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Short communication

E. coli outer membrane with autodisplayed Z-domain as a molecular recognition layer of SPR biosensor

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ABSTRACT

In this work, the Z-domain of protein A was expressed as a recombinant-fusion protein on the outer membrane of *Escherichia coli* by using "Autodisplay" technology, and the outer membrane with Z-domains was layered on a SPR biosensor as a highly sensitive molecular recognition layer. The outer membrane layer formation was demonstrated by using various surfaces with controlled hydrophobicity. The effect of orientation control by the outer membrane with Z-domains was estimated to have far higher sensitivity and far improved limit of detection (LOD) in comparison to the conventional immunoassay by using a model analyte, horseradish peroxidase (HRP). The SPR biosensor with the outer membrane layer was applied to the detection of C-reactive protein (CRP). From these experiments, the LOD of SPR biosensor was estimated to improve more than 100-fold compared to the SPR biosensor with the antibody-layer by physical adsorption.

1. Introduction

Generally, the immunoaffinity (IA) biosensors utilize the highly selective binding affinity of antibodies for the molecular recognition of a target analyte in a complex mixture such as serum. The antigen binding sites of antibodies (IgG's) are known to be localized at F_{ab} region, which is a relatively small part compared with the whole antibody structure (Liddell, 2001). Therefore, the antigen binding sites (F_{ab} region) of antibodies should be exposed to the analyte solution for the analytes to bind effectively to the IA biosensor (Luppa et al., 2001). Additionally, the antibodies should be immobilized with a high density for the sensitive detection of a target analyte at a very low concentration. These requirements are called 'orientation control' and 'density control' of antibodies, respectively (Chung et al., 2006).

In the previous work, we expressed Z-domain of protein A on the surface of the outer membrane of *Escherichia coli* by using "Autodisplay" technology. The autodisplayed Z-domain was analyzed to have the IgG-binding activity by using fluorescence labeled antibodies (IgG's). The ratio of *E. coli* with active Z-domain was estimated to be over 95% by using FACS analysis. Such *E. coli* with autodisplayed Z-domain could be applied to the sandwich-type immunoassay as a signal amplifier of SPR biosensor for the improvement of the sensitivity (Jose et al., 2009).

In this work, the outer membrane with Z-domains was layered on a SPR biosensor as a highly sensitive molecular recognition layer. The layer formation of the outer membrane is presented on the transducer surface by using different surfaces with controlled hydrophobicity. The effect of orientation control and density control of antibodies by the outer membrane with Z-domain on the transducer was estimated in comparison to antibody-layers prepared by the conventional adsorption method. For the feasibility test for SPR biosensors, C-reactive protein (CRP) and hIgG detection was demonstrated.

2. Materials and methods

2.1. Materials

Anti-CRP antibodies (polyclonal), anti-mIgG antibodies labeled with fluorescein (polyclonal), anti-mIgG antibodies labeled with FITC (polyclonal), anti-mIgG antibodies, hIgG, anti-mIgG antibodies labeled with horseradish peroxidase (HRP) (polyclonal), anti-hCG antibodies conjugated with 40 nm gold nano particles (monoclonal) and purified CRP were bought from AbCam (Cambridge, UK). Phenylmethanesulfonyl fluoride and Aprotinin were purchased from Roche Korea (Seoul, Korea). Bovine serum albumin (BSA), lysozyme, DNase, HRP and all of the other chemicals (of analytical grade) were purchased from Sigma–Aldrich Korea (Seoul, Korea).

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2.2. Preparation of outer membrane particle with autodisplayed Z-domain

The vector for autodisplay was constructed by the cloning of the antibody binding Z-domain from *Staphylococcus aureus* with PCR amplification and *E. coli* cells were routinely cultured as described in the previous work (Jose et al., 2009). The outer membrane was isolated by using the cultured *E. coli* cells by the lysozyme reaction and several times of centrifugation as described in the previous work (Hantke, 1981; Schulthesis et al., 2002).

2.3. SPR measurement

A Spreeta™ chip from Texas Instruments Co. (Dallas, TX) was used for the SPR measurements. This SPR chip is equipped with a flow cell with a capacity of 5 μl and the measurements were performed using a home-made control circuit board, which is designed according to the technical specification from Texas Instruments Co. In each case, the sample and the washing solution were injected into the flow cell using an integrated injection valve and a peristaltic pump (Jose et al., 2009; Jeon and Pyun, 2008).

