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Nucleic acid quantification using nicking–displacement, rolling circle amplification and bio-bar-code mediated triple-amplification

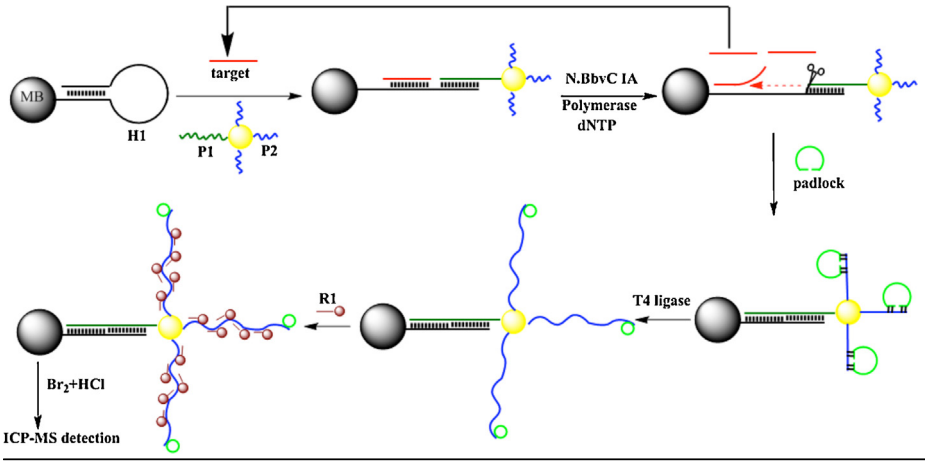
Xue-Mei Li [a,*], Jie Luo [b], Ning-Bo Zhang [a,b], Qing-Li Wei [b]

[a] School of Chemistry and Chemical Engineering, Linyi University, Linyi 276005, PR China
[b] State Key Laboratory Base of Eco-chemical Engineering, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, PR China

HIGHLIGHTS

- ICP-MS-based system was fabricated for the detection of DNA target.
- Triple-amplification by combination of nicking–displacement, RCA and bio-bar-code probes was coupled with ICP-MS.
- The strategy exhibits good sensitivity and high specificity.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present study, an inductively-coupled plasma-mass spectrometry (ICP-MS)-based triple-amplification system, by combination of nicking–displacement, rolling circle amplification (RCA) and bio-bar-code probes, was fabricated for the detection of DNA target. By using this system, hepatitis B virus (HBV) DNA target down to 3.2×10^{-17} M was detected by DNA probes labeled with Au nanoparticles (AuNPs). Single nucleotide polymorphisms in genes can also be effectively discriminated. In addition, we proved that this strategy is capable of detecting the target in complicated biological samples and holds great potential application in biomedical research.

1. Introduction

Ultrasensitive assay of nucleic acid have attracted substantial research efforts due to their broad applications in virus detection, transgenic detection, and early diagnosis of diseases [1,2]. A number of signal amplification strategies have been applied in

* Corresponding author. Tel.: +86 539 8766867; fax: +86 539 8766867.
E-mail address: xuemei_li@yeah.net (X.-M. Li).

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order to achieve the ultrasensitive detection of DNA at a low level, even in single molecule detection [3]. The most widely used amplification techniques for bioanalysis are based on enzyme cycle enhancement. These techniques include polymerase chain reaction (PCR) [4,5], ligase chain reaction (LCR) [6], rolling circle amplification (RCA) [7,8], Loop-mediated isothermal amplification (LAMP) [9,10], self-sustained sequence replication [11], endonuclease [12], exonuclease [13] and duplex-specific nuclease (DSN) [14,15]—induced amplification. Recently, multiple amplification approaches have developed to further improve the sensitivity [16,17]. Among the methods developed for the sensitive detection of DNAs, the fluorescence [18], electrochemistry [19] and surface-enhanced Raman scattering (SERS) [20] are the most widely used approach.

Inductively coupled plasma mass spectrometry (ICP-MS) is a promising tool in bioassay and clinical diagnosis with mass-based individual element and/or isotope resolution [21]. ICP-MS has unique features including a broad dynamic range, a signal that is independent of the chemical species, multi-elements detection capabilities, excellent stability and excellent mass resolution for metallic ions, making it an attractive detector for the quantification of nucleic acids [22,23]. Recently, oligonucleotide hybridization-based biospecific element/isotope-encoding strategy together with bio-amplification strategies, using ICP-MS have been reported to further improve the limit of detection of DNA. For example, Luo et al. have reported an effective ICP-MS-based multiplex detection of viral DNAs with lanthanide-coded oligonucleotide hybridization and RCA strategies [24]. Bruckner et al. demonstrated DNA quantification via ICP-MS using lanthanide-labeled Probes and LCR amplification, resulting in a 6000-fold increase of sensitivity [25]. However, there are few reports on the combination of multiple amplification methods with ICP-MS detection. In the present study, an ultra-sensitive triple-amplification system based on ICP-MS detection together with nicking–displacement, RCA and bio-bar-code probes, was developed for the detection of DNA. The combination of several molecular biological methods improved the sensitivity and the strategy was capable of detecting the target in complicated biological samples.

