Biosensors and Bioelectronics 75 (2016) 55–58

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

SERS encoded nanoparticle heterodimers for the ultrasensitive detection of folic acid

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ARTICLE INFO

Article history:
Received 1 June 2015
Received in revised form
6 August 2015
Accepted 7 August 2015
Available online 8 August 2015

Keywords:
Folic acid
Biosensing
SERS
Heterodimer
Nanoparticles

ABSTRACT

In this paper, gold–silver nanoparticle (AuNP–AgNP) heterodimers were assembled with highly yield as an active SERS substrate, based on antigen–antibody immunoreaction. The developed SERS sensor has successful achieved the ultrasensitive detection of folic acid (FA) with the limit of detection (LOD) as 0.86 pg/mL. And the linear range was from 0.005 ng/mL to 1 ng/mL. The results also demonstrated that this developed method showed high specificity and excellent recovery for the human serum samples, indicating its promising potential in bio-diagnosis and the environmental monitoring.

1. Introduction

Folic acid (FA), known as Vitamin B9, pteroylglutamic acid (PGA) or folate, is a water-soluble vitamin and has been confirmed as a significant nutrient in humans and animals (Ren et al., 2011). In humans, the lack of FA may cause physical weakness, irritability and loss of appetite, even leading to megaloblastic anemia and leucopenia, and an increased risk of cancer. Adequate FA also plays an important role in pregnant women helping to prevent some birth defects such as infant cleft palate and brain malformations (Branum et al., 2013). Therefore, FA detection is critical in preventing diseases related to FA deficiency.

A large number of methods have been developed to detect FA, including enzyme-linked immunosorbent assays (Zhang et al., 2012), electrochemistry (Karimi-Maleh et al., 2013), high performance liquid chromatography (HPLC) (Araya-Farias et al., 2014), chemiluminescence (Wabaidur et al., 2013), electrochemical nanosensors. However, those assays are either expensive, time consuming or not sensitive enough for the detection of low FA concentrations.

SERS is widely used in chemical and biological analysis (Indrasekara et al., 2014; Kneipp et al., 2008; Kodiyath et al., 2013; Palonpon et al., 2013), and has high sensitivity due to an electromagnetic enhancement mechanism in the hot spot between metal NPs for SERS (Kleinman et al., 2013; Wei and Xu, 2013; Ma et al., 2013). In addition, a SERS reporter (an organic Raman reporter molecule) conjugated to nanosubstrates can provide a strongly enhanced SERS signal which may improve the sensitivity (Wang et al., 2013).

SERS activity is largely dependent on the substrate with high electromagnetic enhancement. Our recent study demonstrated the strong plasmonic coupling of the NPs assemblies, especially for the AgNPs nanostructures, which could realize the ultrasensitive biosensing (Wu et al., 2013; Xu et al., 2015). In the present study, SERS encoded AuNP–AgNP heterodimers were controllably assembled with high yield, taking the advantage of strong optical coupling and intense hot spots of NPs heterodimers (Campion and Kambhampati, 1998; Kleinman et al., 2013; Nie and Emory, 1997; Xu et al., 2015), as well as the high affinity of antigen–antibody immune recognition (Wu et al., 2013). This developed SERS sensor based on the AuNP–AgNP heterodimers substrate has achieved the the analysis of FA for the first time, with the lowest LOD of 0.86 pg/mL reported so far.

2. Experimental section

2.1. Materials

Tetrachloroauric acid, sodium citrate, silver nitrate, bis(p-sulfonatophenyl) phenyl-phosphine dihydrate, dipotassium salt, Polyvinyl Pyrrolidone (PVP), and sodium borohydride were purchased

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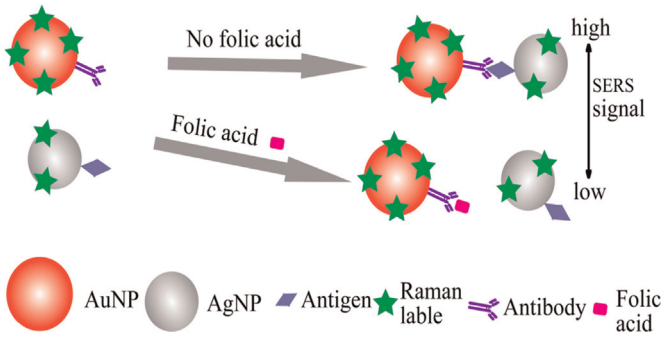
http://dx.doi.org/10.1016/j.bios.2015.08.009
0956-5663/

from Sigma-Aldrich (St. Louis, MO, USA). All the chemicals were used as received. The monoclonal antibody against FA and antigen to FA were prepared by our laboratory according to the classic procedure (Kohler and Milstein, 1975; Wu et al., 2015; Yan et al., 2015). The antigen was the conjugates of FA-BSA, and the half-maximal inhibitory concentration (IC50) of the monoclonal antibody against FA by ELISA was 0.2 ng/mL.

2.2. Apparatus

Deionized (DI) water from a Milli-Q device was used throughout this study. Transmission electron microscopy images were acquired by using JEOL JEM-2100 transmission electron microscope. A Lab-Ram-HR800 Micro-Raman spectrometer was used to obtain the SERS spectra, and assembly sizes were achieved using dynamic light scattering (DLS, Malvern Instruments). UV–vis spectra were measured via a UNICO 2100PC UV/vis spectrophotometer. All the data were processed using Origin Lab software.



