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Fabrication of DNA–protein conjugate layer on gold-substrate and its application to immunosensor

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Abstract

The fabrication of antibody thin film using both protein G and oligonucleotide was carried out by self-assembly (SA) technique for immunosensor. A mixture of 11-mercaptoundecanoic acid (MUA) and oligonucleotide with thiol (−SH) end group was self-assembled of gold (Au) surface for two-dimensional (2D) configuration. Protein G was chemically adsorbed on the 11-MUA surface, and then the antibody was immobilized on the protein G region. On the immobilized single-stranded DNA, the complementary DNA–antibody conjugate was hybridized for the oriented immobilization of antibody. The formation of self-assembled 11-MUA/oligonucleotide layer, protein G immobilization, antibody layer, and antigen binding was investigated using surface plasmon resonance (SPR). The topographies of the fabricated surfaces were observed by atomic force microscopy (AFM). When compared with the amount of antigen binding on the antibody thin film fabricated by protein G only, the proposed biosurface fabricated with both protein G and oligonucleotide showed better binding capacity, which implicates the improvement of the detection limit.

Keywords: Steric-hindrance effect; Surface plasmon resonance; Protein G; Oligonucleotide; Immunosensor

1. Introduction

Antibodies have been used extensively as diagnostic tools in many different formats. Antibody-based bioassays are the most commonly used type of diagnostic assay and still one of the fastest growing technologies for the analysis of biomolecules. Owing to antibodies' high specificity to the target antigen, immobilized antibodies on various solid surfaces have been widely used in many fields, such as purification of materials, diagnostic immunoassays and immunosensors [1,2].

Recently, antibody based immunosensors using surface plasmon resonance (SPR) have been developed for the measurement of antigens binding to antibody immobilized on the SPR sensor surface, which are capable of detecting analytes in complex biological media with high sensitivity, with a short detection time, and with simplicity as a local increase in the refractive index [3,4]. However, as the concentrations of analytes in a biological system are extremely low, the enhancement of sensitivity is required to detect biological materials [5].

The sensitivity of a SPR immunosensor for the detection of antigens with a very low concentration can be increased by control of the orientation of antibodies immobilized on the SPR sensor surface and with decreasing the steric-hindrance effect by controlling structural configuration of immobilized antibodies on the SPR surface [6,7]. When antibodies are immobilized on a solid-phase surface, their binding activity is usually less than that of soluble antibodies. One of the main reasons for this reduction of binding activity is due to steric-hindrance of the molecules in the solid-phase compared with that in solution. Another possible reason is attributed to the random orientation of the antibody molecules on the solid-phase surface. Therefore, the development of the immobilization method for antibodies in a highly oriented manner is strongly required.

In order to construct a well-defined antibody surface, protein G, a cell wall protein found in most species of *Streptococci*, can be used for proper orientation of antibody. Since

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protein G exhibits a specific interaction with the Fc portion of immunoglobulin G (IgG), the paratope of IgG can face the opposite side of the protein G-immobilized solid support [8]. As a result, protein G mediated antibody immobilization can lead to a highly efficient immunoreaction. However, the molecular size difference between protein G and immunoglobulin still induces the steric-hindrance effect which prevents the antigen binding by neighboring immunoglobulin. Therefore, the amount of immobilized antibodies can be decreased by controlling the two-dimensional (2D) structure [9]. A way to formulate this structure is by controlling the differences of heights of the immobilized antibodies, which can be implemented by the formation of mixture composed of such as 11-mercaptoundecanoic acid (MUA) and the proposed oligonucleotide. Although the fabrication of protein G [7] or oligonucleotide film using antibody–oligonucleotide conjugate [10–12] had been individually reported, the immobilization method using both protein G and oligonucleotide simultaneously to decrease the steric-hindrance effect and to control 2D structure has not been reported.

