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Piezoelectric immunosensor for direct and rapid detection of staphylococcal enterotoxin A (SEA) at the ng level

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ABSTRACT

A direct, label-free immunosensor was designed for the rapid detection and quantification of staphylococcal enterotoxin A (SEA) in buffered solutions using quartz crystal microbalance with dissipation (QCM-D) as transduction method. The sensing layer including the anti-SEA antibody was constructed by chemisorption of a self-assembled monolayer of cysteamine on the gold electrodes placed over the quartz crystal sensor followed by activation of the surface amino groups with the rigid homobifunctional cross-linker 1,4-phenylene diisothiocyanate (PDITC) and covalent linking of binding protein (protein A or protein G). Four anti-SEA antibodies (two of which from commercial source) have been selected to set up the most sensitive detection device. With the optimized sensing layer, a standard curve for the direct assay of SEA was established from QCM-D responses within a working range of 50–1000 or 2000 ng ml^{-1} with a detection limit of 20 ng ml^{-1}. The total time for analysis was 15 min. Using a sandwich type assay, the response was ca. twice higher and consequently the lowest measurable concentration dropped down to 7 ng ml^{-1} for a total assay time of 25 min.

1. Introduction

Food borne diseases, i.e. illnesses caused by the consumption of food or water contaminated by pathogens and/or toxins, are a major concern worldwide. There is a need for analytical methods to assay these species in a rapid and sensitive way. Contamination of food (dairy products, meat) by *Staphylococcus aureus* (*S. aureus*) is one of the prevalent causes of gastroenteritis (Archer and Young, 1988). This occurs because some *S. aureus* strains produce so-called enterotoxins (SEs) that are the agents responsible for intoxication. Among the 21 different SEs described to date (Cretenet et al., 2011), staphylococcal enterotoxin A (SEA) is the most commonly encountered toxin in food poisoning outbreaks (Medina, 2006). It is generally admitted that ingestion of 100 ng of toxin is sufficient to cause intoxication symptoms. SEA is a small monomeric protein (28 kDa) with high thermal and proteolytic stability. Immunoanalytical kits are available from various companies to assay SEs in foodstuffs (Brett, 1998). These immunological assays, of the ELISA type, though sensitive, take at least 3 h 30 min for completion (excluding sample extraction) because of their multi-step procedure (Raujel, 1999).

Within the last ten years, a significant number of papers reported the set up of label-free immunosensing devices for food pathogen detection (Ricci et al., 2007). Compared to ELISA-type assays, these biosensing devices can provide a quantitative response in a much shorter time. In the case of SEs, immunosensors operating with optical (Gupta et al., 2010; Homola et al., 2002; Medina, 2005, 2006; Naimushin et al., 2002; Rasooly and Rasooly, 1999; Soelberg et al., 2009; Tsai and Li, 2009; Tsai and Rini Pai, 2009), acoustic (Campbell et al., 2007; Gao et al., 2000; Harteveld et al., 1997; Lin and Tsai, 2003; Maraldo and Mutharasan, 2007; Natesan et al., 2009) and electric (Dong et al., 2001; Yang et al.,

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2010) transduction modes have been described with variable working ranges and sensitivity. In the case of SEA, the immunosensing systems described in the literature employed an optical transduction (Rasooly and Rasooly, 1999; Tsai and Li, 2009) allowing the toxin to be detected down to 100 ng ml^{-1}.

This paper reports the design of a direct, label-free immunosensor for real-time detection and quantification of SEA in buffered solutions based on the monitoring of the association between anti-SEA and SEA at the liquid–solid interface by quartz crystal microbalance with dissipation measurements (QCM-D). With the optimized sensing layer, the direct assay of SEA was achieved with a working range of 50–1000 or 2000 ng ml^{-1}.

2. Experimental part

2.1. Reagents

Cysteamine, 1,4-phenylene diisothiocyanate (PDITC), staphylococcal enterotoxin A and recombinant protein G′ were purchased from Sigma–Aldrich. Recombinant protein A was purchased from Pierce Chemicals. A stock solution of SEA was prepared in water and its actual concentration was determined by UV measurement taking ε_{280} = 38,000 M^{-1} cm^{-1}. Affinity purified rabbit anti-SEA antibody (anti-SEA$_{TT}$) was purchased from Toxin Technology (Sarasota, FL, USA). Monoclonal mouse anti-SEA antibody (ref. C86205M, anti-SEA$_{MLS}$) was purchased from Meridian Life Science (Saco, ME, USA). Additionally, in house anti-SEA antibodies were used in this study, i.e. polyclonal mouse anti-SEA antibody (anti-SEA$_p$) and monoclonal mouse anti-SEA antibody (anti-SEA$_m$) (Techer et al.). The IgG fraction of anti-SEA$_p$ was purified by affinity chromatography on a 1-ml Hitrap protein G column (GE Healthcare) following the procedure provided by the supplier. Phosphate-buffered saline pH 7.4 (PBS) (0.71 g $Na_2HPO_4{\cdot}2H_2O$, 0.17 g KH_2PO_4, 4 g NaCl and 0.1 KCl in 0.5 l water) or PBS containing 0.1% BSA (w/v) (PBS–BSA) were used as running buffers as indicated below.

