Biotechnology and Bioprocess Engineering **2004**, 9: 143-146

Surface Plasmon Resonance Imaging Analysis of Hexahistidine-tagged Protein on the Gold Thin Film Coated with a Calix Crown Derivative

Seung-Hak Baek[1,2], Yong-Beom Shin[1], Min-Gon Kim[1], Hyeon-Su Ro[1], Eun-Ki Kim[2], and Bong Hyun Chung[1]*

[1] BioNanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-600, Korea
[2] Department of Biological Engineering, Inha University, Incheon 402-751, Korea

Abstract A surface plasmon resonance (SPR) imaging system was constructed and used to detect the hexahistidine-ubiquitin-tagged human parathyroid hormone fragment (His_6-Ub-hPTHF(1-34)) expressed in *Escherichia coli*. The hexahistidine-specific antibody was immobilized on a thin gold film coated with ProLinker™ B, a novel calixcrown derivative with a bifunctional coupling property that permits efficient immobilization of capture proteins on solid matrices. The soluble and insoluble fractions of an *E. coli* cell lysate were spotted onto the antibody-coated gold chip, which was then washed with buffer (pH 7.4) solution and dried. SPR imaging measurements were carried out to detect the expressed His_6-Ub-hPTHF(1-34). There was no discernible protein image in the uninduced cell lysate, indicating that non-specific binding of contaminant proteins did not occur on the gold chip surface. It is expected that the approach used here to detect affinity-tagged recombinant proteins using an SPR imaging technique could be used as a powerful tool for the analyses of a number of proteins in a high-throughput mode.

Keywords: calixcrown derivative, gold chip, hexahistidine-tagged protein, surface plasmon resonance imaging (SPRI)

INTRODUCTION

Surface plasmon resonance (SPR) is an optical technique that is used as a valuable tool for the investigation of molecular interactions. SPR allows for the sensitive detection of molecular interactions in real time, without the use of labels [1]. The method uses a quantum phenomenon that arises at the interface between two media of different refractive indices when light is reflected under certain conditions from a thin metal film. When the SPR is linked to imaging, the SPR imaging (SPRI) becomes an ideal surface-sensitive optical technique for the detection of the affinity binding of unlabeled biomolecules onto arrays of molecules immobilized on chemically modified gold surfaces. SPRI utilizes collimated illumination of the entire surface, with the reflected beam imaged onto a two-dimensional array detector [2]. So far, SPRI has been applied to a variety of biomolecular interaction studies such as DNA [3,4], peptide [5], protein [6] and carbohydrate [7].

Proteomics has become the focus of major research to understand the vast amount of genomic information as a gene function is derived from the protein product it encodes. The challenge of studying proteomics requires a high-throughput approach for protein expression and purification [8]. In addition, the rapid detection of protein expression is especially required to determine whether the desired protein is successfully expressed or not. After this, purification can be performed to obtain the desired protein in high purity. To facilitate high-throughput expression and purification of proteins, affinity fusion tags are commonly used. In this study, an efficient method for the rapid detection of the hexahistidine-tagged proteins expressed in *E. coli* is reported using a SPR imaging measurement.

***Corresponding author**
Tel: +82-42-860-4442 Fax: +82-42-879-8594
e-mail: chungbh@kribb.re.kr

MATERIALS AND METHODS

Construction of Plasmids

A 246 bp fragment encoding the hexahistidine-ubiquitin (His_6-Ub) fusion protein was amplified by polymerase chain reaction (PCR) using *Saccharomyces cerevisiae* genomic DNA as template and two primers: 5'-CTA GGC CAT ATG CAT CAC CAT CAC CAC CAT CAA ATT TTC GTC AAA ACT CTA ACA GGG -3' (new *Nde* I site, 5' to start codon) and 5'-ACC ACC TCT CAG TCT CAA CAC CAA-3'. The PCR product was purified

using a QIAEX II gel extraction kit (Qiagen, Germany), and digested with *Nde* I (Boehringer Mannheim, Germany). A gene encoding the human parathyroid hormone fragment(1-34) was amplified using a PCR of the plasmid pECPYE-hPTHF [9]. The primers used were 5'-TCT GTT TCT GAA ATT CAA TTG-3' and 5'-GGG CTC GAG TCA GAA GTT GTG AAC GTC-3', which adds a *Xho* I site to it. The PCR product was purified and digested with *Xho* I. The fragment (102 bp) was ligated into pET22b(+) with a piece of the His_6-Ub-coding gene cut with *Nde* I. The construct was designated pEHUb-hPTHF.

