Available online at www.sciencedirect.com
ScienceDirect
Sensors and Actuators B 121 (2007) 606–615
SENSORS AND ACTUATORS B CHEMICAL
www.elsevier.com/locate/snb

Salmonella detection with a direct-binding optical grating coupler immunosensor

Namsoo Kim [a,*], In-Seon Park [a], Woo-Yeon Kim [b]

[a] *Food Function Research Division, Korea Food Research Institute, San 46-1, Baekhyun-dong, Bundang-gu, Songnam-si, Kyonggi-do 463-746, Republic of Korea*
[b] *Department of Biotechnology, Chung-Ang University, Ansung-si, Kyonggi-do 456-756, Republic of Korea*

Received 17 November 2005; received in revised form 25 April 2006; accepted 26 April 2006
Available online 5 June 2006

Abstract

Four methods of *in situ* immobilization over an optical grating coupler (OGC) sensor chip were developed for an anti-*Salmonella* antibody which was raised against the common structural antigen of *Salmonella* spp., followed by the determination of binding characteristics of *S. typhimurium* with the aim of a label-free detection in direct-binding strategy. In this sensor system, the sensor chip in a flow-through cell was rotated in a defined angle by a goniometer and the photon signals, diffracted and propagated inside the waveguide film were measured with two photodiode detectors. The anti-*Salmonella* antibody was diluted with 4 mM Tris–HCl buffer (pH 7.2) to 50 μg/ml prior to immobilization and the binding ratios of it over the sensor surface were in the range of 19.74–58.41%, with the highest value in the case of 3-aminopropyltriethoxysilane (APTS) protocol. The sensitivity of the current OGC immunosensor was found reasonably good with the limit of detection of 1.3×10^3 CFU/ml. The sensor prepared by APTS protocol was quite specific to *Salmonella* binding and was also repeatable even after ten cycles of measurement with the coefficient of variability of 2.87%. The selectivity of it against the microorganisms of hygienic importance was also remarkably good.

Keywords: *In situ* antibody immobilization; Direct-binding OGC immunosensor; *Salmonella* detection

1. Introduction

An optical grating coupler (OGC) biosensor based on optical waveguide lightmode spectroscopy (OWLS) is a recently developed device in the sector of integrated optics, exploiting the science of light guided in structures smaller than the wavelength of the light [1]. As a technique for studying processes at the solid/liquid interface, it has been reported to push the sensitivity (as well as the convenience and wealth of obtainable information) to levels even higher than the already reported achievements of the former techniques of ellipsometry [2,3], scanning angle reflectometry (SAR) [4] and surface plasmon resonance (SPR) [5]. Although each method has its unique property: ellipsometry is usable with both transparent and non-transparent substrates; SAR is suitable for theoretical works, especially regarding the property of thin films; and SPR, showing a good sensitivity, is used with a noble metal substrate, the OGC biosensor has been reported not only to have superior intrinsic sensitivity but also to have convenience and versatility, especially for biological applications including protein–DNA interaction [6,7], receptor–ligand interaction in a biomembrane [8,9], biomaterial-surface-induced blood coagulation and thrombosis [10], interactions with cells like cell adhesion and spreading, cellular response to toxic compounds [11,12] and monitoring of environmental pollution [13].

Labeled-immunosensors are similar to immunoassays such as enzyme-linked immunosorbent assay (ELISA) concerning that they exploit antibody–antigen complexation and use enzyme or fluorescent labels for signal production in direct-competitive, indirect-competitive or sandwich assay format [14,15]. The main advantages of them such as fiber-optic, particle-based and membrane-based immunosensors are present at their intrinsic high-sensitivity and versatility in system setup. Whereas, they are labile to interference caused by the coloring substances in samples and are normally suffering from complex measurement procedure found in the case of ELISA [16]. On the other hand, many immunosensors called label-free ones such as quartz crystal microbalance (QCM) and SPR immunosensors

* Corresponding author. Tel.: +82 31 780 9131; fax: +82 31 709 9876.
E-mail address: k9130sen@hanmail.net (N. Kim).

0925-4005/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.snb.2006.04.094

do not require probe molecules for signal transduction. Instead, they measure the changes in physical parameters like frequency and refractive index caused by immune responses [16,17]. In consequence, they are normally simple in measurement procedure and inert to such interference found in the case of labeled ones without losing highly sensitive aspect in signal transduction. Considering that immunosensors eventually aim at better simplicity and rapidity in measurement, and simultaneous sensitivity as comparable as those of the conventional methods like ELISA, the development of label-free immunosensors with an arrayed system will be the main trend in immunosensor study in the near future, and the OGC immunosensor is expected to be a potent repertoire for this trend [18].