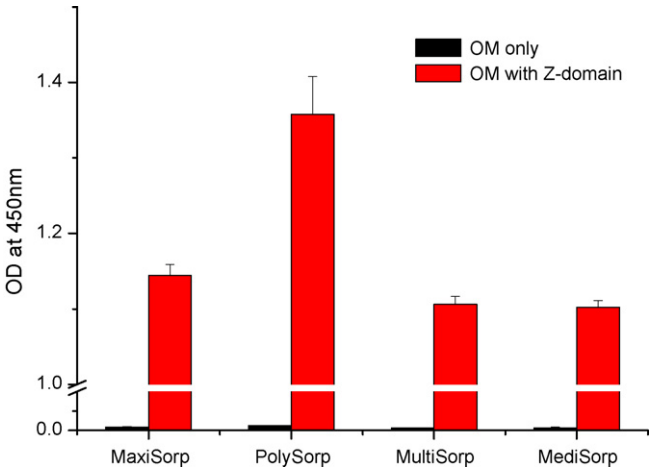

Fig. 1. Outer membrane layer on different surfaces with controlled hydrophobicity. Outer membrane layers with Z-domain and without Z-domain were separately prepared on the microplates, and then HRP-labeled antibodies were treated. The 'OD' at the *Y*-axis means the chromogenic signal at the wavelength of 450 nm by the treatment of TMB. The 'OM' represents the outer membrane.

3. Results and discussion

3.1. Formation of outer membrane layer

The outer membrane particle was prepared by the hydrolysis of the peptidoglycan layer using lysozyme. Outer membrane particles have hydrophilic surface composed of lipopolysaccharide (LPS) in order to interact with the aqueous environment, and the core part of outer membrane particles are composed of hydrophobic lipoproteins and peptidoglycans (Hiroshi, 1996).

Usually, such vesicles with hydrophilic surface and hydrophobic core are known to make a two-dimensional layer on a hydrophobic surface in an aqueous environment (Salafsky et al., 1996). In the case of the outer membrane particle, the hydrophobic core part makes interaction with the hydrophilic surface, and the outer side of outer membrane particle, which is hydrophobic, is directed to the aqueous environment in the outer membrane layer.

To observe the outer membrane layer formation on the hydrophobic surface, the outer membrane particle was treated to differently modified surfaces with controlled hydrophobicity. The contact angles of the commercially available microplates called Maxisorp, Polysorp, Multisorp, Medisorp from Nunc Co (USA) were estimated to be 64.8°, 77.6°, 44.7°, 66.7°, respectively. In this work, the application of the outer membrane with autodisplayed Z-domains was focused on the immunoaffinity biosensors, and the surface with controlled hydrophobicity was prepared at the contact angle range for immunoassays, In order to compare the outer membrane formation on the surfaces with different hydrophobicity, antibodies labeled with HRP were treated, and then the chromogenic reaction of HRP was performed with TMB.

As shown in Fig. 1, the most hydrophobic surface of Polysorp showed the highest chromogenic signal which means that the outer membrane layer on the Polysorp surface had the most dense distribution of Z-domains for the binding of HRP-labeled antibodies (Josephy et al., 1982). The outer membrane layer formed by the particles from the intact *E. coli* (negative control) showed no comparable the chromogenic reaction signal, which means that the level of non-specific binding of the HRP-labeled antibodies was negligible.

Usually, biosensors have used the surface of gold for the preparation of molecular recognition layer, that is, for the immobilization of biomolecules such as enzymes, antibodies, etc. The outer membrane layer formation was tested on the surface of gold for the application to biosensors. As the surface is also quite hydrophobic with a contact angle of approximately 70°, the outer membrane layer was expected to be formed by the same procedure with outer membrane particle treatment. The outer membrane layer prepared with the outer membrane particle with Z-domain shows a strong green fluorescence signal after the treatment with the fluorescein-labeled antibodies (see supplement). However, the outer membrane layer prepared from the intact *E. coli* (negative control) shows no significant fluorescence signal, and the BSA coated gold's surface also shows no significant fluorescence signal. These results imply that outer membrane layer can be formed on the surface of gold and the autodisplayed Z-domain has IgG-binding activities. Same as in the case of microplate experiments, the non-specific binding of antibodies could be prevented by using outer membrane layer from intact *E. coli* without Z-domain.

For the observation of surface morphology, AFM analysis of the outer membrane layer on the gold's surface was performed before and after the treatment of antibodies. The surface of the outer membranes was estimated to be relatively even and flat in comparison to the height of the iceberg structures as high as approximately 100 nm (see supplement).

3.2. Effect of orientation control by outer membrane layer

The OM layer with Z-domain aims to improve the sensitivity of the immunoassay with biosensors, which is realized by the orientation and the density controls of the antibodies for the detection of a target analyte. To evaluate these effects, the immunoassay was carried out by using two kinds of IA layers: the antibody-layer of outer membrane layer with Z-domain and the antibody-layer by physical adsorption. The C-reactive protein was used as a target analyte and anti-CRP antibodies at the same concentration of 0.5 μg/ml were treated for both IA layers.

