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Improved activity of immobilized antibody by paratope orientation controller: Probing paratope orientation by electrochemical strategy and surface plasmon resonance spectroscopy

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ABSTRACT

Electrochemical method and surface plasmon resonance (SPR) spectroscopic analysis are utilized herein to investigate antibody immobilization without and with orientation control for site-positioning paratopes (antigen binding site) of the antibody molecules. Biotin and its antibody were selected in current study as model. Such an approach employed thiophene-3-boronic acid (T3BA) as paratope orientation controller, (i) enabled site orientation of the antibody molecules reducing the hiding of paratopes, and (ii) maintained the activity of the captured antibodies, as confirmed by electrochemical and SPR anlysis. Anti-biotin antibody (a glycoprotein) was covalently bound to a self-assembled monolayer of T3BA modified on a nanogold-electrodeposited screen-printed electrode through boronic acid–saccharide interactions, with the boronic acid units specifically binding to the glycosylation sites of the antibody molecules. The immunosensor functioned based on competition between the analyte biotin and biotin-tagged, potassium hexacyanoferrate (II)-encapsulated liposomes. The current signal produced by the released liposomal $Fe(CN)_6^{4-}$, measured using square wave voltammetry, yielded a sigmoidally shaped dose–response curve that was linear over eight orders of magnitude (from 10^{-11} to 10^{-3} M). Furthermore this biosensing system fabricated based on T3BA approach was found to possess significantly improved sensitivity, and the limit of detection toward biotin was calculated as 0.102 ng mL^{-1} (equivalent to 6 μL of 4.19×10^{-10} M biotin).

1. Introduction

Rapid and sensitive immunoassays are in demand because of the ever-increasing testing for biological analytes in clinical (Malhotra et al., 2010; Wu et al., 2007), environmental (Ahn et al., 2009; Medintz et al., 2003; Zhang et al., 2010), and bio-industrial fields (Luong et al., 2008; Rivas et al., 2008). One of the main issues in the development of an immunosensor is maintaining the immunorecognition capability of the antibody after it has been immobilized on the sensing surface. The available methods for attaching antibodies onto solid surfaces are mainly based on three interactions (Camarero, 2008; Danczyk et al., 2003; Rusmini et al., 2007; Willner and Katz 2000): (i) adsorption, (ii) covalent attachment, and (iii) affinity binding. When antibody molecules are adsorbed onto surfaces and stabilized through electrostatic, hydrophobic, and polar intermolecular interactions (Rusmini et al., 2007), the attachment layer is likely to be heterogeneous and weakly bound, resulting in a random orientation of the paratopes. Covalent methods for immobilizing proteins onto solid substrates usually involve the use of available functional groups (e.g., amino, carboxyl) of accessible amino acids or the formation of gold–thiol bonds to construct self-assembled monolayers (SAMs). Although the formation of a covalent bond between an immobilized protein and a solid substrate surface can improve the reproducibility of protein immobilization to some extent, the available functional groups are presented randomly on the antibody molecule and the attachment may occur simultaneously through many available residues, thereby producing a high degree of heterogeneity in the population of immobilized proteins (Rusmini et al., 2007). In addition, covalent approaches often lead to loss of the biological function of the antibody because of either denaturing under the relatively harsh chemical conditions used in the attachment process or decreased immunorecognition ability resulting from the inappropriate orientation (e.g., paratopes facing away from the solution).

Abbreviations: CV, cyclic voltammetry; SWV, square wave voltammetry; SPE, screen-printed electrode; SPR, surface plasmon resonance; Ab, antibody; HRP, horseradish peroxidase; Fab, fragment antigen-binding

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Development of antibody immobilization methods with orientation control (Camarero et al., 2004; Mouri et al., 2010; Tajima et al., 2011; Werner and Machleidt, 1978) and evaluation of antibody orientation on surface (Chen et al., 2003; Liu et al., 2010; Song et al., 2011; Zhou et al., 2004) has been subjects of relentless efforts and endless discussions among many groups. It was exploited previously that the formation of reversible cyclic esters from boronic acids and the cis-diol units of saccharides in nonaqueous and aqueous media at ambient temperature (Ho et al., 2010; Springsteen and Wang, 2002) and could be used as intermediate molecules for protein orientation control. Moreover boronate affinity has been used widely for the development of aqueous sugar sensors (Akay et al., 2007; Edwards et al., 2007; James et al., 1995), carbohydrate and glycoprotein separation systems (Bossi et al., 2001, 2004; Jackson et al., 2008), glycoprotein (Abad et al., 2002; Chen et al., 2008; Lin et al., 2009; Liu et al., 2005, 2006; Liu and Scouten ,1996) and cell (Polsky et al., 2008) immobilization systems, transporters of ribonucleotides and carbohydrates through lipid membranes (Paugam et al., 1996; Westmark et al., 1996; Westmark and Smith, 1996), and glucose-responsive controlled drug delivery systems (De Geest et al., 2006; Zhao et al., 2009). To minimize the loss of activity of the antibody resulting from structural deformation and to increase the analyte binding capacity of the immunosensor, we became interested in fabricating immunosensors with a site-specific orientation of the antibody units—based on boronic acid–saccharide interactions—aligned on the sensing surface, and evaluate the immobilization performance by measuring the conformation of the antibody on the surface.

