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Microbiological Applications of Surface Plasmon Resonance (SPR) Imaging-based Protein Array Chip System

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A surface plasmon resonance (SPR) imaging system was constructed and used to detect the affinity-tagged recombinant proteins (6xHis, GST, or MBP tags) expressed in *Escherichia coli*. The cell lysates were spotted on gold thin films coated with 11-mercaptoundecanol (MUOH)/dextran derivatized with Ni^{+2}-iminodiacetic acid (IDA- Ni^{+2}), glutathione, or cyclodextrin. After briefly washing the gold chip, SPR imaging measurements were carried out in order to detect the bound affinity-tagged fusion proteins. Using this new approach, rapid high-throughput expression analyses of the affinity-tagged proteins were obtained. The SPR imaging protein chip system used to measure the expression of affinity-tagged proteins in a high-throughput manner is expected to be an attractive alternative to traditional laborious and time-consuming methods, such as SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and western blots.

Introduction

With the current expansion of sequenced microbial genomes as well as genomes from eukaryotic organisms, proteomic approaches in the field of biological sciences become increasingly important for the better understanding of life in the context that life itself can be accounted for the functions of various cellular proteins. In this regard, the concept of high-throughput process in the expression, purification and analysis of target proteins is one critical demand to the technology developing sector to handle hundreds of, even thousands of, proteins within a given short period of timeframe. In this study, we developed a high-throughput tool to detect the expressed proteins based on Surface plasmon resonance (SPR) imaging protein chip system. SPR is an optical technique used to detect the specific binding of unlabeled bio-molecules onto molecules attached to chemically modified gold thin films by measuring changes in the index of refraction upon adsorption [1,2]. Furthermore, it has also been used as a valuable tool to investigate molecular interactions in real time without the use of labels [3]. SPR sensors have also been used for the rapid monitoring of recombinant proteins [4]. The SPR technique can be expanded to SPR imaging and used for the high-throughput analysis of bioaffinity interactions by fabricating protein arrays on gold surfaces. SPR imaging utilizes collimated illumination of the entire surface, with the reflected beam imaged onto a two-dimensional array detector [5]. Since the SPR imaging protein array requires no labels

for the detection of capture proteins, it is considered as an ideal surface-sensitive optical technique that directly detects the multiple interactions of molecules on a two-dimensional gold surface. SPR imaging has successfully been applied to the measurements of bioaffinity interactions using DNA [6-8], peptide [9], protein [10], and carbohydrate arrays [11].

In this study, we introduce an efficient technique to rapidly detect the 6xHis-, glutathione-S-transferase (GST)-, and maltose-binding protein (MBP)-fused proteins expressed in *E. coli* using SPR imaging measurement.

Construction of SPR imaging system

A two-dimensional surface plasmon resonance (SPR) imaging system was constructed to obtain microarray images of the protein spots. A 150 W quartz tungsten-halogen lamp (Schott, Germany) was used as the light source and the light was delivered to a goniometer arm (Physik Instrumente, Germany) using a liquid light guide (Oriel Instruments, USA). The light collimated by the lenses was passed through a narrow interference filter (647.1 nm, $\Delta\lambda$=1 nm; Oriel Instruments) and a polarizer (Newport, USA) in order to convert a monochromatic and a linear polarized beam, respectively. The diameter of this beam was about 2 cm. The gold sensor chip on which proteins were spotted, 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. The goniometer was electronically controlled by a DC servo motor controller (Physik Instrumente). The reflected images from the gold chip were taken by a 1/2 inch charge coupled device (CCD) camera (Sony, Japan), while the contrast images were monitored by a personal computer. A combination of lenses was placed in front of the bare CCD chip in order to obtain a clear image. The images were then stored digitally in a personal computer using a B/W frame grabber (National Instrument, USA).

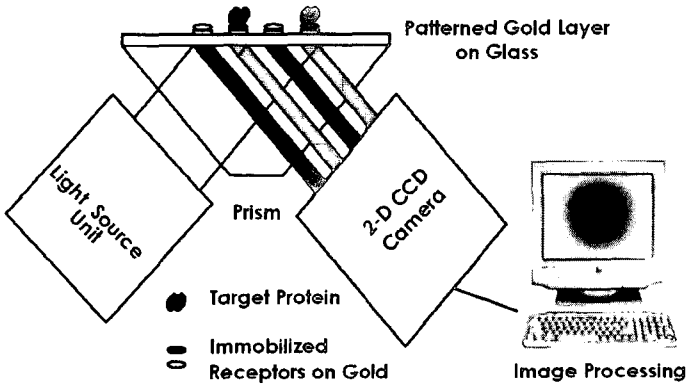

Fig. 1. Schematic view of the SPR imaging protein chip system.

