BIOCHIP JOURNAL, Vol. 2, No. 4, 269-273, December 2008

# Reconstruction of the Immunoaffinity Layer of SPR Biosensor by Using Proteolytic Enzyme

**Byoung-Jin Jeon**[1] **& Jae-Chul Pyun**[1]

[1]School of Materials and Sciences, College of Engineering, Yonsei University, Seoul, Korea
Correspondence and requests for materials should be addressed to J.C. Pyun (jcpyun@yonsei.ac.kr)

Accepted 2 October 2008

## Abstract

**Usually, SPR biosensor has been applied for the detection of a target analyte in a sample by using the highly specific antigen-antibody interaction. For the specific binding of the target analyte in a sample, suitable antibodies are usually immobilized on the sensor surface, which is called 'immunoaffinity (IA) layer'. After repeated sample detection and regeneration processes, the activity of IA layer is damaged and thrown away. In this work, a reconstruction method of IA is presented by using a proteolytic enzyme. By analysis of the amount of immobilized antibodies to the reconstructed IA layer as well as the activity of reconstructed IA layer of the reconstructed IA layers, the feasibility of reconstruction method was demonstrated.**

**Keywords:** Surface plasmon resonance (SPR), Regeneration, Immunoaffinity sensor

## Introduction

Immunoaffinity biosensors employ antibodies (or sometimes antigens) for the specific molecular recognition of a target analyte. Such immunoaffintiy biosensors based on the highly specific antigen-antibody interaction are usually applied for the detection of a target analyte at extremely low concentration in a complex mixture like blood sample. The surface of immunoaffinity biosensors for the molecular recognition of the target analyte have been generally called as an immunoaffinity layer (IA layer)[1-3].

Usually, the target analytes bound to the IA layer and they should be removed for the next measurement. The conventional way to reuse such an IA layer is called 'regeneration', which involves removing the already bound analyte from the IA layer by using high or low pH reagents. Chaotropic agents or ions at high concentration, various kinds of detergents, and nonpolar, water soluble solvents in diluted form have been also used for the regeneration of the IA layer. In rare cases, reducing agents such as DTT are reported to have been used[4]. Recently, several research groups have reported success in regenerating IA layers for reuse of many cycles (50-60 cycles) by treatment of acidic or basic solutions or competitor solutions[5-8]. Such regeneration methods based on chemical treatment can increase the possible number of measurement per immunoaffinity sensor. However, each regeneration cycle partially or completely denature the antigens or the antibodies on the IA layer. Finally, the IA layer on the IA sensor does not bind the target analyte and the IA sensor should be thrown away.

The "reconstruction of IA layer" means the proteolytic digestion of target analyte bound IA layer and re-immobilization of the IA layer. As the basement of such an IA layer, proteins such as protein A or bovine serum albumin (BSA) layer were prepared on the SPR sensor surface. The protein layer was digested by using proteolytic enzyme so that primary amine groups were exposed on the IA layer. These amine groups were used for the covalent coupling of antibodies which consist of the IA layer. For the reconstruction of IA layer, the proteolytic digestion of trypsin and antibody coupling can be performed repeatedly as follows (the procedure in following parenthesis is carried out for reconstruction of IA layer): BSA coating → [trypsin treatment → antibody coupling → blocking → measurement].

In this work, a reconstruction method of the IA layer by using a proteolytic enzyme called trypsin is presented. Anti-HRP antibodies was used as model IA layer on an SPR chip called Spreeta™ which can directly detect the antigen-antibody interaction without any additional label[9-13].

