer and ssDNA to connect to AgNPs without modulating pH or other complex conditions by using poly-adenine as stabilizer linked to one end of ssDNA. The greatest problem for the functionalization of AgNPs is its instability in the presence of NaCl. However, NaCl is always necessary in the functionalization process, so the functionalization of AgNPs has always been a major challenge for researchers.

Recent report has shown that peptide nucleic acid (PNA) oligomer was capable of altering the agglomeration properties of AgNPs [15], which serving as a very simple and promising colorimetric probe. PNA has the same hydrogen-bonding nucleobases as DNA, but they are attached to an uncharged *N*-(2-aminoethyl) glycine backbone [16], which confers superior target-binding characteristics and physicochemical robustness over native DNA probes [17]. Since the use of metal nanoparticles' color changes by aggregation/dispersion as a rapid colorimetric indicator of the PNA–DNA hybridization was reported by the Eritja group [18], label-free colorimetric assays based on PNA/metal nanoparticles have been well developed in biomarker detection [19].

The above-mentioned methods, however, employ PNA/AgNPs to perform the assay, and analytes are reported to be DNA only [15]. Herein, we design a label-free colorimetric sensor based on PNA/AgNPs for mRNA. The proof of concept is demonstrated using a human *c-Myc* mRNA initiation region as a model target, which is always found overexpressed in colon tumors [20]. Careful study of proto-oncogene *c-Myc* mRNA at early stages of tumor development assumes great importance in tumor therapy and diagnosis [21, 22]. In our proposed approach, making use of the controlled silver nanoparticles aggregation/dispersion by PNA/PNA–RNA complex, proto-oncogene *c-Myc* mRNA detection can be achieved. Moreover, the PNA/AgNPs platform can undergo color change in response to target *c-Myc* mRNA with single-base-mismatch sensitivity, which could further help in visually identify single nucleotide differences in target genes.

2 Experimental

2.1 Reagents

12-mer PNA (N′-GCA TCG TCG CGG-C′) with no modification at C- and N-terminals, target RNA of fully complementary (5′-CCG CGA CGA UGC-3′, RNAcom) and single-base-mismatch sequence (5′-CCG CGA GGA UGC-3′, RNAmis) were from KareBay. Biochem, Inc. 12-mer DNA (5′-GCA TCG TCG CGG-3′) was purchased from Sangon Biotech Co., Ltd. $HAuCl_4{\cdot}3H_2O$ (99.99 %) and $AgNO_3$ (99.9 %) were obtained from Alfa Aesar. Murine RNase inhibitor was from J&K Scientific Ltd. Trisodium citrate dihydrate (99.9 %) was obtained from Aldrich and $NaBH_4$ from Fluka. NaCl and EDTA were all of analytical grade and used without further purification.

2.2 Colloidal silver preparation

Prior to the colloidal preparation, the glassware was treated with aqua regia (1:3 HNO_3/HCl). Citrate-stabilized silver nanoparticles were prepared by the reduction of silver nitrate using sodium borohydride. Silver nanoparticles of (16.2 ± 0.5) nm were observed under transmission electron microscope (TEM) (Figs. S1 and S2 online), and concentrations of the AgNPs were 0.45 nmol/L, calculated according to Beer's law.

2.3 Characterization

Ninety-six-well microplates were used as reaction carrier. UV–Vis absorption characterization was performed using a Lambda 750 UV/Vis/NIR Spectrophotometer (PerkinElmer). The reproducibility of the UV absorption spectrum measurement (at room temperature) was evaluated through multiple scans of a given AgNPs solution aliquot in different wells. ζ potential measurements were taken on bare AgNPs and PNA-, DNA–RNA complex-, ssRNA- and PNA–RNA-coated AgNPs in water or in 0.20 mol/L NaCl, using a ZetaPLUS zeta potential analyzer (Brookhaven Instruments).

2.4 Assay procedure

Different concentrations of PNA probes were added to 200 μL of AgNPs solution to test the coagulating property of PNA and then allowed to stand for 10 min at the same temperature before recording of solutions color. The absorption spectra shown represent the average of four scans recorded in a wavelength range of 300–800 nm.

To evaluate the ability of PNA–RNA complex and DNA–RNA to protect AgNPs, the PNA (or DNA) probe and RNA target of equal amount were annealed in 10 mmol/L phosphate buffer (pH 7.2, containing 100 mmol/L NaCl and 0.1 mmol/L EDTA) for 10 min at 37 °C (final PNA concentration 1 μmol/L). In addition, to test PNA hybridization with RNA inside AgNPs solution, RNA was first mixed with AgNPs with 10-min incubation, and then, equal amount PNA was added.