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Enzymatically biocatalytic precipitates amplified antibody–antigen interaction for super low level immunoassay: An investigation combined surface plasmon resonance with electrochemistry

Hao Tang [a,*], Qiong Wang [a], Qingji Xie [a], Youyu Zhang [a], Liang Tan [a,b], Shouzhuo Yao [a,b,*]

[a] *Ministry of Education Key Laboratory of Chemical Biology and Traditional Chinese Medicine Research, College of Chemistry and Chemical Engineering, Hunan Normal University, Changsha 410081, PR China*
[b] *State Key Laboratory of Chemo/Biosensing and Chemometrics, Hunan University, Changsha 410082, PR China*

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Abstract

We demonstrated a simple and efficient strategy, which based on the enzymatically biocatalytic precipitates amplified antibody–antigen interaction, for improving the response signals of surface plasmon resonance (SPR) immunosensing. The antibody–antigen-alkaline phosphatase (AP) labeled secondary antibody sandwich were successfully prepared and characterized by SPR, cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The SPR signal amplification was accomplished through probing resonance angle shift and Faradaic electron impedance of $[Fe(CN)_6]^{3-/4-}$ redox pair after the enzymatically biocatalytic products precipitating on the immunosensing electrode surface. As a result, the accumulation of the enzymatically biocatalytic precipitates leads to significantly resonance angle shift and increase of electron transfer impedance of $[Fe(CN)_6]^{3-/4-}$ probe. The precipitates-enhanced sandwich SPR immunoassay for mouse immunoglobulin G (m-IgG) can easily detect solution protein concentrations in the linear range of 0.02–40 ng mL^{-1} and with a detection limit of 200 fg mL^{-1}, which is more than four-orders and 10 times better compared with the values using streptavidin–biotinylated protein complex and biotinylated HRP biocatalyzation amplification methods. Moreover, this method is generally applicable to other sandwich immunoassays and also can be expanded to monitor other antibody–antigen interaction for immunosensing detection at low concentrations.

Keywords: Alkaline phosphatase; Precipitates; Surface plasmon resonance; Electrochemistry; Immunoassay

1. Introduction

The immunoassay based on the detection of antibody–antigen interaction is one of the most important analytical techniques that have good application in clinical diagnoses for certain tumor-associated disease. In general, there are four types of immunosensors: electrochemical, microgravimetric, thermometric and optical immunosensors (Luppa et al., 2001; Alfonta et al., 2001; Bakker and Telting-Diaz, 2002). Surface plasmon resonance is an optical phenomenon that occurs at total internal reflection when a fraction of energy of the incident light is absorbed by plasmon, resulting in a decrease of the reflected light at a certain angle. The biosensor based on SPR is an affinity optical sensor (Tang et al., 2006; Lee et al., 2006; Yang et al., 2006; Xu et al., 2005; Arnell et al., 2006; Komolov et al., 2006; Nath and Chilkoti, 2004) and demonstrates some advantages such as real-time, non-label for analysis of biological interaction (Yano et al., 2003; Jonsson and Malmqvist, 1992; Karlsson, 2004; Schuck, 1997; Garland, 1996; Heyse et al., 1998; Tung et al., 1998; Lange and Koch, 1997) and provided good potential application for immunoassay (EI-Sayed et al., 2005; Boozer et al., 2006; Singh and Hillier, 2006; Pearson et al., 1998; Raitman et al., 2002; Toda et al., 2002; Karlsson et al., 1991). However, lack of enough high sensitivity (detection limit) for detecting analytes at low concentration is a major impediment to development of SPR immunosensor. The detection limit is ca. 1–10 nM for a 20-kDa molecule and even higher for smaller molecules (Kooyman et al., 1988). The utility of SPR immunosensor would

* Corresponding authors. Tel.: +86 731 8865515; fax: +86 731 8865515.
E-mail address: tanghao@hunnu.edu.cn (H. Tang).

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be greatly expanded if proper strategy was used to amplify the immunological interaction resulting in more pronounced changes in refractive index.

In order to improve the detection limit or sensitivity of the SPR biosensing, many works have been carried out on an extended coupling matrix (DeLano et al., 2000; Cooper et al., 2000; Adamczyck et al., 1997; Karlsson and Falt, 1997) (such as dextran) to increase reasonably the surface loading of biomolecules. Although compared to a monolayer of proteins immobilized directly on Au surface, the SPR signal was enhanced by the dextran matrix, the detection of super-low concentration or low molecular mass analyte was still a challenge even kinds of sensor chips were used. Therefore, other strategies have been proposed to increase the response signals of SPR. Latex particles (Severs and Schasfoort, 1993), liposome (Wink et al., 1998) and streptavidin–biotinylated protein complex (Pei et al., 2001) were utilized to improve the effective surface area and amplify the SPR signals. And the results demonstrated that a substantial enhancement of the detection limit (picomolar) was achieved. Recently, the antigen bioconjugated gold nanoparticles (AuNPs) or AuNPs-based sandwich have been also used to strengthen the SPR response (Lyon et al., 1998). This proposal resulted in a tremendous signal amplification and the detection limit of h-IgG achieved picomolar. However, the preparation of antibody or antigen conjugated AuNPs is relative complex and the non-specific adsorption of biomolecules on AuNPs could result in reduced sensitivity and specificity when used as sensing system in crude samples, these limitations somehow have prevented versatile applications of AuNPs from being implemented.