## Results and Discussion

### SPR Measurement

In this work, a home-made SPR biosensor system based on Spreeta™ chip from Icx Technologies, Inc (Arlington, VA, USA) was used for all sensor measurement. As shown in Figure 1(a), the SPR chip is equipped with an integrated flow cell with a capacity

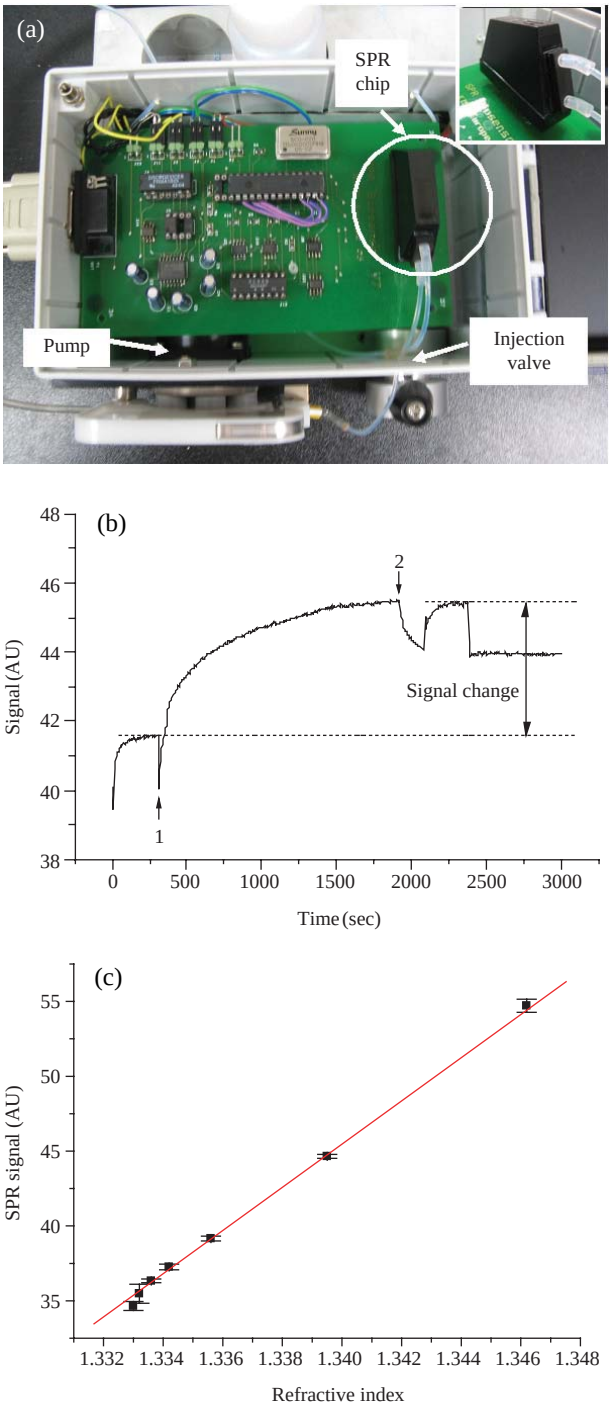


**Figure 1.** Home-made SPR system. (a) Picture of SPR system. The enclosed picture shows the SPR chip equipped with a flow cell. (b) Typical sensor response of sample injection, incubation, and washing. (c) Sensor response according to different RI samples (n=3).

of 5 μL. The sample and the washing solution were injected into the flow cell through an injection valve, and buffer flows by an integrated peristaltic pump. The pumping rate was set to be 1.0 mL/min. The peristaltic pump and the injection valve were controlled using a custom-made microprocessor board. The flow of solution was programmed to stop during the incubation step. The response of the SPR chip was transferred to a PC through 12-bit analog-to-digital converter.

A typical sensor signal from the SPR biosensor is shown in Figure 1(b). Each measurement step consists of sample injection (indicated as '1'), incubation (30 min), washing with 0.1% Tween 20 (indicated as '2'), and incubation (5 min). The signal was calculated by the difference of the SPR angles after washing procedure as indicated by the arrow. The sensor signal was calibrated by using sucrose solution with different refractive index (RI) values. The standard RI value of sucrose solution was determined by using a conventional refractometer from Leica Co. (Germany), and the SPR sensor response was correlated to the RI values by using the same sucrose solution as shown in Figure 1(c).