Scheme 1. Scheme for SERS detection of FA based on AuNP–AgNP heterodimers.

2.3. Synthesis of AuNPs

AuNPs (25 ± 2 nm) were prepared by the reduction of $HAuCl_4$ (Ji et al., 2007). In brief, 1.25 mL of 4 g/L $HAuCl_4$ solution was added to 48.75 mL Milli-Q water under gentle stirring, then was heated to 350 °C followed by the boiling for 2–3 min. Then, 0.8 mL of 10 mg/mL sodium citrate was then injected into the boiling solution under vigorous stirring until the color of the solution turned to reddish-orange. After the solution was cooled to ambient temperature, 1.5 mg of bis(p-sulfonatophenyl) phenyl-phosphine dihydrate, dipotassium salt (BPS) was added to 50 mL of AuNPs solution and then stirred at room temperature for 10 h, to ensure that the AuNPs were well-dispersed in a high ionic strength solution. The prepared AuNPs were then stored at 4 °C (Loweth et al., 1999; Schmid and Lehnert, 1989).

2.4. Synthesis of AgNPs

AgNPs (10 ± 2 nm) were synthesized following a previously described method with some modifications (Liu et al., 2009; Sun and Xia, 2002; Xia et al., 2003). Fresh iced $NaBH_4$ (0.6 mL of 0.1 M) and of PVP (5 mL, 1%) were added to 20 mL Milli-Q water with stirring in an external ice bath. Then 5 mL of 1% PVP and 5 mL of 10 mM $AgNO_3$ were injected into the prepared solution by two constant-flow pumps at the speed of 30 mL/h. The obtained solution was kept at 80 °C in a water bath for 2 h and turned bright yellow in color. The solution was maintained at 4 °C.

2.5. Assembly of AuNP–AgNP heterodimers

To form the AuNP–AgNP heterodimers, the concentration of AuNPs and AgNPs were both adjusted to 30 nM in 0.01 M phosphate buffer (pH=7.5). Under the slight stirring, the monoclonal antibody against FA (1.6 μL, 5 mg/mL) and antigen (0.8 μL, 4 mg/mL) were added into the AuNPs and AgNPs, respectively (Wu et al., 2013). Next, 2 μL of Raman reporter molecule, 4-aminothiophenol

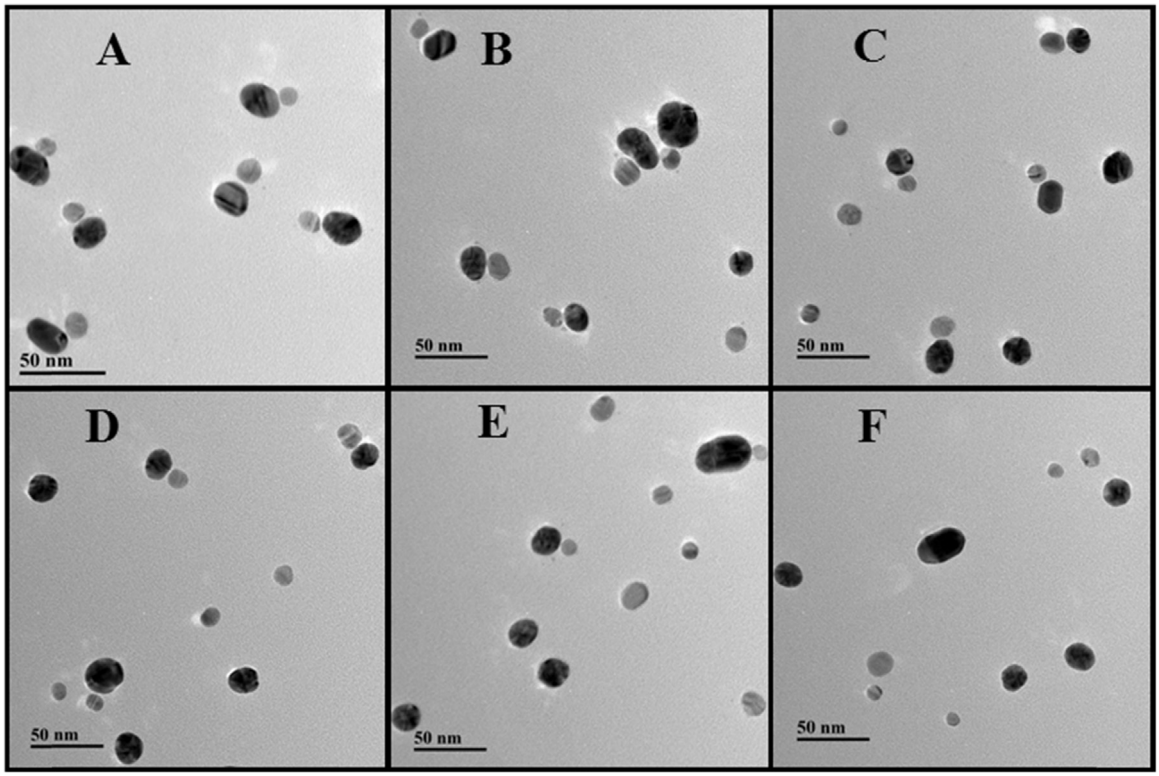


Fig. 1. TEM images of AuNP–AgNP heterodimers with different concentration of FA. (A) 0 ng/mL, (B) 0.005 ng/mL, (C) 0.01 ng/mL, (D) 0.05 ng/mL, (E) 0.1 ng/mL, (F) 1 ng/mL.