On the other hand, enzymatically catalyzed amplification is very effective for sensitivity enhancement in various biosensing methods such as quartz crystal microbalance (Su and O'Shea, 2001), electrochemical sensing (Ruan et al., 2002) and SPR (Su and O'Shea, 2001; Lee et al., 2005; Goodrich et al., 2004; Kim et al., 2005). Although prominent performance enhancement has been demonstrated in SPR detection, enzymatically catalyzed precipitates mainly utilized in oligonucleotide-DAN biosensing processes (Lee et al., 2005; Goodrich et al., 2004) and its application for immunosensing was very few and preliminary (Kim et al., 2005; Cao and Sim, 2007). In this paper, we demonstrated a simple and efficient strategy for improving the sensitivity of SPR immunoassay through amplifying the resonance angle shift, which resulted from the precipitation of insoluble products during enzymatic (alkaline phosphatase, AP) biocatalytic reaction. The anti-mouse immunoglobulin G (a-m-IgG) antibody, used as a model antibody, was covalently immobilized on a glutathione (GSH) modified SPR Au film surface. Immobilization of antibody, formation of antibody–antigen complexes and antibody–antigen-AP labeled secondary antibody sandwich on the Au film electrode were characterized in detail by SPR, CV and EIS. The signal amplification was accomplished through probing the resonance angle shift and Faradaic electron impedance of the $[Fe(CN)_6]^{3-/4-}$ pair after the enzymatically biocatalytic products precipitating on the Au electrode surface. The substrate of alkaline phosphatase, 5-bromo-4-chloro-3-indolyl phosphate (BCIP), was transformed to enzymatic reaction products, 5-bromo-4-chloro-3-indolylphenol, which precipitated on the immunosensing surface. The preliminary application of this SPR signal amplification method for the detection of m-IgG and related analytical performance were also evaluated.

2. Materials and methods

2.1. Chemicals and instruments

All reagents were used as received without further purification. The glutathione (GSH) were purchased from Xiasi Biotechnology Inc. (Beijing). The following biomolecules and chemicals were obtained from Dingguo Biotechnology Co. Ltd. (Beijing, China): the mouse immunoglobulin G (m-IgG), anti-mouse immunoglobulin G antibody (a-m-IgG, isolated from goat) and BCIP/NBT alkaline phosphatase substrate solution (0.02% 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and 0.03% nitro blue tetrazolium (NBT) in 0.1 M tris buffer saline (TBS, pH 9.5)). Alkaline phosphatase labeled anti-mouse immunoglobulin G antibody (APL-a-m-IgG, isolated from horse) solution (pH 7.5, 10 mM HEPES, 0.15 M NaCl, 0.08% sodium azide and 0.1% Tween 20) was purchased from Vecto Laboratories, Inc. (USA). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl (EDC) and *N*-hydroxy-succinimide (NHS) were purchased from Merck and Sigma, respectively. All other reagents were of analytical grade or better. Double-distilled water was used throughout. The a-m-IgG and m-IgG stock solutions were prepared with phosphate buffer solution (PBS, 1/15 M, pH 7.0) and stored at 4 °C.

Autolab electrochemical/SPR (Eco Chemie, Utrecht, Netherlands), PGSTAT30 digital potentiosta/galvanostat (Eco Chemie, Utrecht, Netherlands) and a standard three-electrode cell were employed for SPR and electrochemical studies (Kang et al., 2002). Thin Au film (with a thickness of 50 nm) on the glass slide was used as both the SPR-measurement electrode and electrochemical working electrode. The apparent surface area exposing to the solution is 3.14 mm^2. A platinum foil served as counter electrode and an Ag/AgCl electrode was used as reference electrode. The SPR/electrochemistry flow cell was specially designed for carrying out SPR and electrochemical measurements simultaneously (Bart et al., 2002). All potential values given below refer to Ag/AgCl reference electrode. All experiments were performed at room temperature.

2.2. Au film derivitization and construction of the sandwich immunoassay

Before further experiment, the Au electrode was pretreated by piranha solution (3:1 mixture of 98% H_2SO_4 and 30% H_2O_2) for 30 s and was carefully rinsed by a copious amount of double-distilled water. *Caution*: Piranha solution reacts violently with organic material and should be handled carefully. Fig. 1 shows the schematic diagram of the Au film derivitization and construction of the APL-a-m-IgG/m-IgG/a-m-IgG/Au sandwich immunoassay. The treated Au film was modified with glutathione (GSH) in N_2-saturated PBS contain-