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Titania nanotube-modified screen printed carbon electrodes enhance the sensitivity in the electrochemical detection of proteins

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ABSTRACT

The use of titania nanotubes (TiO_2-NT) as the working electrode provides a substantial improvement in the electrochemical detection of proteins. A biosensor designed using this strategy provided a robust method to detect protein samples at very low concentrations ($C_{protein}$ ca 1 ng/μl). Reproducible measurements on protein samples at this concentration ($I_{p,a}$ of 80 ± 1.2 μA) could be achieved using a sample volume of ca 30 μl. We demonstrate the feasibility of this strategy for the accurate detection of penicillin binding protein, PBP2a, a marker for methicillin resistant *Staphylococcus aureus* (MRSA). The selectivity and efficiency of this sensor were also validated using other diverse protein preparations such as a recombinant protein tyrosine phosphatase (PTP10D) and bovine serum albumin (BSA). This electrochemical method also presents a substantial improvement in the time taken (few minutes) when compared to conventional enzyme-linked immunosorbent assay (ELISA) protocols. It is envisaged that this sensor could substantially aid in the rapid diagnosis of bacterial infections in resource strapped environments.

1. Introduction

The rapid and accurate identification of drug resistant bacteria is crucial to control the outbreak of bacterial infection in hospitals. This is particularly relevant in the case of *Staphylococcus aureus*, a leading cause of high morbidity and mortality in both community and hospital associated infections [1]. An acquired penicillin binding protein, PBP2a, is a prominent biomarker to distinguish Methicillin-resistant *Staphylococcus aureus* (MRSA) from Methicillin-sensitive *S. aureus* (MSSA) [2]. The detection of PBP2a at the gene, mRNA and protein levels has been extensively examined to distinguish MRSA from MSSA. These detection strategies vary substantially in terms of the duration, sensitivity, sophistication and the infrastructure requirements. One of the methods for the detection of PBP2a at the DNA level involves an integrated microfludic system that performs a multistep assay in a single disposable fluidic cartridge, resulting in simultaneous detection of the genes encoding the virulence factor Panton Valentine leukocidin (PVL), femA protein, protein A and PBP2a [3]. Detection of mRNAs from bacterial cultures is another approach that aids in evaluating the expression levels of representative biomarkers [4]. The most cost-effective method however is protein based. This involves the detection of PBP2a from MRSA cell extracts. Almost all currently available protocols for PBP2a identification rely on enzyme linked immunosorbent assays (ELISA) [5] or variants like the bioluminescent enzyme immunoassay (BLEIA) [6], rapid latex agglutination assay [7], radioimmunoassay [8], chemiluminescence assay [9] or the immune-polymerase chain reaction assay [10]. Protein based methods have an inherent drawback due to the stability of the protein sample and the dynamic range of detection [11–13]. Indeed protein levels vary widely between cells and are sensitive to changes in the sample preparation protocol. (See Scheme 1.)

Electrochemical methods for the detection of proteins are faster than conventional biochemical strategies. The small detection volumes, reusability and ease of storage render these methods simple and cost effective. Furthermore, electrochemical methods are amenable for high throughput measurements as the efficiency of electrochemical detection can be substantially enhanced by the use of appropriate nanostructured materials [14]. The integration of nanostructured metals or metal oxides on the carbon layer of the working electrode introduces diversity in the physical (electronic, photonic and catalytic) properties. These variations can be exploited to optimize the performance of the working electrode [15–19]. The nanostructured metal/metal oxide particles also have a very high surface to volume ratio. The larger effective surface area allows more biomolecules to be immobilized at or near the electrode surface. This reduces the distance for electron transfer between the biomolecule and the metal/metal oxide particles. As a result, the charge transfer to the electrodes becomes easier. In addition, the strong interactions between the biomolecule and modified electrode surface increase the surface density of the adsorbed protein [20].

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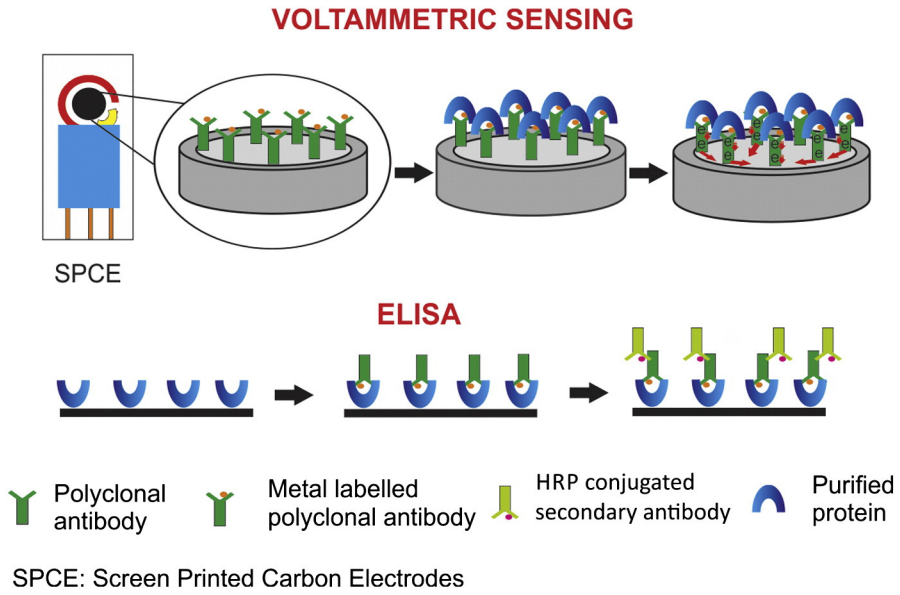