Surface modification of gold chips for the specific binding of fusion proteins

A gold chip (2 nm of chromium as an adhesion layer and 45 nm of gold deposited on an 18 x 18 x 0.3 mm glass) was immersed in a 10 mM ethanolic solution of MUOH at room temperature for 20 - 24 h to

assure the formation of a 11-mercaptoundecanol (MUOH) self-assembled monolayer on the gold surface. To activate the hydroxyl group of the MUOH, the gold chip was treated with 0.6 M epichlorohydrin in a 1:1 mixture of 0.4 M NaOH and 2-methoxyethyl ether for 4 h. After being washed with ethanol and water, the gold chip was immersed in a 0.3 mg/ml dextran solution in 0.1 M NaOH for 20 h. The dextran-coated surface was once again treated with the 0.6 M solution of the epichlorohydrin in the 1:1 mixture of the 0.4 M NaOH and 2-methoxyethyl ether for 4 h to activate the dextran's hydroxyl group. This epichlorohydrin-activated dextran chip was further functionalized in order to give it a specific affinity for the recombinant fusion proteins. It was treated with a 1.7 M solution of iminodiacetic acid (IDA) in 2 M sodium carbonate at 60℃ for 20 h (IDA-Ni(II) chip), a solution of 0.1 mg/ml L-glutathione (reduced form) in pH 7.0 and 44 mM phosphate buffer at 40℃ for 20 h (glutathione chip), and a 70 mg/ml solution of β -cyclodextrin in 0.1 M NaOH at 40℃ for 20 h (cyclodextrin chip), respectively. The IDA-functionalized chip, after being immersed in a 50 mM nickel (II) chloride solution for 3 - 4 h and washed with water, was used as a hexahistidine fusion protein-binding chip (IDA-Ni(II) chip).

Detection of fusion proteins

The hexahistidine-ubiquitin-tagged hGH (His_6-Ub-hGH), which was expressed in a soluble form, was partially purified using a one-step metal affinity chromatography, and analyzed using both SPR imaging and SDS-PAGE (Fig. 2). The SPR images were captured at an incident angle that was lower than the SPR angle of the background surface. The brighter spots are indications of the affinity binding of the target proteins on the IDA-Ni(II) chip. Protein microarray images with varying protein concentrations are shown in Fig. 2(b). The brightness of the SPR image increased as the protein concentration increased. As a control, bovine serum albumin (BSA) with a concentration of 100 μg/ml was spotted on the IDA-Ni(II) gold chip. As shown in Fig. 2(b), the control spots are almost invisible on the SPR image, thus indicating that the protein binding is specific.

