Analytica Chimica Acta 853 (2015) 533–540

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Pathogen detection in complex samples by quartz crystal microbalance sensor coupled to aptamer functionalized core–shell type magnetic separation

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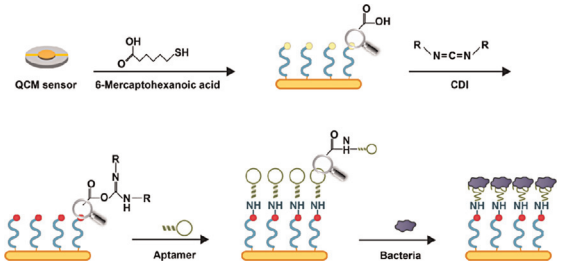
HIGHLIGHTS

- An aptamer based QCM biosensor is developed for the determination of *Salmonella*.
- The aptamers on magnetic beads and QCM crystal chips has high specificity to *Salmonella* cells.
- The detection limit of the proposed method was observed about 10 CFU mL^{-1}.
- This method with conjunction with magnetic beads system offers a rapid, and sensitive detection of *Salmonella* in complex medium.
- The binding of *Salmonella* cells to aptamer-sensor chip was well described Langmuir model.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 18 September 2014
Accepted 9 October 2014
Available online 16 October 2014

Keywords:
Magnetic beads
Biosensors
Aptamers
Quartz crystal microbalance
Food control

ABSTRACT

A quartz crystal microbalance sensor (QCM) was developed for sensitive and specific detection of *Salmonella enterica* serovar typhimurium cells in food samples by integrating a magnetic bead purification system. Although many sensor formats based on bioaffinity agents have been developed for sensitive and specific detection of bacterial cells, the development of robust sensor applications for food samples remained a challenging issue. A viable strategy would be to integrate QCM to a pre-purification system. Here, we report a novel and sensitive high throughput strategy which combines an aptamer-based magnetic separation system for rapid enrichment of target pathogens and a QCM analysis for specific and real-time monitoring. As a proof-of-concept study, the integration of *Salmonella* binding aptamer immobilized magnetic beads to the aptamer-based QCM system was reported in order to develop a method for selective detection of *Salmonella*. Since our magnetic separation system can efficiently capture cells in a relatively short processing time (less than 10 min), feeding captured bacteria to a QCM flow cell system showed specific detection of *Salmonella* cells at 100 CFU mL^{-1} from model food sample (i.e., milk). Subsequent treatment of the QCM crystal surface with NaOH solution regenerated the aptamer-sensor allowing each crystal to be used several times.

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http://dx.doi.org/10.1016/j.aca.2014.10.010
0003-2670/

1. Introduction

Salmonella, a facultative intracellular pathogen, is the causative agent of gastroenteritis in humans and other mammals. When the bacteria enter epithelial cells lining the intestine, they cause temporary damages to microvilli on the surface of host cells [1]. Many *Salmonella* infections are due to ingestion of contaminated food [1–3]. Contamination of foods by *Salmonella* is still one of the most important problems. Therefore, sensitive and accurate detection systems are essential in securing contamination-free food and water supplies. For this purpose, several different methods have been employed such as colony counting, quantitative PCR, electrochemistry and optical methods [4–12]. Surface plasmon resonance (SPR) and quartz crystal microbalance (QCM) are reagent free techniques that can be used for direct measurement of *Salmonella* in food products. In such reagent free systems, specific bio-affinity molecules such as antibody and/or aptamers are used for specific binding to target molecules via non-covalent interactions in real-time [13]. A number of QCM and SPR sensors have been reported for quantitative determination of target pathogens in the food and environmental samples [14–16]. QCM based sensor applications span from small molecules to whole cell detection such as bacterial and eukaryotic cells [1]. The principle of detection in QCM system depends on a decrease in frequency change linearly proportional to added mass on the crystal, which is known as Sauberry relation [2]. In such methods, specific recognition molecules (such as aptamer or antibody) are immobilized on the sensor surface for specific interaction with the target bacteria, the non-covalent interaction between specific functional QCM surface and target organism cause a detectable frequency change even at low concentrations. By this way, simple and specific molecular interactions can be established for the rapid determination of pathogens in food products.

Although many sensitive detection systems were developed for detection of microorganisms, interference from the complex samples has been the major drawback that hindered real-life applications. In recent years, magnetic separation technologies have been adapted for enzyme immobilization [17–21], biological macromolecules separations (such as protein and cells) [22–24] and pre-concentration of target molecules from complex mixture in a rapid way. The unique and attractive property of magnetic carrier materials is that bacteria cells can readily be isolated from sample solutions by the application of an external magnetic field [25]. The magnetic materials also can be reused or recycled because they do not agglomerate after the removal of external magnetic field. There are a few reports that introduce the strategy for the determination of pathogens in food products by combining magnetic pre-concentration system with QCM. For example, multiple methods for the determination of pathogen by magnetic separation and PCR analysis were reported [5,8,26]. However, one substantial drawback of this technique is the time interval required to complete PCR reactions.