Expression of His_6-Ub-hPTHF(1-34)

The transformed *E. coli* cells were cultured in Luria-Bertani (LB) medium and allowed to grow at 30°C to OD 0.6, followed by the addition of 1 mM isopropyl β-D-thiogalactopyranoside (IPTG). After IPTG induction, the cells were cultured for an additional 4 h followed by centrifugation at 5,000 rpm for 5 min. The resulting pellets were suspended in 50 mM Tris-HCl buffer (pH 8.0) and lysed by sonication for 5 min. After sonication, the cell lysate was again centrifuged at 5,000 rpm for 5 min.

Surface Modification of Gold Chip

A gold chip (2 nm of chromium as an adhesion layer and 45 nm of gold deposited on an 18 × 18 × 0.3 mm section of glass) was obtained from K-MAC Co. (Korea). Which was cleaned with a solution of H_2SO_4/H_2O_2 (3 v/v) at 50°C for 30 min. The cleaned gold chip was then immersed overnight in a $CHCl_3$ solution containing 5 mM Prolinker™ B (Proteogen Co., Korea) at room temperature to assure the formation of a calixcrown derivative self-assembled monolayer on the gold surface. It was then washed sequentially with $CHCl_3$, acetone, ethanol and deionized water. The Prolinker™ B-coated surface was immersed overnight in 10 mM PBS (pH 7.4) solution containing the His_6-specific antibody (Novagen Co., Germany) at room temperature. After washing the gold chip with PBST (10 mM PBS containing 0.5% Tween 20, pH 7.4) solution, it was immersed in 3% BSA in PBS solution for 3 h to block the chip surface. The chip was washed with deionized water to remove the excessive BSA, and then used for the detection of the hexahistidine fusion protein.

Detection of Hexahistidine Fusion Protein

The insoluble and soluble fractions were printed on the antibody-coated gold chip using a microarrayer (Proteogen Co., Korea) equipped with an SMP10 Stealth pin (Telechem Co., USA). Printing was carried out in an atmospherically controlled chamber with a relative humidity of 70-80% at room temperature. The gold chip was washed sequentially with PBST solution and deionized water to remove unbound proteins, and then dried under N_2 gas. The dried gold chip was subjected to SPRI measurement.

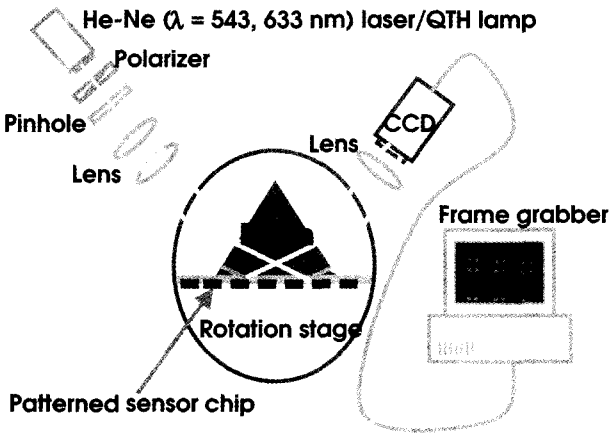

Fig. 1. The optical layout for the surface plasmon resonance imaging (SPRI) system.

RESULTS AND DISCUSSION

A two-dimensional surface plasmon resonance (2D-SPR) imaging system was constructed to obtain microarray images of protein spots on a gold thin film. Fig. 1 shows a schematic view of the SPRI system manufactured in our laboratory. The proteins spotted gold chip was optically coupled with a prism coupler (Korea Electro-optics Co., Korea) via an index matching oil (n_D = 1.517), and placed on the center of the goniometer controlled by a DC servo motor controller. When a biomolecular interaction takes place on the surface of the thin gold film, the change in the intensity of the reflected light at a fixed angle was measured. The resulting changes in the light intensity were displayed as images by a CCD camera.

Unlike DNA, proteins are physically unstable and have three-dimensional structures that are critical to their functions. Thus, the development of surface chemistry is especially required for the fabrication of protein microarrays onto a solid matrix. Recently, two different calixcrown-5 derivatives, novel and bifunctional molecular linkers, have been prepared and used to immobilize proteins for protein-protein interaction studies [10]. Of the two derivatives, ProLinker™ B, which was synthesized to contain SH groups for gold-coated substrates, was used in this study. This novel linker molecule has a great advantage over other linker molecules, with respect to the direct immobilization of proteins, without chemical modification and the correct orientation of antibodies on the ProLinker™ surface.

