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Highly sensitive DNA detection using cascade amplification strategy based on hybridization chain reaction and enzyme-induced metallization

Xu Yu [a], Zhi-Ling Zhang [b], Si-Yang Zheng [a,*]

[a] Micro & Nano Integrated Biosystem (MINIBio) Laboratory, Department of Biomedical Engineering, The Pennsylvania State University, University Park, PA 16802, USA

[b] Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), College of Chemistry and Molecular Sciences, and State Key Laboratory of Virology, Wuhan University, Wuhan, Hubei 430072, China

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ABSTRACT

A novel highly sensitive colorimetric assay for DNA detection using cascade amplification strategy based on hybridization chain reaction and enzyme-induced metallization was established. The DNA modified superparamagnetic beads were demonstrated to capture and enrich the target DNA in the hybridization buffer or human plasma. The hybridization chain reaction and enzyme-induced silver metallization on the gold nanoparticles were used as cascade signal amplification for the detection of target DNA. The metalization of silver on the gold nanoparticles induced a significant color change from red to yellow until black depending on the concentration of the target DNA, which could be recognized by naked eyes. This method showed a good specificity for the target DNA detection, with the capabilty to discriminate single-base-pair mismatched DNA mutation (single nucleotide polymorphism). Meanwhile, this approach exhibited an excellent anti-interference capability with the convenience of the magentic seperation and washing, which enabled its usage in complex biological systems such as human blood plasma. As an added benefit, the utilization of hybridization chain reaction and enzyme-induced metallization improved detection sensitivity down to 10 pM, which is about 100-fold lower than that of traditional unamplified homogeneous assays.

1. Introduction

In recent years, highly sensitive detection of specific DNA sequences associated with genetic and infectious diseases in complex media has become significantly important for early clinical diagnosis and gene therapy (Liu et al., 2013b; Ren et al., 2013). Due to the low abundance of disease specific DNA and the complexity of the biological samples, highly sensitive and selective approaches for DNA detection are required in clinical applications. To meet this challenge, many methods have been well developed, for example, electrochemical sensing (Kong et al., 2014; Liu et al., 2013a), fluorescence (Hu et al., 2013; Niu et al., 2010) and chemiluminescence detection (Li and He, 2009; Wang et al., 2013c), and föster resonance energy resonance energy transfer (FRET) (Liu et al., 2013b; Su et al., 2014; Xing et al. 2013). To further boost the performance, signal amplification strategies with various enzymes are usually adopted, such as exonuclease III-assisted amplification (Gao and Li, 2013; Luo et al., 2012), rolling circle amplification (Xu et al., 2012), and strand displacement amplification (Wang et al., 2011; Zhang et al., 2013). Among the various signal amplification strategies, the hybridization chain reaction (HCR) attracted particular attention because it does not need any enzymes or labeling processes (Dirks and Pierce, 2004; Huang et al., 2011; Niu et al., 2010; Wang et al., 2013a). Huang et al. developed a DNA-amplified detection method, which combined the amplification of HCR and the fluorescence emission-switching property of the hairpin probes that were modified with the pyrene molecules. In their system, two hairpin probes H1* and H2* dual labeled with pyrene moieties at each end were used. Chain reactions of hybridization between alternating H1* and H2* can propagate along the target DNA to form nicked double-helix. In this case, a pyrene moiety on one probe was brought into close proximity to a pyrene moiety on the neighboring probe, which could produce strong fluorescence.

The signal amplification based on metallization, especially silver metallization on the gold nanoparticles (AuNPs) have been used in the electrochemical biosensors and colorimetric detections (Lai et al., 2012; Nam et al., 2003; Xianyu et al., 2013). Mirkin and co-authors (Nam et al., 2003) developed a bio-barcode technology

* Corresponding author. Fax: +1 814 863 0490.
E-mail address: sxz10@psu.edu (S.-Y. Zheng).

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based on the polymerase chain reaction (PCR) amplification and silver metallization on the AuNPs for ultrasensitive detection of prostate-specific antigen (PSA). Jiang and co-authors (Xianyu et al., 2013) used glucose to reduce the growth of silver nanoparticles under the catalysis of the negatively charged AuNPs. Based on this, glucose detection in human plasma without any enzyme involvement was realized colorimetrically due to the generation of silver nanoparticles. Recently, a signal amplification strategy for highly sensitive colorimetric detection of avian influenza virus particles was reported (Zhou et al., 2014). The biometallization-based colorimetric assay was developed by combining the highly specific enzyme-induced silver metallization with the highly sensitive AuNPs induced silver deposition. The method was applied for the detection of alkaline phosphatase (ALP) and H_9N_2 avian influenza virus.

Magnetic beads have been widely used in biological sample preparation and detection assays due to their benefits of large surface-to-volume ratio, easy manipulation, flexible functionalization, and excellent biocompatibility (Chen et al., 2010; Yu et al., 2011, 2014, 2013). Owing to the ease of separation and enrichment of the targets, magnetic beads were widely used to improve the selectivity and sensitivity of various analytical methods. For example, they were successfully used in cell sorting (Adams et al., 2008), protein (Csordas et al., 2010) and virus detection (Ferguson et al., 2011; Zhao et al., 2012), and aptamer screening (He et al., 2014). Meanwhile, the magnetic beads modified with DNA could be used for detection of the complementary target DNA.