## Reconstruction of IA Layer

As described in Introduction, the reconstruction procedure consists of 1) the proteolytic digestion of IA layer, and 2) re-immobilization of the IA layer as follows (the procedure in parenthesis is carried out for reconstruction of IA layer): BSA coating → [trypsin treatment → antibody coupling → blocking → measurement].

In this work, bovine serum albumin (BSA) layer was prepared as the basement of IA layer on the SPR sensor surface and then digested by using trypsin so that primary amine groups were exposed on the IA layer. These amine groups were used for the coupling of antibodies which compose the IA layer. In this work, anti-HRP antibodies were immobilized on the IA layer by covalent coupling and the rest functional groups were blocked by treatment of BSA solution. The prepared IA layer of anti-HRP layer was applied to detect the HRP in samples at three different concentrations. By using the SPR biosensor, the construction process as well as the HPP sample treatment was monitored as shown in Figure 2(a).

The SPR response after trypsin treatment shows that the basement BSA layer was slightly digested. To ensure the high immobilization density of antibodies, an ideal basement layer should expose amine groups as much as possible. Trypsin is well known to digest specifically the peptide bond between arginine and lysine[14]. For the adjustment of the amine group concentration, the selection of proteolytic enzyme can be also considered to be changed. For the demonst-

ration of the reconstruction process of IA layer, the proteolytic digestion of the IA layer was carried out by treatment of trypsin as shown in Figure 2(b).

After proteolytic digestion of the basement BSA layer, anti-HRP antibodies were immobilized by covalent coupling. The activity of so-prepared IA layer was tested by injection of HRP samples at three different concentrations. In comparison to the signal increase by the binding of anti-HRP antibodies (SPR signal=1.369) and by three HRP samlples (additive SPR signal=0.755), the proteolytic digestion by trypsin resulted in the SPR signal of 0.366. This result shows that the proteolytic digestion removes only the IA layer which consisted of anti-HRP antibodies, the bound HRP. This means that the basement layer becomes thicker and thicker after reconstruction process.

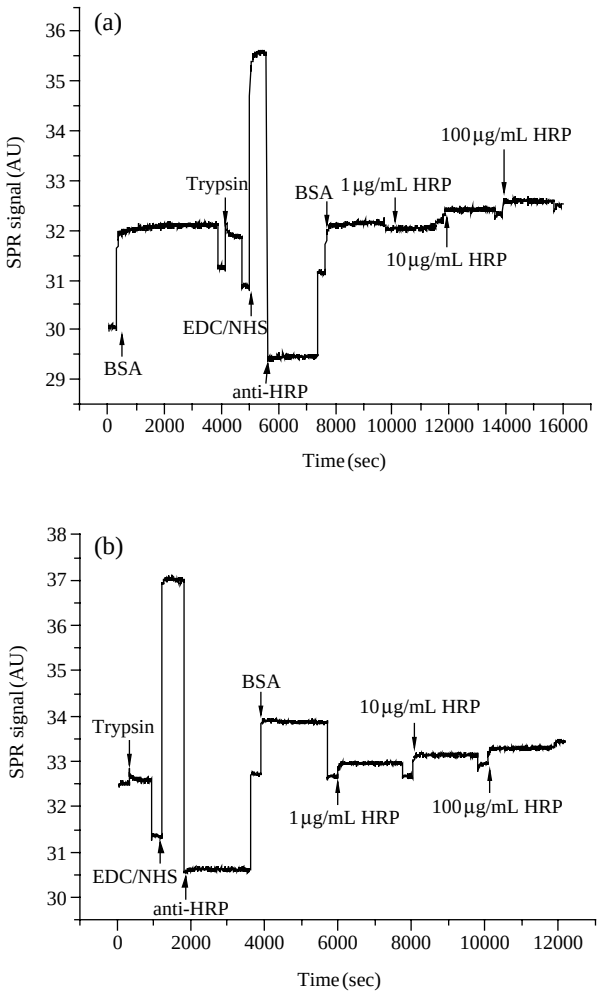


**Figure 2.** SPR sensorgram for IA layer preparation and reconstruction process. (a) IA layer preparation on BSA basement layer. (b) Reconstruction of IA layer and sequential injection of HRP samples.

