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Immobilization of enzymes to silver island films for enhanced enzymatic activity

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ABSTRACT

Hypothesis: The performance of the enzyme-based biosensors depends on the enzymatic activity and the use of an appropriate technique for immobilization of enzymes. The incorporation of silver island films (SIFs) into the enzyme-based biosensors is expected to enhance the enzymatic activity and to increase the detectability of analytes of interest.

Experiments: Two enzymes, β-galactosidase (β-Gal) and alkaline phosphatase (AP) were immobilized onto SIFs using the interactions of avidin-modified enzymes with (i) a monolayer of biotinylated bovine serum albumin (b-BSA) and/or (ii) a monolayer of biotinylated poly(ethylene-glycol)-amine (BEA molecular weight: 550–10,000 Da). To confirm the effect of SIFs on enzymatic activity, two control surfaces (no silver) were also employed.

Findings: No enhancement in enzymatic activity for β-Gal on all SIFs was observed, which was attributed to the inhibition of β-Gal activity due to direct interactions of β-Gal with SIFs. The AP activity on SIFs with BEA was significantly larger than that observed on SIFs with b-BSA, where a 300% increase in AP activity was observed as compared to control surfaces. These observations suggest that SIFs can significantly enhance AP activity, which could help improve the detection limits of ELISAs and immunoassays that employ AP.

1. Introduction

The specific, selective, and catalytic properties of enzymes have led to their use in diverse applications in biotechnology and biomedical technology [1]. For example, in biosensors, enzymes are employed as recognition and signaling elements for the detection of specific molecular analyte of interest [2–4]. In this regard, enzymes are immobilized on to surfaces through covalent binding [4], direct crosslinking [5], and encapsulation [6] of enzymes on different platforms such as alumina [7], silica [8], electrode [4] and nanoparticles [9]. The extent of enzymatic activity after surface immobilization depends on the binding procedure and on the availability of enzymes to substrates.

Since 1990s, plasmonic nanostructures have received increased attention due to their utility in the detection of biomolecular interactions [10,11] Salamon et. al. recently demonstrated that plasmonic nanoparticles can be used as a solid-supported planar proteolipid membranes, which can be a good tool for studying the biochemistry and biophysics of membrane-associated receptors and enzymes using surface plasmon resonance (SPR) spectroscopy [10]. Plasmonic nanoparticles have also been used as a platform in the quantitative study of protein-protein interactions with peptides arrays using SPR imaging [11]. In addition, one can create hybrid systems by combining the plasmonic nanoparticles with enzymes, and make use of the dual biological and electronic functions at the same time. Moreover, these hybrid systems can enhance one or both of the functions of its components. For example, Jena et al has demonstrated the use of a highly sensitive nano-architectured amperometric sensor based on platinum nanoparticles and enzyme for the detection of hydrogen peroxide, uric acid, cholesterol and glucose [12]. They have found out that by combining nanomaterials and enzymes the analytical performance of their sensor in terms of sensitivity, selectivity, and limit of detection was improved. It was also shown to exhibit a fast and stable response, and did not undergo deactivation as compared to the unmodified sensors. In another study, Kirchhoff et al has studied the electrodeposition of colloidal gold nanoparticles on gold electrodes for the attachment of acetylcholinesterase, which was then used in the electrochemical detection of thiocholine [13]. Gold nanoparticles on gold electrodes were found to enhance the adsorption and stability of acetylcholinesterase, making it highly sensitive and selective in the detection of thiocholine and acetylcholinesterase inhibitors at low inhibitor concentrations while maintaining the performance of the enzyme upon immobilization for up to 1 week. However, a significant decrease in sensor

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response was observed in the absence of the nanoparticle layer [13]. Most recently, Jia and co-workers has described the detection of carcinoembryonic antigen [14], using enzyme-labeled gold nanoparticle probes. Gold nanoparticle probes were developed by binding gold nanoparticles with a detection antibody, single-stranded DNA, and streptavidin-HRP, which was then immobilized onto a magnetic microparticle probe that contains a capture antibody. Their results showed an improvement in detection limit with high sensitivity and specificity than the conventional enzyme-linked immunosorbent assay (ELISA).

The Aslan Research Group has recently demonstrated the combined use of plasmonic nanoparticles, i.e., SIFs, with horse radish peroxidase (HRP) to increase the HRP activity in a biosensing scheme. Abel et al. [12] in this work, three different SIFs with different extent of loading on the glass slide (based on the SPR peak at 420 nm for silver: low loading: $A = 0.38$, medium loading: $A = 0.55$ and high loading: $A = 1.1$) and four different enzyme immobilization strategies: (i) a biotin-avidin protein assay: (for protein assays), (ii) self-assembled monolayer of hexamethylene diamine: (for covalent binding of enzymes), (iii) poly-L-lysine layer: (for covalent binding of enzymes), and (iv) Biotin-Poly (Ethylene-glycol)-Amine (BEA): (for protein assays) were used [13]. This work enabled us to investigate the effect of enzyme immobilization on the enzymatic activity on SIFs. The comparison of the enzymatic activity on SIFs using all four immobilization strategies demonstrated that an increase of ~300% in enzymatic conversion of organic substrate by HRP was observed from SIFs with high loading using only strategies (i) and (iv) [13]. These results also provided direct evidence that the enzymatic activity is affected by the enzyme-nanoparticle distance and the extent of loading of silver nanoparticles.