The hexahistidine-ubiquitin-tagged human parathyroid hormone fragment (His_6-Ub-hPTHF(1-34)), which was expressed in a soluble form, was partially purified using one-step metal affinity chromatography, spotted onto the antibody-coated gold chip using a microarrayer, and analyzed by SPRI. Fig. 2 shows a schematic view of the analytical procedure used in this study. The SPR images were captured at an incident angle lower than the SPR angle of the background surface. The protein microarray

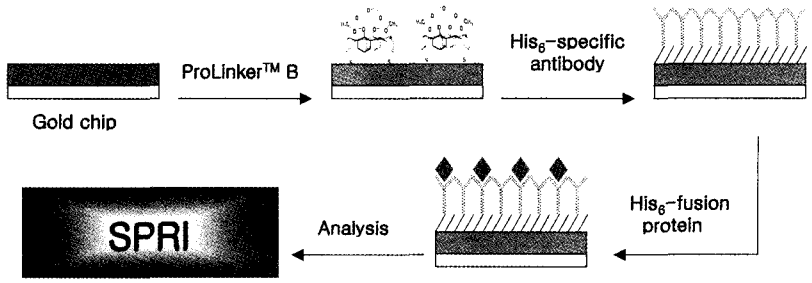

Fig. 2. A schematic view of the procedure for detection of His_6-tagged recombinant proteins using SPRI imaging.

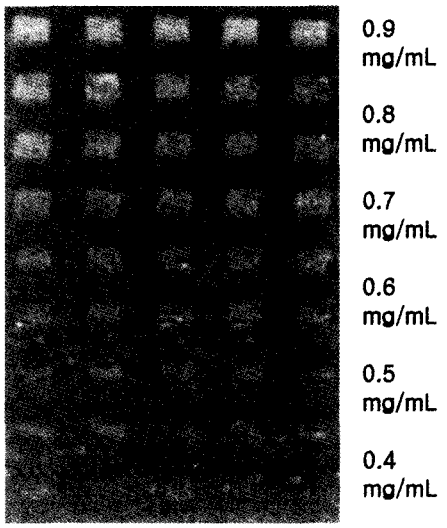

Fig. 3. SPRI analysis of purified His_6-Ub-hPTHF(1-34) at various concentrations.

images, with varying protein concentrations, are shown in Fig. 3. The brighter spots indicate the affinity binding of His_6-Ub-hPTHF(1-34) with the His_6-specific antibody. The brightness of the SPR image increased with increasing protein concentration.

Using SPR imaging, a direct analysis of the recombinant proteins, without purification, was carried out. After the recombinant *E. coli* cells expressing His_6-Ub-hPTHF (1-34) were lysed by sonication, the soluble and insoluble fractions were separated by centrifugation, and directly spotted onto the antibody-coated chip. After 30 min incubation of the spotted gold chip, to induce the affinity binding between His_6-Ub-hPTHF(1-34) and antibody, it was washed with the washing solution and deionized distilled water. After drying, the expressed protein spots bound to the antibody were measured using SPR imaging. Fig. 4 shows the result of the SDS-PAGE and SPR imaging analyses. Lanes 1, 2, 3 and 4 in the SDS-PAGE correspond to lanes 1', 2', 3' and 4' in the SPR imaging, respectively. Brighter SPR spot images were observed with the induced soluble cell lysates, whereas no visible