## Repeated Reconstruction of IA Layer

The reconstruction of IA layer was repeatedly carried out by treatment of trypsin and then immobilization of anti-HRP antibodies. To confirm the feasibility of reconstruction process, the following parameters were compared: 1) the amount of anti-HRP antibodies which were immobilized on the SPR biosensor, 2) the binding activity of reconstructed IA layer when HRP sample at three different concentrations were injected to the SPR biosensor.

The amount of immobilized anti-HRP antibodies on the SPR biosensor indicates the amount of amine groups which were produced after proteolytic digestion of IA layer. As shown in Table 1, the SPR signal by the immobilized anti-HRP antibodies was increased as the reconstruction of IA layer was repeatedly carried out. In comparison to the 1st layer, far more amount of anti-HRP antibodies were observed to be immobilized on the 2nd IA layer. This result shows that anti-HRP bound IA layer supplied far more amount of amine groups than the BSA layer digested by trypsin. From the same reason, the 3rd IA layer was observed to be also supplied similar amount of anti-HRP antibody immobilization.

The second parameter for the binding activity of reconstructed IA layer was also related to the amount of anti-HRP antibodies. However, this parameter represents more directly the feasibility of the reconstruction method. As shown in Table 1, the activity of reconstructed IA layer was observed to be increased when the reconstruction process was repeated. This result shows that IA layer can be prepared repeatedly at the same sensor element by using reconstruction method based on proteolytic enzyme.

However, the SPR signal by proteolytic digestion is always smaller than the increased SPR signal by binding of anti-HRP antibodies and HRP. This means that

**Table 1.** SPR signal parameters of repeated reconstruction of IA layer.

| | SPR signal by anti-HRP antibodies | SPR signal by HRP samples | | | SPR signal by proteolytic digestion |
|---|---|---|---|---|---|
| | | 1 μg/mL | 10 μg/mL | 100 μg/mL | |
| 1st IA layer | 0.3064 | 0.0511 | 0.2146 | 0.3815 | 0.3659 |
| 2nd IA layer | 1.3690 | 0.1115 | 0.2657 | 0.7552 | 1.1667 |
| 3rd IA layer | 1.6409 | 0.3388 | 1.2063 | 1.0482 | 1.2553 |

the basement layer becomes thicker and thicker after reconstruction process. From this reason, the SPR signal saturation at 3rd IA layer was reached sooner than 2nd IA layer by the same HRP injection.

In summary, the reconstruction results show that the reconstruction method of IA layer is feasible for IA sensor application. The repeatable number of reconstruction is expected to be dependent to the selection of adequate basement proteins as well as the proteolytic enzymes.

## Conclusions

Usually, SPR biosensor has been applied for the detection of a target analyte in a sample by using the highly specific antigen-antibody interaction. For the specific binding of the target analyte in a sample, suitable antibodies are usually immobilized on the sensor surface, which is called 'immunoaffinity (IA) layer'. After repeated sample detection and regeneration processes, the activity of IA layer is damaged and thrown away. In this work, a reconstruction method of IA is presented by using a proteolytic enzyme. By analysis of the amount of immobilized antibodies by the SPR measurement, the amount of immobilized anti-HRP antibodies was observed to be increased as the reconstruction of IA layer was repeatedly carried out. Additionally, the binding activity of reconstructed IA layer was observed to be increased when HRP samples at three different concentrations are injected. From these results, the IA layer can be prepared repeatedly at the same sensor element by using reconstruction method based on proteolytic enzyme.

## Materials and Methods

### Materials

Bovine serum albumin (BSA), trypsin and sucrose were purchased from Sigma-Aldrich Korea (Seoul, Korea). Spreeta™ chip was purchased from Icx Technologies, Inc (Arlington, VA, USA).