In this study, we investigated the use of SIFs with other enzymes, namely alkaline phosphatase (AP) and β-Galactosidase (β-Gal). AP and β-Gal are typically used in conjunction with a biotinylated antibody or avidin (in excess) and a substrate for the detection of an antigen [14]. Recently, AP was used in an enzyme-based amplification to demonstrate the electrochemical sensing of DNA sequences [15], in the detection of glucose and in the determination of cell growth using an acid assay [16,17]. β-Gal is a well-known enzyme that hydrolyses lactose into its monosaccharide units of glucose and galactose, which has been used in biosensors [18,19]. SIFs were chemically deposited on to silanized glass slides with different extent of surface loading. In addition, two control surfaces were also employed to investigate the effect of plasmonic surfaces on the enzyme activity: (1) nano-glass beads (~22 ± 3.5 nm) on glass slides were used as a platform for the quantitative comparison of protein surface coverage onto roughened surface similar to that SIFs generates on glass slides and (2) blank glass slides. In a previous publication [20], our laboratory reported that a similar protein (b-BSA) surface coverage is obtained on SIFs and blank glass slide [20].

We observed a significant increase in AP activity on all BEA-modified SIFs, where the largest increase (~300%) in AP activity was observed on SIFs (high loading) using BEA-5000 Da, as compared to the two control surfaces. Since the extent of enzyme on all surfaces was comparable and similar chemical environment was maintained on all surfaces, the increase in AP activity is directly attributed to the SIFs. It was also found that the loss of SIFs from the glass surface was negligible when BEA is used to immobilize AP. On the other hand, no enhancement was observed for β-Gal on all SIFs, where the loss of SIFs from the surface was significant. These results showed that SIFs can be incorporated into the current enzyme-based biosensing applications for the detection of biotinylated compounds, where the observed colorimetric response is significantly enhanced. The enhancement of colorimetric response has implications in increased detectability of a target biomolecule.

2. Materials and methods

2.1. Materials

Sodium hydroxide (anhydrous, 98%), D-glucose, silver nitrate, ammonium hydroxide (30%), sulfuric acid (A.C.S reagent grade), 3-aminopropyltriethoxysilane (APS) (99%), hydrogen peroxide (30 wt.% solution in water), albumin from bovine serum (99%) (BSA), albumin biotin labeled bovine (b-BSA), alkaline phosphatase yellow (pNPP) liquid substrate system for ELISA, avidin-alkaline phosphatase from streptomyces avidinii (AP-avidin), and 2-nitrophenyl β-D-galactopyranoside (ONPG) were all obtained from Sigma–Aldrich. Avidin-β-galactosidase conjugate (β-Gal-avidin) was purchased from Invitrogen (USA). Sodium phosphate dibasic and monobasic anhydrous, Potassium phosphate dibasic, 2-Mercaptoethanol, sodium carbonate, magnesium chloride (A.C.S certified) was purchased from Fisher Scientific. Citric acid (analytical reagent grade) was purchased from Mallinckrodt Chemical Works, and sulfuric acid (A.C.S reagent grade), and ethyl alcohol (200 proof) were obtained from PHARMCO. Tri(hydroxymethyl) aminomethane was purchased from Research Organics, Inc. Glass slides (micro slides, thickness: 0.96–1.06 mm) used in this work was obtained from Corning Inc. Silicone isolator adhesive (2.0 mm deep and 4.5 mm diameter) were all obtained from Electron Microscopy Sciences (Hatfield, PA). Microtest plate 96 well high throughput screening (HTS) plates were obtained from Sarstedt, Inc., and biotin-polyethylene glycol amine (BEA) was brought from Layson Bio, Inc. All reagents were used as received. Nano-glass beads (22 ± 3.5 nm) were purchased from Microsphere-Nanospheres Inc. Triethoxysilylbutyraldehyde and bis(trimethoxysily) octanes were bought from Gelest, Inc. All aqueous solutions were prepared using deionized water (>18.0 MΩ cm resistivity at 25 °C) obtained from Millipore Direct Q3 system.

2.2. Methods

2.2.1. Deposition of silver island films (SIFs) onto APS-coated glass slides

The silanization of the glass slides and deposition of SIFs were performed using previously described procedures [12]. The extent of SIFs on glass slides were varied by keeping the glass slides in the solution at different time intervals; low loading (for ~50 s), medium loading (for ~2 minutes), and high loading (for ~4 minutes). A silicone isolator (2.0 mm deep and 4.5 mm diameter) was used to cover SIFs, before proceeding to the next steps in this work.

2.2.2. Surface modification of glass slides using organosilanes (A control surface)

We adopted a previously described procedure for the surface modification of glass slides using an organosilane [21]. In this regard, an organosilane (i.e., triethoxysilybutyraldehyde) solution was prepared using ethanol 95%, water 3%, and organosilane 2% all in a closed glass bottle containing a magnetic stirrer. The mixed solution was allowed to sit for 5 minutes before transferring it into a closed vial, which the clean glass slides were immersed. [*Note: The glass slides were cleaned using piranha solution (H_2SO_4: H_2O_2) (3:1). Piranha solution is extremely dangerous and must be handled with care*]. The glass slides were then incubated in a closed vial containing 50 mL of the prepared organosilane solution, which was allowed to incubate for 30 minutes. The slides were later rinsed with ethanol to remove the unbounded materials and dried using air. Annealing of the silanes was achieved by placing the slides in the oven for 30 minutes at 100 °C, before use.