In this study, we implemented an easier and more convenient approach to better control paratope orientation and to facilitate the fabrication of immunosensing surface. Futhermore, we attempted to employ electrochemical method and SPR analysis to probe the antibody orientation/conformation on either a nanogold-electrodeposited screen-printed electrode or planar gold surface, that were pre-assembled a monolayer of thiophene-3-boronic acid (T3BA) (Park et al., 2008), stabilized through gold–thiol linkages, as compared with the orientations of antibody units surface-immobilized using (i) direct adsorption, and (ii) gold–thiol linkage. As a result of boronate affinity, the boronic acid moiety of T3BA binds covalently with oligosaccharides, which are attached to the C_{H_2} domains in most immunoglobulins (Suzuki et al., 2003; Yoo et al., 2002), leading to site-positioning of paratopes and preservation of the binding ability of paratopes (Lin et al., 2009). The new immobilization method described herein is confirmed to be less laborious, less time-consuming, and minimizes the probability of hiding the binding sites of antibody (i.e., it regulates the orientation of the paratopes in the antibody molecules).

2. Materials and methods

2.1. Reagents and materials

All chemicals and organic solvents were of reagent grade or better. Potassium chloride, sodium chloride, acetic acid, potassium ferrocyanide, biotin, thionin acetate, hydrochloric acid, 2-mercaptoethylamine hydrochloride (2-MEA), and ethylenediaminetetraacetic acid tetrasodium salt hydrate (EDTA) were purchased from Sigma-Aldrich (St. Louis, MO). Hydrogen tetrachloroaurate(III) was obtained from Alfa Aesar (Ward Hill, MA). Tris (base), HEPES, potassium phosphate monobasic (KH_2PO_4), and sodium bicarbonate ($NaHCO_3$) were obtained from J. T. Baker (Phillipsburg, NJ). Potassium phosphate dibasic (K_2HPO_4) and sodium carbonate (Na_2CO_3) was purchased from Riedel-de Haën (Seelze, Germany). Thiophene-3-boronic acid (T3BA) was obtained from Frontier Scientific (Logan, UT). The enzyme substrate 3,3′,5,5′-tetramethylbenzidine (TMB, Neogen K-blue enhanced activity substrate, containing H_2O_2) was obtained from Neogen (Lexington, KY). Dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylglycerol (DPPG) were acquired from Avanti Polar Lipids (Alabaster, AL). N-((6-(biotinoyl) amino)hexanoyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (Biotin-X-DHPE) was purchased from Molecular Probes (Eugene, OR). The IgG (immunoglobulin G) fraction of antibiotin (rabbit) was acquired from Rockland Immunochemicals (Gilbertsville, PA). Goat F(ab')2 polyclonal secondary antibody to rabbit IgG-(Fab')2 (HRP) (HRP-conjugated anti-Fab antibody) was obtained from Abcam (Cambridge, UK). Biotin-HRP was acquired from Invitrogen (Carlsbad, CA). Dialysis tubing (MWCO: 12,000–14,000) was purchased from Spectrum Laboratories (Rancho Dominguez, CA). All solutions were prepared with deionized water having a resistivity not less than 18 MΩ cm (Milli-Q, Bedford, MA).

2.2. Apparatus

Cyclic voltammetry (CV), square wave voltammetry (SWV) and amperometric *i–t* curve measurements were performed using a CHI 633 electrochemical analyzer/workstation (CH Instruments, Austin, TX). Disposable electrochemical screen-printed electrodes (SPEs), comprising a carbon working electrode (3 mm diameter), carbon counter electrode, and Ag/AgCl pseudo-reference electrode, were purchased from Zensor R&D (Taichung, Taiwan). The effective diameter and zeta potential of the liposomes were measured using a Brookhaven 90Plus Nanoparticle Size Analyzer and Zeta Potential Analyzer, respectively (Brookhaven Instruments, Holtsville, NY). Surface plasmon resonance (SPR) experiments were performed using a Biacore T100 system (Biacore, Uppsala, Sweden).

2.3. Fabrication of Ab/T3BA/nanoAu/SPE

Prior to modification, the working electrode of the SPE was preconditioned electrochemically by cycling the potential repeatedly between −0.6 and +0.6 V at 0.5 V s^{-1} in PBS buffer (0.1 M potassium phosphate, 0.15 M NaCl, pH 7.2). The pre-conditioned SPE was then immersed in a solution of 10 mM $HAuCl_4$ containing 0.1 M KCl, followed by electrodeposition (at −0.66 V for 10 s) to fabricate nanostructured gold on the electrode surface (Ho et al., 2009). Six microliters of 50 mM T3BA in distilled deionized (D.D.) water was placed onto the working electrode and left to react for 6 h at ambient temperature. After rinsing with D.D. water, the SPE was dried in air. Subsequently, a droplet (6 μL) of 0.2 mg mL^{-1} antibiotin antibody (Ab) in 0.1 M carbonate buffer (pH 8.5) was applied on the T3BA-modified surface and incubated at 4 °C overnight to complete the preparation of the Ab-modified SPEs. The blocking of non-specific binding sites on the immunosensing surface was initiated by rinsing with PBS buffer to remove the noncovalently bound antibody; subsequently, 1% casein (10 mg mL^{-1} in PBS, 6 μL) was placed on the working electrode and left to react for 10 min at room temperature. Finally, the electrode was rinsed thoroughly with PBS buffer and stored at 4 °C until required for use.

2.4. Evaluation of orientation of immobilized antibody using three different approaches

Electrochemical strategy adopted herein employed the HRP-conjugated anti-Fab antibody to evaluate the spatial position of the paratopes on the immobilized antibody. The boronate affinity immobilization procedure, approach with orientation control, was compared with two other antibody immobilization procedures—direct adsorption (one without orientation control) and gold–thiol assembly (one with orientation control)—in terms of the resulting binding capacity of the immobilized antibody, where the sensing