Sensors and Actuators B 265 (2018) 285–292

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Colorimetric detection of *Listeria monocytogenes* using one-pot biosynthesized flower-shaped gold nanoparticles

Juan Du [a,b,c,d], Hina Singh [b], Wei-jie Dong [e], Yan-hong Bai [a,c,d], Tae-Hoo Yi [b,*]

[a] College of Food and Bioengineering, Zhengzhou University of Light Industry, Zhengzhou, 450001, China
[b] Department of Oriental Medicinal Biotechnology, College of Life Science, Kyung Hee University Global Campus, 1732 Deokyoungdaero, Giheung-gu, Yongin-si, Gyeonggi-do, 446-701, Republic of Korea
[c] Henan Key Laboratory of Cold Chain Food Quality and Safety Control, Zhengzhou, China
[d] Henan collaborative Innovation Center of Food Production and Safety, Zhengzhou, China
[e] The Isotope Research Institute Ltd of Henan Academy of Science, Zhengzhou, 450001, China

ARTICLE INFO

Article history:
Received 3 July 2017
Received in revised form 8 March 2018
Accepted 13 March 2018
Available online 14 March 2018

Keywords:
Colorimetric assay
Flower-shaped gold nanoparticles
Listeria monocytogenes
Biosynthesis
One-pot

ABSTRACT

The present study described a novel fast, simple and green one-pot synthesis approach of flower-shaped gold nanoparticles (flower-shaped AuNPs) by using antioxidant plant extracts. The AuNPs synthesized by *Alchemilla mollis* extract showed maximum absorbance at 610 nm and were polydispersed flower-shaped with average size (±SD) of 68.7 ± 2.8 nm. We have explored a colorimetric sensing assay for rapid detecting *Listeria monocytogenes* by using single-stranded DNA (ssDNA) conjugated flower-shaped AuNPs. ssDNA were used as probe for DNA detection and the synthesized flower-shaped AuNPs were used as indicators. The limit of detection for *hlyA* gene and genomic DNA of *Listeria monocytogenes* were 48.4 ng and 100.4 ng, respectively. The proposed assay is fast and specific for *Listeria monocytogenes* detection, with the improved color change and stability.

1. Introduction

Nanoparticles have been rapidly developed and widely applied in various fields due to their specific characteristics such as relatively large surface area as compared with bulk materials, highly optical, electronic, and catalytic properties. Among which, gold nanoparticles (AuNPs) have been extensively attractive because they possess a unique size- and shape-dependent optical property known as localized surface plasmon resonance (LSPR) and their excellent suitability for fabrication of surface-enhanced Raman scattering (SERS) substrates [1–3]. AuNPs offer strong and well-defined colors, and can be aggregated to induce a color change and red shift of the SPR depending on the aggregation degree [4]. Based on these exhibiting optical properties, AuNPs have been used extensively as colorimetric biosensors, which have attracted considerable attention as a simple and rapid visual detection assay [5–9]. The key to the AuNP-based colorimetric sensing platform is the control of AuNPs aggregation and dispersion stages by using certain ligand, for instance, antibodies and aptamers. Previously reported colorimetric biosensors are mostly relying on sphere-shaped AuNPs which usually synthesized by classical Turkevich method using sodium citrate [10]. The optical properties of AuNPs can be further exploited by varying their shape, size and surface characteristics. Special interest has been focused on anisotropic shapes, especially flower-shaped AuNPs owing to the high electromagnetic field localized at their branches, which influences the plasmon resonances and SERS properties [11,12]. However, there are rarely reports about using flower-shaped AuNPs as indicators in colorimetric assay [13]. As far as we are aware, the possibility of using flower-shaped AuNPs functionalized with single-strand DNA (ssDNA) for colorimetric sensing has not been explored yet.