Scheme 1. Schematic representation of the electrochemical sensor. (A). Nanostructured oxide modified screen printed carbon electrodes coated with metal labeled antibodies. Two proteins (*S. aureus* PBP2a and *D. melanogaster* PTP10D) were examined. (B) Detection of identically prepared protein samples using ELISA. For ease in comparison, metal tagged antibodies were employed in this assay.

In this study, we demonstrate the feasibility of a titania-nanotube (TiO_2-NT) modified carbon electrode as the working electrode in an electrochemical biosensor to enhance the sensitivity in protein detection. Titania was chosen for the modification of the working electrode surface due to its biocompatibility and exceptional electrical properties [21]. The performance of this titania nanotube biosensor was found to be comparable or better than ELISA-based methods with the added advantage of much lower sample volumes and rapid detection.

2. Experimental: materials and methods

2.1. Protein purification

The recombinants *S. aureus* PBP2a and *Drosophila melanogaster* PTP10D were obtained by overexpression in *Escherichia coli*. The plasmids encoding PBP2a and PTP10D were transformed into BL21(DE3) cells. The recombinant proteins from these expression cells were purified by immobilized metal affinity chromatography (IMAC) using Ni^{2+}-nitrilotriacetic acid (Ni^{2+}-NTA) affinity beads (Sigma-Aldrich Co.) and ion exchange chromatography. The subsequent steps of purification and variations in the buffer composition in these steps are described in relevant references [22,23]. The purity of recombinant protein was examined by sodium dodecyl sulfate poly acrylamide gel electrophoresis (SDS-PAGE) (Supplementary Fig. 1).

2.2. Preparation of E. coli cell free lysate

The plasmid containing the gene encoding *S. aureus* PBP2a was transformed into *E. coli* BL21(DE3) pLysS cells. A single transformed colony was inoculated into 7 ml of LB media containing kanamycin. Cells were grown at 37 °C to an optical density of 0.6 at 600 nm. The cells were subsequently distributed into seven aliquots of 1 ml each. The *E. coli* cultures were induced with varying concentrations of Isopropyl β-D-1-thiogalactopyranoside (IPTG) (C_{IPTG}; 0–0.4 mM final concentration). Post induction, the cells were further grown at 37 °C for 2–3 h. Simultaneously, 1 ml of untransformed culture was also grown under similar experimental conditions. The cells were pelleted and resuspended in 100 μl of buffer (100 mM Tris-HCl, pH 7.5). The resuspended culture was lysed, centrifuged and the supernatant was collected and used in the electrochemical experiments.

2.3. Preparation of DTPA anhydride

Diethylene triamine pentaacetic acid (DTPA) anhydride was prepared as described earlier [24]. DTPA (1.96 g, 0.01 mol) and triethylamine (3.5 ml, 0.05 mol) were dissolved in dry acetonitrile by stirring at 60 °C for 1 h. The reaction mixture was cooled to room temperature and 5 ml of this mixture was further cooled to 0 °C in a vial with a rubber stopper. Isobutyl chloroformate (0.5 mmol) was added to the cooled reaction mixture. The solution was allowed to stand in an ice bath for 1.5 h after shaking it well. Later, the sample was flash frozen and stored at −80 °C.

2.4. Polyclonal antibody generation, purification and labeling

The purified recombinant proteins were used to raise polyclonal antibodies in New Zealand white rabbits. Briefly, blood samples were collected and allowed to clot at 4 °C for 24–30 h and the serum was retrieved by centrifugation at 3000 rpm for 30 min. The serum was stored at −20 °C. Antibodies from serum were purified by ammonium sulfate precipitation and were subsequently tagged with the metal ion. The procedure adapted for metal ion conjugation to antibody was adapted from a protocol described earlier [24]. Briefly, Diethylene triamine pentaacetic acid (DTPA) anhydride was reacted with 300 μg of antibody in 0.1 M phosphate buffer at pH 7.5 for 2 h at room temperature. The reaction mixture was later cooled in an ice bath and 50 μl of 50 mM $FeCl_3$ was added to the 1 ml cooled mixture and allowed to stand in an ice bath for 10 min. The reaction mixture was then neutralized with 300 μl of 0.1 M phosphate buffer (pH 7.5) for 5 min and the antibody thus labeled was further purified by passing it through a 10 ml Sephadex G-50 size exclusion chromatography column (Sigma-Aldrich Co.) (Supplementary Fig. 2). The purified metal tagged antibodies were subjected to inductively coupled plasma atomic emission spectroscopy (ICPAES) analysis and the presence of Fe (III) was confirmed (Supplementary Fig. 4). These modified antibodies are referred to as Fe (III)-tagged anti-PBP2a and Fe (III)-tagged anti-PTP10D in the subsequent discussion.