The immunoassays by using the antibody-layer of outer membrane layer with Z-domain (■) and the antibody-layer by physical adsorption (●) were performed by using CRP samples at the concentration range of 0.1–200 ng/ml as shown in Fig. 2. The limit of detection (LOD) was evaluated as the concentration where the response is three times higher than that of the standard deviation from the baseline response by a blank sample (Ezan and Jacques, 2000). In the use of the antibody-layer of outer membrane layer with Z-domain (■) and the antibody-layer by physical adsorption (●), the LOD was calculated to be approximately 1.5 ng/ml

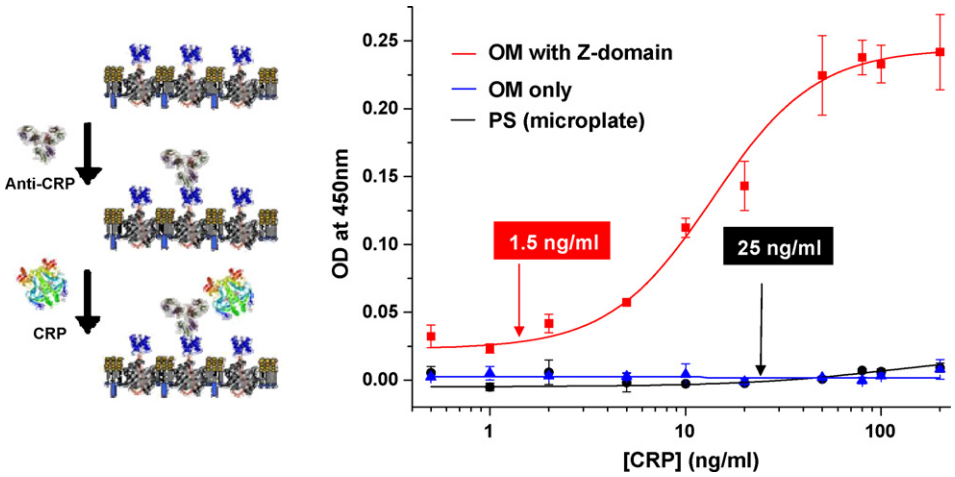

Fig. 2. Immunoassays based on outer membrane layer with autodisplayed Z-domains. Comparison of ELISA based on outer membrane layer (OM) with conventional method. 'PS (microplate)' represents a polystyrene microplate for the conventional ELISA.

and 25 ng/ml, respectively. This result shows that the LOD of the antibody-layer of outer membrane layer with Z-domain (■) was 15-fold more sensitive than the antibody-layer by physical adsorption (●), which indicates that the antibody-layer of outer membrane layer with Z-domain (■) has a significantly larger number of analyte binding sites at the same unit area than the antibody-layer by physical adsorption (●). The analyte binding site represents the well-oriented antibodies suitable for the analyte binding, which were made by the orientation and density control of the anti-CRP antibodies by the Z-domains.

Usually, a saturated response at a certain analyte concentration is found at the response curve of immunoassays (Ezan and Jacques, 2000). The antibody-layer of outer membrane layer with Z-domain (■, OD unit: 0.25) shows far higher saturation response than the antibody-layer by physical adsorption (●, OD unit: 0.02) as shown in Fig. 2. This result shows that the outer membrane layer with Z-domain has far higher binding capacity at the unit area in comparison to the antibody-layer by physical adsorption. Additionally, the outer membrane layer without Z-domain (negative control) shows no significant non-specific binding after the treatment of antibodies as well as CRP samples.

3.3. SPR measurement with outer membrane layer as molecular recognition layer

For the feasibility test for SPR biosensor, the outer membrane layer with Z-domain was prepared on the gold's surface of SPR biosensor, and then the antibodies against CRP were sequentially treated. As shown in Fig. 3, the SPR signal was monitored during the whole sensing process from the outer membrane layer formation step to the analyte binding steps.

When the outer membrane particle was injected, the SPR signal was observed to have increased significantly increased during the incubation step (see supplement). After the washing step, the SPR signal by the outer membrane layer preparation was calculated to be 2.3 (A.U.). Then, the anti-CRP antibodies were treated and the SPR signal was calculated to be 1.1 (A.U.). These results illustrates that the SPR signal could be monitored during the outer membrane layer formation and during the followed immobilization step of antibodies, which indicates that the sensitive layer of SPR biosensor was not saturated by the outer membrane layer with Z-domain.

