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A strategy for sensitivity and specificity enhancements in prostate specific antigen-α_1-antichymotrypsin detection based on surface plasmon resonance

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Abstract

A biochip based on surface plasmon resonance was fabricated to detect prostate specific antigen-α_1-antichymotrypsin (PSA-ACT complex) in both HBS buffer and human serum. To reduce non-specific binding and steric hindrance effect, the chemical surface of the sensor chips was constructed by using various oligo(ethylene glycol) mixtures of different molar ratios of $HS(CH_2)_{11}(OCH_2CH_2)_6OCH_2COOH$ and $HS(CH_2)_{11}(OCH_2CH_2)_3OH$. The self-assembled monolayers were biotinylated to facilitate the immobilization of streptavidin. Using the chip surfaces, PSA-ACT complex in HBS buffer and human serum was detected at 20.7 and 47.5 ng/ml by primary immunoresponse, respectively. However, the limit of detection could be simply enhanced by a sandwich strategy to improve the sensitivity and specificity of the immunoassay. An intact PSA polyclonal antibody was used as an amplifying agent in the strategy. As a result, PSA-ACT complex concentrations as low as 10.2 and 18.1 ng/ml were found in the HBS buffer and human serum sample, respectively. The result indicates that this approach could satisfy our goal without modifying the secondary interactant.

Keywords: Prostate specific antigen; Surface plasmon resonance; Enhancement; Oligo(ethylene glycol); Biotinylation; Self-assembled monolayer

1. Introduction

Prostate cancer is a major cause of death in the male population. In 2003, approximately 220,900 cases of prostate cancer were detected in the United States. Prostate malignancy exists in 30% of all men who are over the age of 50. This rate increases to 50% for men in their eighties. The disease is increasing rapidly, which has led to the prediction that prostate cancer will become the most common cause of cancer causing death in men by the year 2010 (Savage and Waxman, 1996). At present, no curative therapy is available once the disease metastasizes to other sites in the body. Early and accurate detection of prostate cancer offers the best hope to combat against the disease while it is still localized in the prostate gland.

Prostate specific antigen (PSA), a 33-kDa serin protease, can be used to detect prostate cancer in the early stages. PSA has been recognized as the premier tumor marker for prostate cancer (Armbruster, 1993; Savage and Waxman, 1996). In addition, the major forms of PSA found in serum are complexes with two major extracellular serine protease inhibitors, α_1-antichymotrypsin (PSA-ACT, MW 90 kDa) and α_2-macroglobulin (PSA-AMG), and a free form (f-PSA, MW 34 kDa) (Lilja et al., 1991). PSA-ACT is the predominant form of PSA complex; it is immunoreactive, whereas PSA-AMG is not. The minor forms are constituted by a combination of PSA and protein C inhibitor (PSA-PCI), α_1-antitrypsin (PSA-AT), and α-trypsin (PSA-IT). Therefore, PSA-ACT and f-PSA are two molecules that if measured can be used to determine prostate cancer (Lilja et al., 1991; Savage and Waxman, 1996; Sarkar et al., 2002).

Conventional assays for PSA detection mostly involve a monoclonal or a polyclonal antibody of PSA tagged with an

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enzyme, a fluorophore or a radioactive isotope (Armbruster, 1993). These approaches had several disadvantages, such as being time consuming, inconvenient, and expensive. Furthermore, the immune reactions could not be observed in real time. Moreover, use of a radioactive isotope is very dangerous, and has not been approved by the Food and Drug Administration (FDA) (Armbruster, 1993).

Surface plasmon resonance (SPR) can be used to overcome the disadvantages of traditional methods. SPR is an affinity optical sensor based on the detection of changes in mass concentration at a biospecific interface. The advantages of SPR-based biosensors are that biomolecular reactions can be monitored in real time, there is no chemical labeling, the technology is rapid, the chip is reusable, there is flexible experiment design and only a small sample size is required (Homola, 2003). On the other hand, the major disadvantage is that it is difficult to determine an analyte at low concentration or with a low molecular mass. The detection limit is approximately 1–10 nM for a 20-kDa molecule and is even higher for smaller molecules (Gomes and Andreu, 2002). To improve the detection limit or sensitivity of SPR, sandwich immunoassays using Au or Ag nanoparticles (Gu et al., 1998), latex spheres (Homola, 2003), liposomes (Wink et al., 1998), streptavidin-biotinylated antibody complexes (Pei et al., 2001), or an enzyme precipitation strategy (Kim et al., 2005) have been reported. These assays involve modifying the secondary interactants chemically so that they can be conjugated with the SPR signal enhancers. As a result, this increases the mass concentration at the biochip interface, leading to amplification of the signals. However, chemical modification of the secondary interactants, which are mostly antibodies, would partially or completely change their biological activities. Sometimes the activity loss is caused by physically blocking the antigen binding sites or by conformation changes during conjugation (Hermanson, 1996).