2. Experimental

2.1. Chemicals

All oligonucleotides used in our study were synthesized by SBS Genetech Co., Ltd., and their sequences were provided in Supplementary Table S1. The desired concentrations of DNA were prepared by serially diluting the stock standard solution. The ultrapure water was acquired from a water purification system (resistance $\geq 18\,M\Omega$ Chengdu ultrapure Technology Co. Ltd.) All the glassware was seriously washed with hydrochloric acid and hydrogen peroxide solution, washed with the pure water, then dried in the drying oven. Chloroauric acid ($HAuCl_4{\cdot}4H_2O$) was got from shanghai tops chemistry Co., Ltd. Trisodium-citrate was purchased from Tianjin Bodi Chemical Limited by Share Ltd. Sodium borohydride was purchased from Sinopharm Chemical Reagent Co., Ltd.

T4 DNA ligase, Nb.BbvC IA nicking endonuclease, Phi29 DNA polymerase, deoxyribonucleoside 5′-triphosphates (dNTPs) and bovine serum albumin (BSA) were purchased from Thermo Scientific. The carboxyl modified magnetic beads (MBs) (1 μm diameter, 10 mg/mL) were obtained from Tianjin Baseline Chromtech Research Centre. Tri(2-carboxyethyl) phosphine hydrochloride (TCEP, 98%) was purchased from Alfa Aesar (MA, USA). The buffer solutions are as follows: ligation buffer solution is the mixture of 40 mM Tris–HCl, 10 mM $MgCl_2$, 10 mM DTT and 0.5 mM ATP (pH7.8); reaction buffer is the mixture of 33 mM Tris-acetate, 10 mM Mg-acetate, 66 mM K-acetate, 1 mM DTT and 0.1% Tween 20 (pH7.9).

2.2. Instrumentation

To quantify Au, we used an Agilent 7500ce inductively coupled plasma mass spectro-meter (ICP-MS, Agilent Technologies, USA) equipped with a microconcentric nebulizer (MCN, Agilent Technologies, USA). Non-denaturing polyacrylamide gel electrophoresis (PAGE) was performed on DYCZ-28C electrophoresis power supply equipped with WD-9413A gel documentation & analysis systems from Beijing Liuyi Instrument Factory (Beijing, China). UV–vis absorption spectra were obtained with a Cary 50 Series Spectrophotometer (Varian, Australia). The sizes of the AuNPs were verified by transmission electron micrograph (TEM) using a JEOL JEM-2100EX microscope (Japan).

2.3. Preparation of 30 nm AuNPs

1% (w/v) $HAuCl_4$ solution was added into 50 mL of ultrapure water and heated to boiling for 20 min with stirring, and then appropriate volume of 1% (w/v) sodium citrate was added. The mixture was kept boiling for another 20 min until the color of solution unchanged, then kept stirring for 15 min. After cooling to room temperature, the AuNPs solution was placed in refrigerator. The final gold nanoparticles prepared by this method had an average diameter of approximately 30 nm by TEM (Fig. S1a in Supporting information).

2.4. Preparation of 5 nm AuNPs

Firstly, 0.6 mL of 1% $HAuCl_4$ and 0.2 mL of 0.2 M potassium carbonate solution were added into 40 mL ultrapure water (4 °C), and kept stirring for 5 min. Then 2 mL of 0.5 mg/mL sodium borohydride solution was added into above solution, and kept stirring until the color of solution turned into orange red. The final gold nanoparticles prepared by this method had an average diameter of approximately 5 nm by TEM (Fig. S1b and c in supporting information).

2.5. Preparation of bio-bar-code AuNPs labeled with ***P1*** and ***P2***

The process of bio-bar-coded AuNPs according to our previously reported method [26] was performed as follows: 20 μL of 1×10^{-5} M thiol modified **P1** and 160 μL of 1×10^{-5} M **P2** were mixed together, and then put 40 μL K-acetate buffer (pH 5.6) solution and 20 μL of 10 mM TCEP for 1 h, and then added to 2 mL of freshly prepared 30 nm AuNPs and shaken gently overnight. After reaction of 16 h, the DNA–AuNP conjugates were aged in salts (0.01 M NaCl, 5.0 mM tris-acetate buffer) for another 40 h. Excess reagents were removed by centrifuging at 15,000 rpm for 30 min. The oligonucleotide–AuNPs was then centrifuged at 10,000 rmp for 30 min to remove excess oligonucleotide and washed repeatedly for a total of three, then redispersed in 0.01 M phosphate buffer solution (7.4) for next use.

With reference to the above method, the reporter DNA modified AuNPs (5 nm) was obtained. The bio-bar-code probes and the reporter modified AuNPs were characterized by UV–vis absorbance (Fig. S2 in Supporting information).

2.6. Preparation of ***H1***-immobilized MBs

A 50 μL suspension of carboxyl MBs (10 mg/mL) was washed three times with 0.1 M imidazol-HCl buffer (pH 7.0, 3 × 100 μL). A 0.1 M EDC solution (100 μL) was added to the MB solution and the mixture was incubated at room temperature for 20 min to activate