For the demonstration of orientation control, another SPR biosensor with the antibody-layer by physical adsorption of the

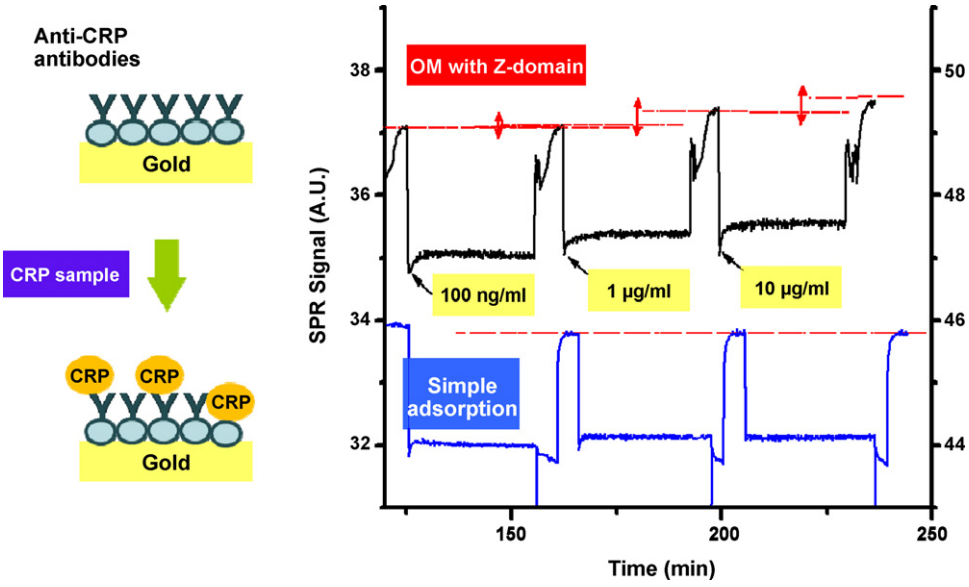

Fig. 3. Response of SPR biosensors for the detection of analyte (CRP) by the outer membrane (OM) layer with Z-domains and by the randomly oriented antibody-layer.

anti-CRP antibodies was prepared. Standard CRP samples were prepared in bovine serum and injected to both biosensors. As shown in Fig. 3, the SPR biosensor with outer membrane layer shows sensor response by the injection of CRP sample at the concentration of 100 ng/ml, 1 μg/ml and 10 μg/ml. These results mean that the LOD of outer membrane layered SPR biosensor was estimated to be less than 100 ng/ml. In the case of SPR biosensor with the antibody-layer by physical adsorption of anti-CRP antibodies, no measurable SPR signal was detected by the injection of the above three standard CRP sample. This means that the LOD of the SPR biosensor with the antibody-layer by physical adsorption is estimated to be more than 10 μg/ml. From the simple comparison, the LOD of SPR biosensor with outer membrane layer with Z-domain is more than 100-fold sensitive than with the antibody-layer by physical adsorption. Such difference in LOD's can be explained by the orientation and density control effects of the Z-domain on outer membrane layer. As in the case of microplate assay, the outer membrane layer with Z-domain supplied more binding sites than the antibody-layer by physical adsorption and the significant improvement of the sensitivity of SPR biosensor was achieved.

As another demonstration of the orientation and density control effects of the outer membrane with Z-domain, SPR biosensor with anti-hIgG antibodies were prepared for the detection of hIgG and the LOD was estimated to be improved more as much as 200-fold compared to the SPR biosensor with the antibody-layer by physical adsorption (see supplement). These results can be also explained by the exceeding number of binding sites for the analyte, which was supplied from the orientation and the density control effects of the outer membrane layer with Z-domain.

4. Conclusions

In this work, the Z-domain of protein A was expressed as a recombinant-fusion protein on the outer membrane of *E. coli* by using "Autodisplay" technology, and the outer membrane with Z-domains was layered on a SPR biosensor as a highly sensitive molecular recognition layer. The outer membrane layer formation was demonstrated by using various surfaces with controlled hydrophobicity. For the evaluation of the orientation effects, the immunoassay was carried out by using the antibody-layer of outer membrane layer with Z-domain and the antibody-layer by physical adsorption, and the LOD was calculated to be approximately 1.5 ng/ml and 25 ng/ml, respectively. These results indicate that the LOD of outer membrane layer can improve the sensitivity up to 15-fold than the antibody-layer by physical adsorption and that the outer membrane layer with Z-domain has a significantly larger number of analyte binding sites at the same unit area by the orientation control effect of Z-domain. The SPR biosensor with the outer membrane layer was applied to the detection of C-reactive protein. From these experiments, the LOD of SPR biosensor was estimated to improve more than 100-fold compared to the SPR biosensor with the antibody-layer by physical adsorption.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2009.09.025.

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