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Development of PEI-GA modified antibody based sensor for the detection of *S. aureus* in food samples

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ABSTRACT

In this article, an amperometric immunosensor based on antibody immobilization onto the platinum (Pt) electrode surface by cross-linkage via glutaraldehyde (GA) pre-coated with polyethyleneimine (PEI) layer for the detection of *Staphylococcus aureus* (*S. aureus*) in food samples has been developed. Immobilization of antibodies on the sensor surfaces lead to a change in response for control (absence of test bacteria) and samples (presence of test bacteria). The changes were quantified by the increase in amperometric response. Response of the sensors to increasing concentrations (10^1–10^8 CFU/ml) of pure culture of *S. aureus* NCIM 2602 as well as *S. aureus* inoculated food samples (milk, cheese and meat) was studied and for all the samples similar response pattern was observed. The amperometric response obtained between the increasing concentrations of test bacteria and current output showed good linearity, achieving detection limit down to 10 CFU/ml. Further Scanning Electron Microscopy studies justified the response obtained for amperometric measurements.

1. Introduction

Bacterial contamination accounts for 91% of total foodborne diseases. *Salmonella sp.*, *Escherichia coli*, *Listeria monocytogenes*, *S. aureus*, *Campylobacter jejuni*, *Campylobacter coli* and *Bacillus cereus* were found to be the main source of bacterial contaminations in our food supply (Potter, Gonzalez-Ayala, & Silarug, 1997; Beran, Shoeman, & Anderson, 1991; Swaminathan & Feng, 1994). *S. aureus*, a major foodborne pathogen causes gastroenteritis on consumption of food contaminated with it. *S. aureus* is also responsible for suppurative infections such as boils, abscesses and wound infections (Le Loir, Baron, & Gautier, 2003). Development of specific molecular methods for detection and control of such foodborne pathogens in contaminated food is necessary as they cause such health risks (Wong et al., 2002). Traditional methods of detection such as enzyme linked immunosorbent assay (ELISA) (Beckers, Tips, Soentoro, Delfgou- Van Asch, & Peters, 1998) and polymerase chain reaction (PCR) (Oliveira et al., 2002) are widely used but they are time consuming, labor intensive and needs pretreatment procedures.

Biosensor technology is a rapid and reproducible approach for food safety. It eases the rapid detection of pathogens and toxins because of its simplicity, flexibility and efficacy (Mello & Kubota, 2002). Immunosensors have been developed by combining ELISA with amperometric, photometric, chromatographic and many other analytical methods of detection (Kalab & Skladal, 1997).

Amperometric immunosensors were initially based on ELISA and the measurements of electrochemically active products were carried out using redox-enzymes (Tiefenauer, Kossek, Padeste, & Thiebaud, 1997). Therefore, amperometric immunosensing requires labeling of either antigen or antibody, since the two reaction partners are electrochemically inert. In

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most of the cases, an enzyme is used as the label, allowing an enzymatic amplification of the primary signal. In the enzymatic reaction, electrochemically active substances are either consumed or released and electrochemically detected in the second step (Wendzinski, Grundig, Rennerberg, & Spener, 1997; Ghindilis, Krishnan, Atanasov, & Wilkins, 1997).

An amperometric immunosensor was designed based on the covalent immobilization of RbIgG at gold electrodes using the heterobifunctional cross-linker 3,3-dithiodipropionic acid di (*N*-succinimidyl ester) (DTSP) for the quantification of *S. aureus*. It has a detection limit of 3.7×10^2 cells/ml (Escamilla, Campuzano, Pedrero, & Pingarrón, 2008a). Another amperometric immunosensor for the quantification of *S. aureus* based on the coimmobilization of rabbit immunoglobulin G (RbIgG) and tyrosinase on a mercaptopropionic acid self-assembled monolayer modified gold electrode was reported. The immunosensor showed a detection limit of 1.7×10^5 cells/ml. The limit of detection was remarkably improved by subjecting *S. aureus* cells to wall lysis by heat treatment. The value obtained was 2.3×10^3 cells/ml, which was adequate for the monitoring of *S. aureus* contamination levels in some foodstuffs (Escamilla, Campuzano, Pedrero, & Pingarrón, 2008b). A disposable amperometric magneto immunosensor, based on the use of functionalized magnetic beads and gold screen-printed electrodes (Au/SPEs) was developed. It is for the specific detection and quantification of Staphylococcal protein A (Prot A) and *S. aureus*. The developed methodology showed very low detection limit of 1 cfu/ml *S. aureus* in raw milk samples with a short analysis time (2 h). A good selectivity was obtained against the most commonly involved foodborne pathogens originating from milk (de Avila, Pedrero, Campuzano, & Escamilla, 2012).