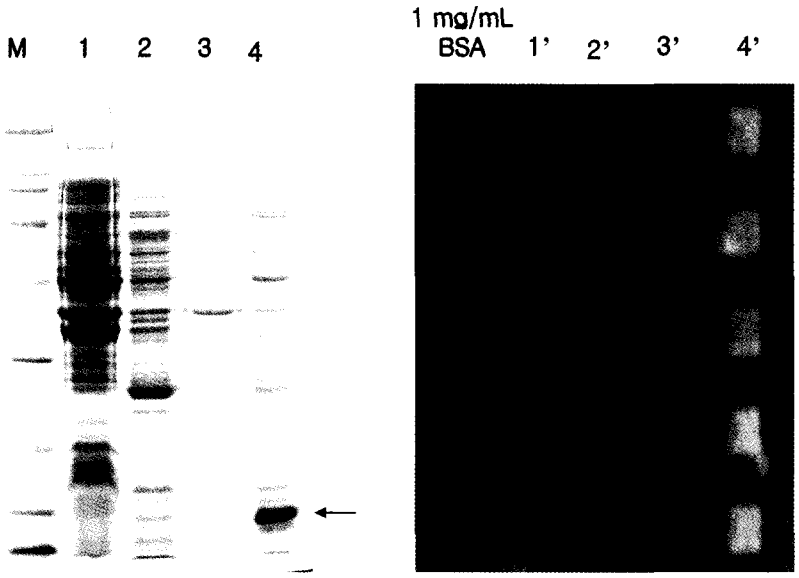

Fig. 4. SDS-PAGE (a) and SPR imaging (b) analyses of *E. coli* cell lysates expressing His_6-Ub-hPTHF(1-34): lane M, protein marker; lanes 1 and 1', insoluble fraction, uninduced; lanes 2 and 2', soluble fraction; lanes 3 and 3', insoluble fraction, induced, induced and lanes 4 and 4', soluble fraction, induced. The arrow indicates a His_6-Ub-hPTHF(1-34) protein band.

images were observed with the uninduced cell lysates. Furthermore, there were no discernible images in the insoluble fraction of the induced cell lysates. This demonstrates that the insoluble inclusion bodies cannot be detected because they do not bind to the antibody. As a control, bovine serum albumin (BSA) at a concentration of 1 mg/mL was also spotted onto the antibody-coated gold chip. The BSA control spots are almost invisible on the SPR imaging chip. This indicates that non-specific binding is negligible on the surface of the antibody/ ProLinker™ B-coated gold chip.

This study has demonstrated the creation of His_6-tagged protein microarrays on a SPR gold chip coated with a His_6-specific antibody/calix crown derivative, and its use to rapidly monitor His_6-tagged recombinant proteins using a SPRI optical detection system. The His_6-specific antibody was successfully immobilized on the self-assembled monolayer of a thiol-functionalized calix-crown, without chemical modifications. It is expected that the approach used here to detect affinity-tagged recombinant proteins using an SPRI technique could be used as a powerful tool for the analyses of a number of proteins in a high-throughput mode.

Acknowledgements This research was supported by a grant from the KRIBB Initiative Research Program and, in part, by the grants from KOSEF through the Center for Advanced Bioseparation Technology (BSEP) and from the Intelligent Microsystem Center.

REFERENCES

[1] Fivash, M., E. M. Towler, and R. J. Fisher (1998) BIAcore for macromolecular interaction. *Curr. Opin. Biotechnol.* 9: 97-101.

[2] Jordan, C. E. and R. M. Corn (1997) Surface plasmon resonance imaging measurements of electrostatic biopolymer adsorption onto chemically modified gold surfaces. *Anal. Chem.* 69: 1449-1456.

[3] Smith, E. A., M. G. Erickson, A. T. Ulijasz, B. Weisblum, and R. M. Corn (2003) Surface plasmon resonance imaging of transcription factor proteins: Interactions of bacterial response regulators with DNA arrays on gold films. *Langmuir* 19: 1486-1492.

[4] Smith, E. A., M. Kyo, H. Kumasawa, K. Nakatani, I. Saito, and R. M. Corn (2002) Chemically induced hairpin formulation in DNA monolayers. *J. Am. Chem. Soc.* 124: 6810-6811.

[5] Wegner, G. J., H. J. Lee, and R. M. Corn (2002) Characterization and optimization of peptide arrays for the study of epitope-antibody interactions using surface plasmon resonance imaging. *Anal. Chem.* 74: 5161-5168.

[6] Wegner, G. J., H. J. Lee, G. Marriott, and R. M. Corn (2003) Fabrication of histidine-tagged fusion protein arrays for surface plasmon resonance imaging studies of protein-protein and protein-DNA interactions. *Anal. Chem.* 75: 4740-4746.

[7] Smith, E. A., W. D. Thomas, L. L. Kiessling, and R. M. Corn (2003) Surface plasmon resonance imaging studies of protein-carbohydrate interactions. *J. Am. Chem. Soc.* 125: 6140-6148.

[8] S. A. Lesley (2001) High-throughput proteomics: protein expression and purification in the postgenomic world. *Protein Expr. Purif.* 22: 159-164.

[9] Oh, G.. H., M. S. Hahm, and B. H. Chung (1999) Use of carboxypeptidase Y propeptide as a fusion partner expression of small polypeptides in *Escherichia coli*. *Protein Expr. Purif.* 17: 428-434.

[10] Lee, Y., E. K. Lee, Y. W. Cho, T. Matsui, I. C. Kang, T. S. Kim, and M. H. Han (2003) Proteochip: A highly sensitive protein microarray prepared by a novel method of protein immobilization for application of protein-protein interaction studies. *Proteomics* 3: 2289-2304.

[Received February 28, 2004; accepted April 15, 2004]