2.2. Methods

2.2.1. SAM formation and activation with PDITC

The sensor chips were dipped in a freshly prepared solution of cysteamine (10 mM in water) for 18–24 h. After washing with water, they were further dipped in a solution of PDITC (0.02%, w/v, in pyridine/DMF 1:9) for 30 min. The sensors were extensively washed in absolute ethanol and dried under a flow of argon (Spadavecchia et al., 2009).

2.2.2. Immunosensor construction

An activated sensor was mounted in the QCM and a solution of protein A or protein G (20 mg l^{-1} in PBS) was flowed for 5 min. After a washing step with PBS, the capture antibody (10 mg l^{-1} or 1/1000 for anti-SEA$_m$ in PBS) was applied for 10 min. After another washing step, non specific binding was blocked with PBS–BSA.

2.2.3. Detection of SEA in the direct assay format

Solutions of SEA at 0, 9.7, 48.5, 194, 485, 970 and 1940 ng ml^{-1} were prepared in PBS–BSA from the stock aqueous solution. These solutions were flowed over immunosensors for 10 min followed by a washing step with PBS–BSA for 5 min. The variation of frequency between the beginning of injection and the end of the washing step was measured on each sensorgram. Alternatively, the slope of the frequency shift was mathematically determined by linear regression on the linear portion of the trace.

2.2.4. Detection of SEA in the amplified sandwich assay format

A solution of anti-SEA$_{MLS}$ (15 mg l^{-1} in PBS–BSA) was flowed over the sensors for 5–8 min, followed by a washing step with PBS–BSA for 5 min. The variation of frequency between the beginning of injection of SEA and the end of the washing step was measured on each sensorgram.

2.2.5. QCM measurements

Piezoelectric measurements were performed with AT-cut gold-coated quartz crystals with nominal frequency f of 5 MHz (Lot-Oriel, France) in a flow-through mode (flow rate = 50 μl min^{-1}) with a quartz crystal microbalance with dissipation monitoring QCM-D (E1 model, Q-sense AB, Sweden) at 22 °C. The experimental set up is described in (Boujday et al., 2010).

Mass uptakes Δm were calculated with the Sauerbrey equation (1) assuming the deposited films behave as an elastic mass:

$$\Delta f = -N \times \frac{\Delta m}{C_f} \quad (1)$$

where Δf is the frequency shift, C_f (=17.7 ng cm^{-2} Hz^{-1} at f = 5 MHz) the mass sensitivity factor and N (=1, 3, 5, 7, . . .) the overtone number.

3. Results

3.1. Construction of the capture layer

The principle of direct, piezoelectric detection of SEA is based on the high affinity association between SEA and anti-SEA antibody immobilized on a quartz crystal resonator. This binding event is transduced by the decrease of the resonance frequency of the sensor chip as a result of mass uptake. The most important stage in the design of an immunosensor is the construction of the so called sensing layer comprising the capture element, i.e. the method by which the antibody is attached to the quartz crystal. Indeed, numerous reports demonstrated that the strategy used for immobilizing the capture antibody onto the transducer had a marked influence on its capacity to capture the target and eventually on the possibility to regenerate the biosensor. Moreover the intrinsic features of the capture antibody (affinity, polyclonal or monoclonal nature) have an equally important influence on the sensor's analytical range and sensitivity. This is why we tested four different anti-SEA antibodies, two monoclonal and two polyclonal, one produced in rabbit and three produced in mouse.

The quartz crystal resonator being sandwiched between two gold electrodes, the first step of capture layer construction consisted in the formation of a self-assembled monolayer (SAM) of cysteamine (2-mercaptoethylamine) to generate surface amine functions. This SAM was further activated by treatment with 1,4-phenylene diisothiocyanate (PDITC). This rigid homobifunctional cross-linker has recently gained popularity for the immobilization of amine-containing biomolecules (proteins, DNA) to aminated surfaces (Michel et al., 2007; Parker et al., 2009; Raj et al., 2009; Spadavecchia et al., 2009). Both these steps were carried out ex situ. To have an efficient control of the antibody orientation with respect to the surface, protein A or protein G′ (a truncated form of protein G lacking the albumin- and F_{ab}-binding sites) was covalently bound to the PDITC-activated surface. Both these bacterial proteins display high affinity for the F_c domain of IgG-type antibodies (Akerstrom and Bjorck, 1986; Hjelm et al., 1975), enabling the two F_{ab} domains to be in the upward position and optimally oriented for antigen capture (Boujday et al., 2008a,b; Caruso et al., 1996). Protein A is typically used for capturing rabbit IgGs while protein G′ displays an optimal affinity for mouse IgGs. Binding of protein A and protein G′ resulted in an average frequency shift of −6.9 ± 2.7 (n = 16) and −6.4 Hz, corresponding to mass uptakes of 122 ± 47 and 113 ng cm^{-2}, respectively. Coverage rates calculated from Stokes' radiuses of 5.0 nm for protein A (Schmid et al.,