Nowadays, the synthesis of flower-shaped AuNPs can be achieved via seeded growth or non-seeded approaches. The seeded pathway requires preformation of small precursor AuNPs as seeds, and then applied to a secondary solution containing an additional metal salt which can promote the growth of branches. This method involves multiple steps and relatively expensive procedures and is time consuming [14–16]. The non-seeded (one-pot) approach can be completed in a single step, but generally, a reagent acting as a shape-controlling surfactant is needed. The drawbacks are the necessity of supplementary procedures for removal of the surfactant from the surface of AuNPs and the difficulty with controlling parameters of the process [17,18]. The two approaches are usually

* Corresponding author.
E-mail address: drhoo@khu.ac.kr (T.-H. Yi).

https://doi.org/10.1016/j.snb.2018.03.067
0925-4005/

prepared by chemical reducing agents at high temperatures. With a growing need of eco-friendly process of nanoparticles synthesis, biological approach of synthesis AuNPs employing plant extract is termed as eco-friendly and received tremendous attention [10]. Considering this, the present study highlighted the one-pot synthesis of flower-shaped AuNPs by plants extract at room temperature without using shape-controlling surfactant.

Listeria monocytogenes (*L. monocytogenes*) is a Gram-positive, facultatively anaerobic bacterium, with the ability to adapt to a wide range of conditions such as acidic foods, high salt foods, refrigeration temperatures (2–4 °C), and within the host immune system [19,20]. *L. monocytogenes* is one of most virulent foodborne pathogens, responsible for many outbreaks related to the food products consumption [21]. Documented food item sources for human disease have included meat products, vegetable and fish, particularly *L. monocytogenes* is an emerging bacterial pathogen in meat and meat product. Taking *L. monocytogenes* contaminated foods may lead to listeriosis, primarily causes infections of the central nervous system, and other symptoms such as, meningitis, encephalitis, septicaemia and miscarriage [22]. *L. monocytogenes* can grow and reproduce inside the host's cells, and the fatality rate is approached 30%, which far exceeds other foodborne pathogens [23]. Therefore, the fast, specific and highly sensitive methods are greatly needed for *L. monocytogenes* detection.

In this study, a specific, rapid and simple method has been established for detection of *L. monocytogenes*. Unlike previously reported works where flower-shaped AuNPs were synthesized by chemical reagents, the synthesis was hereby achieved by means of plant extracts, which used both as reducing and surfactant agent for the synthesis of flower-shaped AuNPs. We evaluated the applicability of single-stranded DNA (ssDNA) functionalized flower-shaped AuNPs as colorimetric sensors for *L. monocytogenes* determination. At the time of writing, this is the first time biosynthesized flower-shaped AuNPs which functionalized with ssDNA are used as a colorimetric sensor to detect bacterial pathogens.

2. Materials and methods

2.1. Materials

$HAuCl_4$ (99.999%), ethanol, sodium chloride (NaCl), ribonuclease A, ribonuclease T1, lysozyme and isopropanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). CTAB extraction solution and phenol/chloroform/isoamyl alcohol solution (PCI; 25:24:1) were purchased from Biosesang (Seoul, Korea). Proteinase K was purchased from Bioshop (Burlington, Ontario, Canada). Primers, PCR PreMix kit and PCR product purification kit were purchased from Bioneer (Daejoun, Korea). The pathogenic species and non-pathogenic species were obtained from Korean Collection for Type Culture (KCTC; Daejoun, Korea) and Korea Agriculture Culture Collection (KACC; Suwon, Korea). Culture media were procured from Oxoid Ltd. (Basingstoke, England). Unless otherwise mentioned, solvents were purchased from Samchun Chemicals (Seoul, Korea). In this work, a total of twenty-five plant extracts (Table S1) were studied to synthesize flower-shaped AuNPs. The selection of those plant was depending on the previous study of our lab, and related papers which reported their antioxidant activities. Dried antioxidant plants were purchased from Mountain Rose Herbs (Eugene, Oregon, USA) and Damaon (Daehan oriental medicine, Uiwang, Korea).

