Biosensors and Bioelectronics 40 (2013) 121–126

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

Electrochemical detection of *Salmonella* using gold nanoparticles

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ARTICLE INFO

Available online 16 July 2012

Keywords:
Gold nanoparticles
Magneto-immunoassay
Salmonella
Electrochemical detection
Label

ABSTRACT

A disposable immunosensor for *Salmonella enterica* subsp. enterica serovar Typhimurium LT2 (S) detection using a magneto-immunoassay and gold nanoparticles (AuNPs) as label for electrochemical detection is developed. The immunosensor is based on the use of a screen-printed carbon electrode (SPCE) that incorporates a permanent magnet underneath. *Salmonella* containing samples (i.e. skimmed milk) have been tested by using anti-*Salmonella* magnetic beads (MBs-pSAb) as capture phase and sandwiching afterwards with AuNPs modified antibodies (sSAb-AuNPs) detected using differential pulse voltammetry (DPV). A detection limit of 143 cells mL^{-1} and a linear range from 10^3 to 10^6 cells mL^{-1} of *Salmonella* was obtained, with a coefficient of variation of about 2.4%. Recoveries of the sensor by spiking skimmed milk with different quantities of *Salmonella* of about 83% and 94% for 1.5×10^3 and 1.5×10^5 cells mL^{-1} were obtained, respectively. This AuNPs detection technology combined with magnetic field application reports a limit of detection lower than the conventional commercial method carried out for comparison purposes in skimmed milk samples

1. Introduction

Foodborne disease has been a serious threat to public health for many years and still remains a public health problem (WHO, 2011). *Salmonella* is one of the most frequently occurring pathogens in food affecting people's health (Newell et al., 2010). This bacteria is transmitted to humans mainly through the consumption of contaminated food of animal origin such as milk, meat and eggs.

According to World Health Organization (WHO) in the United States of America (USA), for instance, around 76 million cases of foodborne diseases, resulting in 325,000 hospitalizations and 5,000 deaths, are estimated to occur yearly (WHO, 2011). In 2011, more than 10 outbreaks comprising hundreds of patients were reported by Centers for Disease Control and Prevention (CDC) originated in the ingestion of *Salmonella*-contaminated food, leading to medical costs of thousands of dollars (CDC, 2011).

The methods recommended by International agencies of food health control and International Organization of Standardization for *Salmonella* detection in food samples (ICMSF, 2002; ISO, 2002) are the classical culture methods. These methods can give qualitative and quantitative information, however, a pre-treatment of the samples is needed; furthermore they are greatly restricted by the assay time at locations in the food processing or distribution network, to achieve an earlier detection. Furthermore, to perform them, it is necessary to employ highly skilled people and more than three days, which exclude their use in field applications. The development of new methodologies with faster response time, better sensitivity and selectivity and easy multiplexing is still a challenge for food hygiene inspection.

In recent years, new technologies have been developed in order to improve the time of analysis of the traditional culture detection. These technologies are mainly based on polymerase chain reaction (PCR) and immunoassays. Moreover, biosensor technologies have been used as potential alternatives to circumvent the bottlenecks of the standard method because they have rapid response time and furthermore they are sensitive, robust, portable and easy to use (Liebana et al., 2009a; Liebana et al., 2009b; Mata et al., 2010; Salam and Tothill, 2009).

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http://dx.doi.org/10.1016/j.bios.2012.06.054

The electrochemical detection methods possess several advantages such as easy operation, low cost, high sensitivity, simple instrument and suitability for portable devices. Currently, we are observing a noticeable growth in AuNPs as electrochemical label for immunoassay (De la Escosura-Muñiz et al., 2010). This electrochemical approach is based on the adsorption of AuNPs on the surface of the electrotransducer, electrooxidation of the AuNPs to Au(III), and reverse electroreduction to Au(0), which generates cathodic peak constituting the analytical signal. The AuNPs as a label in connection to magnetic particles and screen-printed carbon electrodes (SPCEs) was also shown to be a very useful alternative for proteins detection (De la Escosura-Muñiz et al., 2011). However, this technology has not been used for the screening of pathogenic organisms.

Nanomaterials have received special attention in the development of novel biosensing systems (Merkoçi, 2010). Particularly nanoparticles have shown to bring interesting advantages for DNA (Merkoçi et al., 2005), proteins (De la Escosura-Muñiz et al., 2010) and even cells (Perfézou and Merkoçi., 2012) analysis. Our group has already shown the effectiveness of AuNPs for ICP-MS linked (Merkoçi et al., 2005a) and electrochemical (Pumera et al., 2005) DNA assays, electrochemical and optical detections of human IgG (Ambrosi et al., 2007), CA 15-3 glycoprotein (mainly used to watch patients with breast cancer) (Ambrosi et al., 2010) and even of human tumor HMy2 cells (De la Escosura-Muñiz et al., 2009).

Herein, a rapid and sensitive strategy for *Salmonella* detection, that takes advantages of AuNPs used as labels and magnetic particles as preconcentrators, is developed and shown to be effective enough even for real sample applications. In this approach the bacteria are captured from the samples (i.e. skimmed milk) and preconcentrated by immunomagnetic separation, followed by labeling with AuNPs modified with a polyclonal anti-*Salmonella* antibody. Then, the modified MBs are captured by applying a magnetic field below the SPCE used as transducer for the electrochemical detection.