### IA Layer Preparation & Reconstruction

A model IA layer was prepared by using BSA layer on SPR biosensor. The BSA layer was prepared by incubation of BSA solution (10 mg/mL) for 1 hr at room temperature. After rinsing with washing solution (0.1% Tween 20 in PBS), the BSA layer was digested by treatment of trypsin (1 mg/mL in PBS) for 10 min at 37˚C. Then, the proteolytic reaction of trypsin was quenched by rinsing the sensor surface by using the washing solution.

For the coupling of anti-HRP antibodies, the surface was treated with 100 mM EDAC/50 mM NHS in 10 mM sodium phosphate buffer (pH 5.0) for 10 min. In order to block the other reactive sites, BSA solution (1 mg/mL in PBS) was incubated for 30 min at 37 ˚C. After incubation the sensor surface was rinsed by using washing solution (0.1% Tween 20 in PBS).

## Acknowledgements

This work was supported by "System IC 2010" project of Ministry of Knowledge Economy and by KOSEF through National Core Research Center for Nanomedical Technology (R15-2004-024-00000-0) and by Seoul Research and Business Development Program (10816).

## References

1. Luppa, P.B., Sokoll, L.J. & Chan, D.W. Immunosensors-principles and application to clinical chemistry. *Clin. Chim. Acta* **314**, 1-26 (2001).
2. Ekins, R. Immunoassay and other ligand assays: From isotopes to luminescence. *J. Clin. Ligand Assay* **22**, 61-77 (1999).
3. Warsinke, A., Benkert, A. & Scheller, F.W. Electrochemical Immunoassays. *Fresen. J. Anal. Chem.* **366**, 622-634 (2000).
4. Andersson, K., Haemaelaeinen, M. & Malmqvist, M. Identification and optimization of regeneration conditions for affinity-based biosensor assays. A multivariate cocktail approach. *Anal. Chem.* **71**, 2475-2481 (1999).
5. Quinn, J.G., O'Kennedy, R., Smyth, M., Moulds, J. & Frame, T. Detection of blood group antigens utilizing immobilized antibodies and surface plasmon resonance. *J. Imm. Methods* **206**, 87-96 (1997).
6. Svitel, J., Dzgoev, A., Ramanathan, K. & Danielsson, B. Surface plasmon resonance based pesticide assay on a renewable biosensing surface using the reversible concanavalin A monosaccharide interaction. *Biosens. Bioelectron*. **15**, 411-415 (2000).
7. Pei, R., Yang, X. & Wang, E. Enhanced surface plasmon resonance immunoassay for human complement factor 4. *Anal. Chim. Acta* **453**, 173-179 (2002).
8. Dillon, P.P., Daly, S.J., Manning, B.M. & O'Kennedy, R. Immunoassay for the determination of morphine-3-glucuronide using a surface plasmon resonance-based biosensor. *Biosens. Bioelectron.* **18**, 217-227 (2003).
9. Melendez, J. *et al.* A commercial solution for surface plasmon sensing. *Sens. Actuators B* **35**, 212-216 (1996).
10. Melendez, J. *et al*. Development of a surface plasmon resonance sensor for commercial applications. *Sens. Actuators B* **39**, 375-379 (1997).

11. Spangler, B.D., Wilkinson, E.A., Murphy, J.T. & Tyler, B.J. Comparison of the Spreeta surface plasmon resonance and a quartz crystal microbalance for detection of *Escherichia coli* heat-labile enterotoxin. *Anal. Chim. Acta* **444**, 148-161 (2001).
12. Mohammed, I., Mullett, W.M., Lai, E.P.C. & Yeung, J.M. Is biosensor a viable method for food allergen detection? *Anal. Chim. Acta* **444**, 97-102 (2001).
13. Naimushin, A.N. *et al.* Detection of Staphylococcus enterotoxin B at femtomolar levels with a miniature integrated two-channel surface plasmon resonance (SPR) sensor. *Biosens. Bioelectron.* **17**, 573-584 (2002).
14. Stryer, L., Biochemistry (W.H. Freeman and Co., New York, 1988).