The sensitivity of an immunological interaction can be considerably enhanced by a sandwich strategy using an intact polyclonal antibody if the molecular weight of the antigen is smaller than that of the polyclonal antibody. This approach does not change the physical, chemical or biological characteristics of the antibodies. Because polyclonal antibodies contain the entire antigen-specific antibody population, one antigen molecule can form a complex with several antibody molecules. Moreover, use of the sandwich strategy can enhance the specificity of an immune reaction because the overall specificity of the antibody–antigen–antibody sequence is higher than that of the antibody–antigen (Chapman et al., 2000; Cui et al., 2003).

This paper describes a strategy for detection of PSA-ACT complex based on an SPR immunosensor in combination with signal enhancement by polyclonal antibodies. Since SPR-based detection depends on the refractive index of the medium close to the non-illuminated side of a gold layer (Homola, 2003), it is important to employ suitable materials in the construction of a monolayer onto the thin gold surface. Different self-assembled monolayer (SAM) surfaces have been developed to improve the sensitivity of SPR biosensors. Oligo(ethylene glycol) (OEG) has been recognized for its ability to prevent non-specific adsorption of proteins (Chapman et al., 2000; Benesch et al., 2001; Frederix et al., 2004; Chen et al., 2005). Thus, we functionalized a bare gold surface using various OEG mixtures of different molar ratios of $HS(CH_2)_{11}(OCH_2CH_2)_6OCH_2COOH$ and $HS(CH_2)_{11}(OCH_2CH_2)_3OH$ to optimize the surface coverage of protein and reduce non-specific binding. Biotin–streptavidin chemistry has been routinely used as a biomaterial immobilization device (Green et al., 1971; Orth et al., 2003). Their bond formation is very rapid and, once formed, is unaffected by wide extremes of pH, temperature, organic solvents, and other denaturing agents. Therefore, the SAM was biotinylated, followed by SA immobilization. The immobilized surface was used to determine PSA-ACT complex concentration in HBS buffer and human serum. The results show that this real time immunoassay is very simple, effective, and easy to implement. It gives an alternative method for detection of PSA-ACT complex.

2. Materials and methods

2.1. Instrumentation

SPR measurements were performed on a BIAcore 2000 apparatus (Pharmacia Biosensor AB, Uppsala, Sweden). The instrument was operated using the BIAcore 2000 control software; data were evaluated using BIAevaluation 3.2.

2.2. Materials

PSA-ACT complex, PSA-ACT complex monoclonal antibody (PSA-ACT mAb) and goat PSA polyclonal antibody (PSA pAb) were supplied by BiosPacific, Inc. (Emeryville, CA, USA). $HS(CH_2)_{11}(OCH_2CH_2)_6OCH_2COOH$ (EG_6-COOH) and $HS(CH_2)_{11}(OCH_2CH_2)_3OH$ (EG_3-OH) were purchased from Cos Biotech (Korea). Human serum, bovine serum albumin (BSA), human immunoglobulin G (IgG), fibrinogen from human plasma, streptavidin (SA), 2-morpholinoethane sulfonic acid (MES), dimethyl sulfoxide (DMSO) were purchased from Sigma–Aldrich. Biotin hydrazide, EZ-link sulfo-NHS-LC-Biotinylation Kit (including a D-Salt™ dextran desalting column with a molecular weight cut-off 5000 and 2-Hydroxyazobenzen-4′-Carboxylic Acid (HABA) assay reagents) were supplied by Pierce (Rockford, IL, USA). HBS buffer (0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% Surfactant P20), ethanolamine–HCl solution, 50 mM NaOH solution, *N*-hydroxysuccinimide (NHS), *N*-ethyl-*N*′-(3-diethylaminopropyl) carbodiimide (EDC), and the bare gold surface (SIA Kit Au®) were obtained from BIAcore AB (Uppsala, Sweden).

2.3. Formation of oligo(ethylene glycol)-terminated SAMs on the bare gold surface (Fig. 1, step (a))

The gold-coated chips were first modified with a mixture of EG_6-COOH and EG_3-OH to form different mixed SAM surfaces. The clean bare gold chips were separately immersed into total 0.5 mM absolute ethanol solutions containing 1:2, 1:9 and 1:18 molar ratio of EG_6-COOH/EG_3-OH for 24 h to form a