Aptamers are bio-recognition molecules selected from complex libraries of nucleic acids by a combinatorial chemistry method known as SELEX [3]. DNA aptamers have several advantages comparable to antibodies such as better stability, smaller size, and easy chemical modifications. Such advantages make DNA aptamers good capture and detection molecules in sensor development. Although many sensitive detection systems were developed for microorganisms, interference from the complex samples has been the major drawback that hindered applications [27–33]. In a previous study, we have developed a core–shell type magnetic separation system for quick bacteria purification from complex samples and subsequently coupling with real time PCR [5,8]. In this study, sensitive and real time QCM system was used for detection of bacterial cells in combination with a similar aptamer-magnetic pull-down system. *Salmonella* contaminated milk samples were treated with aptamer-immobilized magnetic beads for separation and the captured cells were eluted and fed into an aptamer-based QCM sensor for monitoring the presence of *Salmonella* cells. We showed that whole bacterial cells could be captured from milk samples and the number of cells could be determined by a QCM system.

2. Materials and methods

2.1. Materials

Hydroxypropyl methacrylate (HPMA), ethyleneglycol dimethacrylate (EGDMA) and glycidyl methacrylate (GMA), benzoyl peroxide (BP), bipyridine, CuBr, 2-bromo-2-ethylpropionyl bromide (BMP), triethylamine, and tetrahydrofuran, toluene, ethylene diamine, 6-mercaptohexanoic acid, carbodiimide (1-ethyl-3-3-dimethyl-aminopropyl carbodiimide hydrochloride), and polyvinyl alcohol (PVA) were obtained from Sigma–Aldrich Chemical Co. (Germany). The monomers were distilled under reduced pressure in the presence of hydroquinone and stored at 4 °C until use. Aptamer recognizing *Salmonella,* (NH_2-C6-CTGATGTGTGGG-TAGGTGTCGTTGATTTCTTCTGGTGGGG) was selected by Dwivedi et al. [4] and synthesized by Iontek Ltd. (Istanbul, Turkey). All other chemicals were of analytical grade and purchased from Merck AG (Darmstadt, Germany). Purified water was used in the investigation, and the milk (≥1.5% fat) was obtained from a local store. During the measurements, all set of experiment were triplicate. The standard deviations were determined on the basis of the parallels average.

2.2. Preparation of magnetic p(HPMA/EGDMA)-g-p(GMA) beads

The preparation of magnetic *p*(HPMA/EGDMA)-g-*p*(GMA) beads with immobilized *Salmonella* aptamer was explained in detail previously [25]. Briefly, ferric-*p*(HPMA/EGDMA) beads were prepared via suspension polymerization in presence of $FeCl_3$ (0.3 mol L^{-1}, 300 mL). The organic phase that contained HPMA (7.5 mL), EGDMA (7.5 mL) and PVA (5.0%, 15 mL) were mixed with BP (0.2 g) in 15 mL toluene. The reaction was maintained at 70 °C for 2.0 h and then at 80 °C for 1.0 h. For co-precipitation reaction, ferric-*p*(HPMA/EGDMA) beads (50 g) were transferred into a reactor containing $NH_3{\cdot}H_2O$ (50 mL, 25% w/v), and a solution of $FeCl_2$ (4.0 g) in water (100 mL) was added. The reaction mixture was continuously stirred and refluxed under nitrogen atmosphere at 50 °C for 4.0 h, and then at 90 °C for 2 h. The magnetic beads were separated using a magnet and dried in a vacuum oven at 50 °C and stored at room temperature until use.

The magnetic *p*(HPMA/EGDMA) beads were functionalized with 2-bromo-2-methylpropionyl bromide (BMP) as described previously [5] for creating hairy polymer on the magnetic bead surfaces. The beads with 75–150 μm size fraction were used for grafting with *p*(GMA). A typical procedure is as follows: Br-end functionalized magnetic beads (10.0 g) were transferred into a reactor (100 mL) and the following chemicals were added: GMA (30 mL) as monomer, CuBr (0.6 g) bipyridine (2.81 g), and dioxane 30 mL. The graft polymerization was carried out at 65 °C for 10 h (Fig. 1). After reactions, the magnetic *p*(HPMA/EGDMA)-g-*p*(GMA) beads were washed sequentially with water and ethanol and stored in 70% ethanol at 4 °C.

2.3. Immobilization of Salmonella specific aptamers on magnetic p(HPMA/EGDMA)-g-p(GMA) beads

The magnetic *p*(HPMA/EGDMA)-g-*p*(GMA) beads (about 50 mg) were equilibrated in phosphate buffer (50 mM, pH 8.5) for 2 h, and