Inspired by these works, for the first time, we propose a highly sensitive and selective biosensor that combines HCR signal amplification with enzyme-induced metallization for colorimetric sequence-specific DNA detection. Superparamagnetic beads (SPMBs) were modified with a capture DNA and used as a solid carrier to catch the target DNA. The HCR was carried on with alternative DNA hybridizations of two biotin labeled stem-loop DNAs. The HCR and the following alkaline phosphatase induced silver metallization on the negatively charged gold nanoparticles constituted the cascade signal amplification for the target DNA detection. The metallization of the silver on the gold nanoparticles induced a significant color change from red of pure gold nanoparticles to yellow and black of the silver-covered AuNPs, which could be easily recognized by the naked eyes or measured by a UV–vis spectrometer. This novel approach could discriminate between single-base mismatched DNA and double-base mismatched DNA, which demonstrated a great specificity for target DNA detection. Furthermore, this biosensor showed a great anti-inference capability and could be used in complex biological samples without any sample pre-treatment. We believe this method for the target DNA detection is well suitable for the point-of-care diagnosis in future clinical trials.

2. Materials and methods.

2.1. Chemical reagents

L-glutathione (GSH), hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4$), silver nitrate ($AgNO_3$) and sodium tetrahydridoborate ($NaBH_4$) were purchased from Alfa Aesar. Superparamagnetic beads (SPMBs, 500 nm diameter) with core iron oxide nanoparticles (70% by weight) and carboxyl groups on the surface were obtained from Ademtech SA (Pessac, France). N-(3-dimethyl-aminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) and diethanolamine (DEA) were purchased from Alfa Aesar. Target DNA was purchased from Life technologies. All the other DNA samples were obtained from Bio Basic Inc., Canada. Strepavidin–alkaline phosphatase conjugate (SA–ALP) was purchased from Vector Laboratories Inc. (Burlingame, CA, USA). 4-Aminophenyl phosphate · sodium salt (4-APP) was obtained from Enzo Life Sciences, Inc. (Farmingdale, NY, USA). Bovine serum albumin (BSA), Tween 20 and Tris(hydroxymethyl)aminomethane hydrochloride (Tris · HCl) and 2-(N-morpholino) ethanesulfonic acid hydrate (MES) were obtained from Sigma-Aldrich. The human blood samples were collected in EDTA tubes from healthy donors according to approved institutional review board (IRB) protocol (IRB protocol number: 31216). Plasma was obtained after removal of blood cells and stored at −20 °C until use. All the other chemical reagents not mentioned here were obtained from VWR (Radnor, PA, USA) and used without further purification.

2.2. Conjugating capture DNA (C_{DNA}) to SPMBs

The SPMBs with carboxyl groups were functionalized with C_{DNA} according to our previous reports (He et al., 2014; Yu et al., 2011, 2014, 2013). Briefly, 40 μL of 50.0 mg/mL SPMBs were first washed three times with MES buffer (50.0 mM MES, pH=6.0). Then, 10 μL of 100 μM C_{DNA} was added and incubated for 30 min at room temperature. Afterwards, 60 μL of 10 mg/mL EDC dissolved in cold MES buffer was added into the above mixture solution to the final volume of 460 μL. The reaction of the carboxyl groups and the amine groups was allowed for 12 h with slow tilted rotation of 120 rpm at room temperature. After that, the C_{DNA} modified SPMBs (C_{DNA}–SPMBs) were separated by a magnet (DynaMag™-Spin, Life Technologies), and washed three times with DNA wash buffer (10 mM Tris–HCl, 0.2 M NaCl, 0.05% Tween 20, pH 8.0). The C_{DNA}–SPMBs were kept in 1 mL TE buffer (10 mM Tris–HCl, 1 mM EDTA, 0.05% Tween 20, pH 7.5) at 4 °C until further experiments.

2.3. Synthesis of GSH-capped AuNPs

GSH-capped AuNPs (GSH–AuNPs) was prepared according to the procedure described previously with some minor modification (Brinas et al., 2008; Chai et al., 2010; Kumar et al., 2013). The preparation scheme (Fig. S1) was shown in Supplementary information S2. The as-prepared GSH–AuNPs was purified by Amicon Centrifugal Filter Unit (MWCO 30 kDa) to remove free agents and then dispersed in ultrapure water and stored at 4 °C until further experiments. The TEM characterization of the GSH–AuNPs was shown in Fig. S2 (Supplementary information S3). The size of the GSH–AuNPs was measured to be ~6 nm. The concentration of the GSH–AuNPs was determined by measuring the surface plasmon resonance (SPR) absorption peak at 520 nm as previously reported (Liu et al., 2007). The GSH–AuNPs were stable and could tolerate a high concentration of many metal ions except the Pb^{2+} (Chai et al., 2010).

2.4. Cascade amplification for detection of target DNA (T_{DNA})

A one-step hybridization reaction was performed by mixing the C_{DNA}–SPMBs modified SPMBs (C_{DNA}–SPMBs), the target DNA (T_{DNA}) and the Link DNA (L_{DNA}) together. In a typical experiment, 40 μL of 2 mg/mL C_{DNA}–SPMBs were incubated in 1% BSA (0.01 M PBS, pH 7.4, 0.05% NaN_3) for 5 min in order to block the nonspecific adsorption. After rinsing, 50 μL of T_{DNA} at various concentrations, 10 μL of 10 μM L_{DNA} and 340 μL DNA hybridization buffer were added into the C_{DNA}–SPMBs and incubated for 30 min at room temperature with gentle shaking. The hybridization was carried out for 30 min at room temperature, which should be sufficient for hybridization between short complementary DNA (~30 base pairs) sequences (Chen et al., 2014; Hu et al., 2013; Liu et al., 2013b). Then, the hybrid-conjugated SPMBs were washed three times with the washing buffer (Tris-HCl 10 mM, Tween-20 0.01%, NaCl 100 mM, pH 7.5). Afterwards, the hybrid-conjugated SPMBs were