In this study, an immobilization method for the enhancement of antibody activity by the introduction of 2D configuration is developed on solid-phase surface. In order to select the best method for antibody immobilization, the amounts of antigen bound on the fabricated biosurfaces are compared using SPR spectroscopy. The formation of protein G layer on self-assembled 11-MUA was done, and then binding of antibody and antigen was done in series. Biosurface fabrication using both protein G and oligonucleotide is carried out for the immobilization of native antibody and DNA-conjugated antibody on Au surface. The amount of antigen bound on the fabricated antibody surface is represented as angle shift in the SPR spectroscopy. The morphological characteristics of the fabricated biosurfaces were observed with atomic force microscopy (AFM). Through the performance comparison between two types of molecular layers, the biosurface with better performance is selected and applied for the detection of insulin.

2. Materials and methods

2.1. Immobilization of biomolecules

The 18 mm × 18 mm Au coated glass (BK 7 type cover glass, Superior, Germany) was used as a substrate. Au substrate was cleaned by pirana solution before use, and the immobilizations of biomolecules were performed in similar methods as cited in Ref. [7].

The self-assembled monolayer of 11-MUA on the Au substrate was fabricated by submerging the prepared Au substrate into a 150 mM 11-MUA containing glycerol/ethanol (1:1, v/v) solution for a minimum of 12 h, and then washed by ultra pure ethanol. To activate the carboxylic groups of 11-MUA, the 11-MUA adsorbed Au substrate was then submerged into the 10% 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC) solution for 2 h at room temperature, and then washed by PBS buffer. The self-assembled protein G layer was fabricated by submerging the prepared EDAC activated 11-MUA layer in a 125 nM protein G solution for 2 h at room temperature, and then washed by PBS buffer.

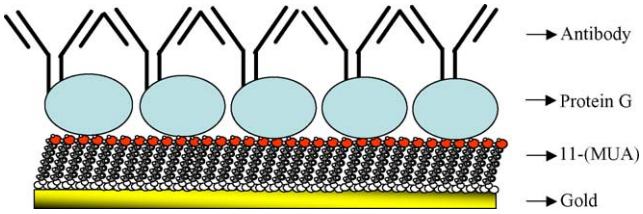

Fig. 1. A schematic diagram of antibody immobilization using protein G as a pre-layer.

For the specific binding of antigen to antibody, monoclonal antibody (Mab) against Bovine Serum Albumin (BSA) was used as a binding antibody. To immobilize the Mab, the protein G layer was immersed in a 50 nM of Mab containing PBS buffer solution. After 4 h incubation at room temperature, the Mab immobilized surface was washed by PBS buffer solution. Finally, for the antigen binding on antibodies, the prepared Mab adsorbed substrate was then immersed into a 50 nM BSA solution. Prepared Mab/Protein G/11-MUA layer is schematically shown in Fig. 1.

2.2. Immobilization of biomolecules by mixed layer

To improve the antigen binding efficiency on antibody, 11-MUA and 5′-thiol modified oligonucleotides (A1) mixed SA layer was fabricated by submerging the Au substrate into a 50 μM Al containing 150 mM 11-MUA solution for 12 h at 40 °C. And then, prepared 11-MAU/A1 SA substrate was submerged into 10% EDAC solution for 2 h at room temperature, and then washed by PBS buffer solution. The 5′-thiol modified oligonucleotide, which used to improve the binding ability, was purchased (Geno-Tech Corp., Seoul, Korea). The sequence of A1 is as follows: DNA (A1); 5′-thiol-GAA-TGA-GAG-AGT-AGG-CAG-AT-3′. The protein G SA layer was fabricated by submerging the prepared substrate in a 125 nM protein G solution for 2 h at room temperature, and then washed by PBS buffer. To immobilize the antibody on A1 surface, amino modified oligonucleotide (A1–C) was conjugated to Mab [14]. The sequence of A1–C was complimentary to the A1 and was as follows: DNA (A1–C); 5′-amine-ATC-TGC-CTA-CTC-TCT-CATTC-3′. The conjugation of Mab and A1–C was performed by the activation of terminal groups of Mab and A1–C. The carboxyl group of Mab was activated by sodium perodate and easily bound to amine group of A1–C. The sodiumperodate, which activates the carboxyl group of Mab was purchased from Alderich Chemical Co., USA. To immobilize the Mab, the protein G layer was immersed in a 50 nM of Mab and A1–C conjugated Mab mixed solution for the hybridization of A1–C and