Protein A (SpA), cell wall protein of *S. aureus* has got high binding affinity for Fc region of human Immunoglobulin G (IgG) and interferes with the immune response (Zacco, Pividori, Llopis, del Valle, & Alegret, 2004) and is present in around 95% of all pathogenic strains of *S. aureus* (Forsgren, 1970). Therefore detection of protein A bearing *S. aureus* is of prime significance.

This paper describes the development and performance of an immunosensor with good stability produced by coating the sensor surface with polyethylenimine polymer via cross-linkage by glutaraldehyde. Further antibody immobilization was done for detection and quantification of *S. aureus* in pure culture and in food samples.

2. Materials and methods

2.1. Reagents and chemicals

Polyethylenimine (PEI) (Sigma), glutaraldehyde (GA) (Loba), Bovine serum albumin (BSA) (sd fine-chem), methanol (Merck), Human Immunoglobulin G (IgG) (Sigma), rabbit anti-protein A antibody (Sigma), anti-rabbit immunoglobulin G alkaline phosphatase (AP) conjugate (Santa cruz Biotechnology), nicotinamide adenine dinucleotide phosphate (NADP) SRL, diaphorase (Sigma), alcohol dehydrogenase (SRL), diethanolamine, Tween 20, lysostaphin (Sigma), Dnase I (Fermentas), phosphate buffer saline (PBS, pH 7.0), phosphate buffer saline—Tween 20 (PBST), sodium citrate buffer (pH 7.5), Potassium ferricyanide ($K_3[Fe(CN)_6]$) (Rankem).

2.2. Electrodes and apparatus

All electrochemical measurements were performed in a two electrode system including a Platinum (Pt) working electrode and Ag/AgCl reference electrode. Electrodes were procured from Bioanalytical Systems (BASI, USA). Surface diameter of Pt working electrode was 1.6 mm. Amperometric method of detection was followed in all analytical measurements. An Amperometric Biosensor Detector (ABD), model no. 3001 (Universal Sensors Inc, USA) was used for the measurement output.

2.3. Food samples

Milk was procured from Mother Dairy, Kolkata, cheese (Amul, Gujarat) and goat meat (local market, Kolkata).

2.4. Sample preparation

Pure cultures of *S. aureus* NCIM 2602 was procured from National Collection of Industrial Microorganisms (NCIM), National chemical Laboratory (NCL) (Pune, India). The culture was maintained on slopes of nutrient agar by monthly subculture and storage at 4 °C. Broth cultures were obtained by overnight (18 h) incubation in sterile nutrient broth at 37 °C. Enumeration of test bacteria was done by colony counting method after incubation at 37 °C for 18 h.

The media and the food samples were all autoclaved to ensure no presence of live bacteria prior to inoculation. This further ensures the right concentration of the bacteria in the sample when artificially inoculated with test bacteria. The bacterial suspension was initially assumed to contain approximately 10^8 CFU/ml of *S. aureus* cells. 0.1 ml of 10^8 CFU/ml of bacterial suspension was inoculated into 10 g or 10 ml of food samples (milk, cheese and goat meat) and then incubated at 37 °C for overnight. Then the inoculated food suspension was serially diluted to have different bacterial cell count and all the samples were plated onto petriplates to get the actual initial concentration of the inoculated bacteria in the food sample.

2.5. Isolation and identification of cell wall protein

Target antigen, protein A (SpA) was isolated from *S. aureus* NCIM 2602 using lysostaphin and Dnase I (Cheung & Fischetti, 1998) and subsequently estimated by the Lowry method (Lowry, Passonneau, Schulz, & Rock, 1961), the molecular weight was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970) and the presence of protein A was confirmed (Majumdar, Agarwal, Chakraborty, & Raychaudhuri, 2012). The protein was stored at − 70 °C for future use.