Although other electrochemical biosensing strategies for *Salmonella* detection based on nanoparticles (Noguera et al., 2011), carbon nanotubes (Zelada-Guillen et al., 2010) etc. have already been developed (see Table SI-1 in Supporting Information Section) the proposed AuNPs electrochemical labeling strategy is previewed to be of special interest for future in field applications given the robustness of the electrochemical system in general and that of nanoparticles particularly.

2. Experimental section

2.1. Materials and apparatus

All voltammetric experiments were performed using an electrochemical analyzer Autolab 20 (Eco-Chemie, The Netherlands) connected to a personal computer using a software package GPS 4.9 (General Purpose Electrochemical System). A thermoshaker TS1 (Biometra) was used to stir the samples operating at controlled temperature. Transmission Electron Microscope (TEM) images were taken with Jeol JEM-2011 (Jeol Ltd., Japan). Scanning electrochemical microscopy (SEM) images were acquired using a Field Emission-Scanning Electron Microscopy (Merlin, Carl Zeiss).

The electrochemical transducers were homemade screen-printed carbon electrode (SPCEs), which are constituted by three electrodes in a single strip: carbon working electrode (WE) with diameter of 3 mm, Ag/AgCl reference electrode (RE) and carbon counter electrode (CE). A magnet (3 mm in diameter), inserted under the WE, was also used to accumulate the complex formed due to magnetic beads modification with anti-*Salmonella* first capturing antibody, *Salmonella* and AuNPs modified with anti-*Salmonella* rabbit polyclonal second antibody (MBs-pSAb/S/sSAb-AuNPs) and used later during the electrochemical measurements.

All glassware used in the synthesis of AuNPs was washed with aqua regia overnight and the rinsed carefully with milli-Q water.

2.2. Reagents and solutions

Anti-*Salmonella* magnetic beads modified with the first capturing antibody (MBs-pSAb) (Prod. no.° 710.02) was purchased from Dynal Biotech ASA (Oslo, Norway) and Anti-*Salmonella* rabbit polyclonal second antibody (sSAb) (Prod. no. 01.91.99) was from Biogen scientific (Madrid, Spain). *Salmonella enterica* subsp. enterica serovar Typhimurium LT2 (CECT 722T) and *Escherichia coli* K-12 (CECT 433) strains were purchased from "Colección Española de Cultivos Tipo (CECT)", Bovine serum albumin, Hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$, 99.9%), trisodium citrate, were purchased from Sigma-Aldrich (St. Louis, MO). Millipore milli-Q water was obtained from purification system (18.2 M cm). The buffers were prepared in deionized water: PBS buffer 10 mM pH 7.4 with 2.7 mM KCl, and 137 mM NaCl; PBS–Tween buffer (PBS buffer pH 7.4 with tween 20% (m/v)). Samples for SEM analysis were prepared by using glutaraldehyde and hexamethyldisilazane (HMDS) microscopy grade solutions, Sigma-Aldrich (Spain). The electrochemical measurements were performed in a 0.2 M HCl solution. Finally, all reagents and other inorganic chemicals were supplied by Sigma-Aldrich or Fluka, unless otherwise stated.

2.3. Bacterial strains, inocula preparation

Freeze-dried cultures of *Salmonella* and *E. coli* were revived in Tryptone Soy Broth (TSB, Oxoid Ltd., Basingstoke, Hampshire, UK). Stock cultures of both strains were prepared on Tryptone Soy Agar (TSA, Oxoid), incubated at 37 °C for 24 h and stored at 4 °C for a maximum time of 9 weeks. Stock cultures were subcultured into 10 mL of TSB and incubated at 37 °C for 20 h. After incubation, the broth was spread using a disposable loop on TSA plates and incubated at 37 °C for 20–24 h. Subsequently, cell suspensions were prepared in 10 mL of PBS–Tween to obtain 9.–9.5 log cells mL^{-1}. Tubes were placed into a boiling water bath (100 °C) for 15 min and they were cooled to room temperature prior to immunological testing. To determine the load of cells before the heat treatment dilutions were prepared in buffered peptone water (Oxoid). Then, 1 mL of these dilutions was placed as duplicate in TSA (Oxoid) and incubated at 37 °C for 24 h.

2.4. Synthesis of gold nanoparticles (AuNPs)

The Turkevich synthesis generates AuNPs of 20 nm (Fig. SI-1). First a solution of 0.508 mL $HAuCl_4$ (1% m/v) in 49.492 milli-Q water was heated at 150 °C and stirred. When the solution was boiling, 5 mL of sodium citrate (40 mmol L^{-1}) were added rapidly. In the next 10 min of heating and stirring the solution changed its color from pale yellow to red; it was stirred for another 15 min at 25 °C (De la Escosura-Muñiz et al., 2009a) and after this step the AuNPs were ready to use. AuNPs were protected from the light and stored at 4 °C.

2.5. Conjugation of anti-Salmonella rabbit polyclonal second antibody with AuNPs

First, 100 μL of anti-*Salmonella* rabbit polyclonal second antibody (sSAb) (1 mg mL^{-1}) was added with gentle stirring in 1.5 mL of colloidal gold suspension with pH adjusted to 9.0 using borate buffer 50 mM. It was incubated for 20 min at 25 °C and 650 rpm