2.2. Preparation of plant extracts and synthesis of AuNPs

Antioxidant plants were extracted for 24 h at room temperature in 50% ethanol with a ratio of 1:10 (w/v) for three times. The extract was centrifuged and the supernatant obtained was filtered (Whatman 5, USA). The filtrate was evaporated under vacuum and then subjected to freeze drying (FDCF-12012; Operon Co., Gimposi, Korea) for 3 days. The plant extracts were then dissolved in tri-distilled water (DDW) at concentration of 2 mg/mL as stock, the solution were filtered through a 0.45 μm PVDF syringe filter (SmartPor, Seoul, Korea). Aqueous solution of $HAuCl_4$ (at a final concentration of 1 mM) and the prepared plant extracts (at a final concentration of 120 μg/mL) were mixed in DDW. The mixtures were shaken at 600 rpm at room temperature for different time periods. The synthesis of AuNPs was indicated by color change of the reaction mixtures, and confirmed by UV–vis spectrophotometer, between the wavelength range of 300–800 nm (Optizen POP; Mecasus, Daejeon, Korea). Unreacted $HAuCl_4$ and plant extracts were removed by pelleting, and purified by repeated centrifugation. The resulting AuNPs were resuspended in DDW, and used for further characterization.

2.3. Characterization of AuNPs

The morphology of the AuNPs was analyzed by using Field emission transmission electron microscopy FE-TEM (JEM-2100F, JEOL), sample was prepared by placing a drop of AuNPs dispersed in water on carbon coated copper grid and subsequently drying at oven, then transferred it to FE-TEM and operated at a voltage of 200 kV. The hydrodynamic size of AuNPs were investigated by dynamic light scattering (DLS analysis), and the charge of the AuNPs were investigated by zeta potential, using Malvern Zetasizer Nano ZS90 (Malvern Instruments, Worcestershire, UK), DDW was used as dispersive medium. The crystal structure of the synthesized AuNPs was detected by a Bruker D8 Advance X-ray diffraction (XRD) operated at 40 kv with Cu Kα radiation. In addition, Fourier Transform infrared spectroscopy (FTIR) was employed to investigate the role of possible functional groups of plant extract that were responsible for the reduction of Au^{3+} to Au^0 and the functional groups capped on surface of the AuNPs, infrared spectrum was recorded from 4000/cm to 450/cm (Spectrum One System, Perkin-Elmer, Waltham, MA, USA). For XRD and FTIR analysis, dried powder of the AuNPs was prepared,

2.4. Preparation of AuNP-probe

A pair of 18 base thiolated oligonucleotide (5′HS-CCT AAG ACG CCA ATC GAA-3′) and (5′ HS-AAG CGC TTG CAA CTG CTC-3′) were designed as the probe based on the *hlyA* gene of *L. monocytogenes*. The conjugation of the flower-shaped AuNPs and probe were performed by low pH-assisted method [24,25]. Briefly, 20 nmol of each thiolated oligonucleotide probes were incubated with 1 mL flower-shaped AuNPs (1 mM), mixed thoroughly and standing for thirty minutes, a final of 10 mM citrate buffer (pH 3) was added to accelerate probe attachment. The mixture was stood for ten minutes and sonicated for one minute. Then the flower-shaped AuNP-probe conjugates were washed three times with DDW by centrifuging, and stored in dark at 4 °C until use.

2.5. Bacterial DNA extraction and amplification of hlyA region

E. coli and *L. monocytogenes* were cultured at 37 °C, genomic DNA of the strains was prepared as described previously [26]. Briefly, bacteria were collected and suspended with Tris-EDTA buffer and 10% SDS, then continuous treated with lysozyme, Proteinase K, Ribonuclease A and Ribonuclease T1 at 37 °C to digest bacteria, protein and RNA. After all the enzyme treatment, the solutions were added with NaCl (5 M/L) and CTAB incubated at 65 °C for 20 min. Then PCI was added to the above solution and centrifuge for 5 min, the supernatant which contains DNA was treated with isopropanol