Although newly emerging pathogens in food safety sector have gained increasing attention, *Salmonella* poisoning is still a primary concern in food safety because it occurs frequently in foods in the marketing procedure and is generally detrimental to human health [19]. Although conventional culturing methods are the best choice for *Salmonella* identification in food products, they are cumbersome and time-consuming, which makes them unsuitable for presumptive test of *Salmonella* spp. in foods. Various immunoassays developed for supplementing them are also inclined to be complex in sample pretreatment and measuring procedure and to be affected with interfering chromogens. Therefore, new techniques of rapid and reliable detection for *Salmonella* spp. have been strongly urged to keep food products safe [20,21]. Nowadays, two main technologies have been developed for this purpose; i.e., polymerase chain reaction (PCR) and immunosensor technology. The former detecting *Salmonella* spp. on a gene level has been diversified to increase sensitivity, specificity and accuracy of measurement. For example, enrichment in Rappaport-Vassiliadis broth [22,23], immunomagnetic bead concentration before PCR amplification [24] and implementation of real-time PCR to tetrathionate broth enrichment [25] have been done, resulting in the establishment of limit of detection (LOD) less than 10^1 CFU/ml [24]. The latter detecting *Salmonella* spp. directly on the transducer surface or in the vicinity of it shows the analytical performance as comparable as that of PCR. Moreover, contrary to most PCR methods requiring enrichment or pre-concentration to increase sensitivity of analysis [23,24], some immunosensors based on SPR, conductometry and impedance behavior of the oscillating QCM, decreasing their sensitivities for this microorganism to the levels for the direct detection on the moderately infected foods with it without the above pretreatment steps [17,26–28], will significantly shorten the time for initial *Salmonella* screening in various samples including meat and poultry products.

As mentioned above, the OGC biosensor has enormous capability as a device for measuring the changes in refractive index in affinity-based processes in bio- and nano-technology. The food industry, as an important sector of biotechnology, requires rapid and reliable screening methods for *Salmonella* spp. to improve the safety status of food products by preventing food poisoning. In this study, our target was to develop a sensitive screening method for this microorganism by preparing a direct-binding, label-free OGC immunosensor. For this purpose, various immobilization methods for an anti-*Salmonella* antibody over the surface of amino-, epoxide- and thiol-terminal silane were developed, followed by the measurement of binding property, sensitivity, specificity and repeatability for the prepared OGC sensor chips. As an important output, the OGC immunosensor of this study was found to be reasonably sensitive enough to detect *Salmonella* spp. in moderately contaminated food samples without any initial enrichment procedure, which might conspicuously reduce the time required for detecting it in the real food samples. The main characteristics of the current immunosensor were also discussed in conjunction with the previous works.

2. Experimental

2.1. Materials

An anti-*Salmonella* antibody and a peroxidase-labeled anti-*Salmonella* antibody, both immunized against *Salmonella* common structural antigen and purified from goat serum, were purchased from Kirkegaard and Perry Laboratories Inc. (MD, USA). The amino-, epoxide- and thiol-terminal silane compounds used for antibody immobilization were 3-aminopropyltriethoxysilane (APTS), 3-glycidoxypropyltrimethoxysilane (GOPS) and 3-mercaptopropyltrimethoxysilane (MTS), respectively, and were from Sigma Chemical Co. (MO, USA). Dimethylformamide (DMF), streptavidin, 3,3′,5,5′-tetramethylbenzidine (TMB) and succinimidyl-6-(biotinamido)hexanoate (NHS-LC-biotin) which was a key compound for producing the biotinylated antibody of this study were also supplied by Sigma Chemical Co. Glutaraldehyde and *N*-γ-maleimidobutyryloxy succinimide ester (GMBS), which were used for the preparation of activated intermediates for antibody binding were separately obtained from Sigma Chemical Co. and Fluka (Switzerland). All other chemicals were the products of Sigma Chemical Co. and double distilled water was used throughout this study. A *S. typhimurium* stock culture (ATCC 14028) was maintained at 1.5% tryptic soy broth (Difco Laboratories, France). For microbial detection with the OGC immunosensor of this study, the bacteria were grown in a static mode in the broth at 37 °C and were diluted serially with 4 mM Tris–HCl buffer (pH 7.2), the reaction buffer, to minimize possible matrix effect.

The OGC sensor chip (OW 2400) as a transducer was purchased from MicroVacuum Ltd. (Budapest, Hungary). The diffraction grating of it has the surface relief depth ∼20 nm, the grating periodicity of 2400 lines/mm, and the grating area dimensions of ∼2 mm (length) and 16 mm (width). The refractive index (n_F) and thickness (d_F) of the waveguide film under the diffraction grating were 1.77 and 170–220 nm, respectively. The substrate glass slide has the dimensions of 48 mm (length), 16 mm (width) and 0.55 mm (thickness), and the refractive index (n_S) of 1.53.

2.2. Construction of the OGC immunosensor system

The schematic diagram of the OGC immunosensor system used, with a magnified inset of the waveguide layer, is depicted