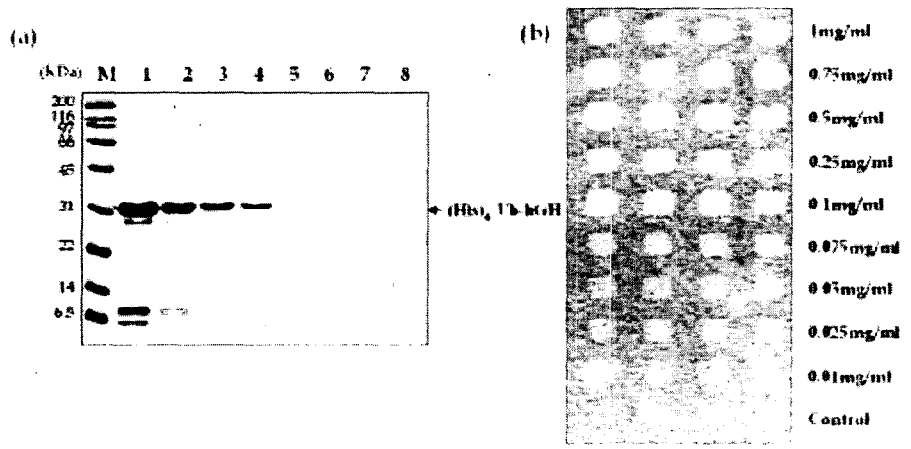

Fig. 2. SDS-PAGE (a) and SPR imaging (b) analyses of partially purified His6-Ub-hGH fusion protein at different concentrations: lane M, protein maker; lane 1, 0.5 mg/ml; lane 3, 0.25 mg/ml; lane 4, 0.1 mg/ml; lane 5, 0.05 mg/ml; lane 6, 0.01 mg/ml; lane 7, 0.005 mg/ml; lane 8, 0.0025 mg/ml; lane 9, 0.001 mg/ml.

Along with hexahistidine residues, GST and MBP are also widely used as an affinity tag and offer the possibility of purifying the recombinant proteins through the use of affinity chromatographies. When the target protein is fused to a highly soluble partner such as the GST or MBP moiety, correctly folded and soluble heterologous proteins are produced intracellularly in the bacterial cytoplasm [12]. Human interleukin 6 fused with GST (GST-hIL6) and MBP (MBP-hIL6) was expressed in *E. coli* in a soluble form, and then the cell lysates were directly spotted onto a glutathione or cyclodextrin-functionalized gold chip. Fig. 3 shows the SDS-PAGE and SPR imaging analyses for GST-hIL6 and MBP-hIL6, respectively. Lane 2 and 3 in the SDS-PAGE correspond to lane 2 and 3 in the SPR imaging, respectively. Unlike the His_6-Ub-hGH expression, the GST-hIL6 and MBP-hIL6 expression in uninduced cells was hardly detected on the SDS-PAGE (Lane 2 in Fig. 3(a) and Fig. 3(c)). The brighter SPR images were clearly observed with induced cell lysates, whereas no visible images were obtained with uninduced cell lysates (Fig. 3(b, d)). This demonstrates that the SPR imaging analysis corresponds to the SDS-PAGE quantitatively.

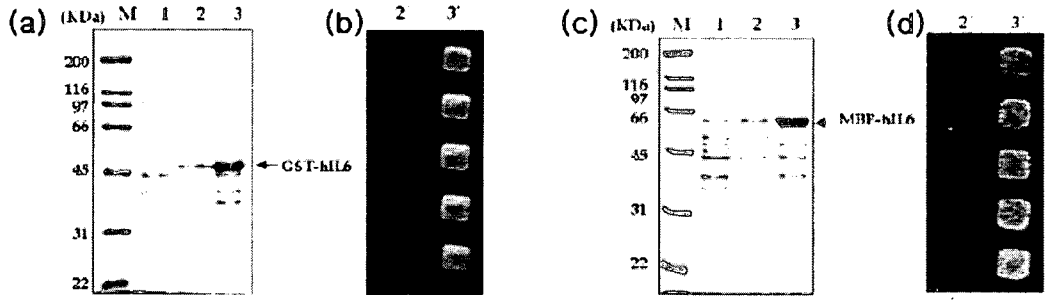

Fig. 3. SDS-PAGE (a, c) and SPR imaging (b, d) analyses of E. coli cell lysates expressing GST-hIL6 (a, b) and MBP-hIL6 (c, d) fusion proteins: lane M, protein marker; lane 1, untransformed cell lysates; lane 2 and 2', transformed cell lysates, uninduced; lane 3 and 3', transformed cell lysates, induced with IPTG addition.

The sequencing of the genomes of various organisms has led to the concept of analyzing protein function on a genome-wide scale. Recently, rapid progress in high-throughput and parallel approaches in protein expression, purification, and detection has been made to study proteins on a global scale. So far, many researches have focused on the development of robust techniques for high-throughput and the parallel expression and purification of multiple proteins. However, the detection of multiple proteins, which are often expressed in 96-well microtiter plates for automation, relies on traditional methods such as the SDS-PAGE and western blots. In this study, therefore, we introduce a novel approach to the rapid and simultaneous detection of multiple recombinant proteins expressed in *E. coli* with affinity tags to facilitate purification. This method enables on-chip purification, label-free detection, and on-chip quantitative analyses of target proteins. It is thus expected that the SPR imaging measurement of protein expression can be an attracting alternative to traditional methods such as the SDS-PAGE and western blots.

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