Surface Plasmon Resonance Imaging-Based Protein Array Chip System for Monitoring a Hexahistidine-Tagged Protein during Expression and Purification

Hyeon-Su Ro,† Sun Ok Jung, Byung Hoon Kho, Hyung Pyo Hong, Jae Sung Lee, Yong-Beom Shin, Min Gon Kim, and Bong Hyun Chung*

BioNanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology, Yuseong, Daejeon, Korea

Received 18 March 2004/Accepted 13 September 2004

A surface plasmon resonance imaging-based Ni2+-iminodiacetic acid-coated gold chip system was developed to enable specific detection of a hexahistidine-tagged recombinant protein in crude extracts or in column chromatography fractions. This system is especially advantageous for high-throughput analysis of multiple proteins.

With the advent of proteomics and the requirement of analysis of a massive variety of expressed proteins, rapid and high-throughput detection of the proteins becomes increasingly important for high-throughput expression and purification of proteins (1–3). Recently we have reported a novel method for rapid detection of affinity-tagged recombinant proteins spotted onto a modified gold thin film by surface plasmon resonance (SPR) imaging (SPRI) (5). SPRI detects the specific binding of unlabeled biomolecules to arrayed capture molecules attached to a modified gold thin film by measuring changes in the local index of refraction upon adsorption (11). It has been proven to be a valuable tool for investigating molecular interactions without the use of any fluorescent or radiochemical labels (4, 6–8). Use of the SPR principle can be further expanded to the detection of microbial cells with immobilized antibodies or protein ligands on an SPR sensor chip (9, 12).

In this report, we demonstrate that SPRI can be used to achieve rapid and high-throughput monitoring of affinity-tagged proteins in the expression and purification processes. We also demonstrate that the SPRI protein array chip technology can be a powerful tool for the simultaneous detection of multiple proteins that are cloned and expressed on a genome-wide scale.

Plasmid construction, protein expression, and preparation of cell extract. The expression vectors used in this study, including the esterase S21 expression vector, were previously constructed (10). Expression and preparation of proteins were performed as previously reported (10). To analyze the solubility of the expressed proteins, cells were harvested at the time intervals shown in Fig. 1. Harvested cells were washed twice with 10 ml of phosphate-buffered saline (NaCl at 8 g/liter, KCl at 0.2 g/liter, Na2HPO4 at 1.44 g/liter, KH2PO4 at 0.24 g/liter, pH 7.4), resuspended in 10 ml of 50 mM Tris-HCl buffer (pH 8.0), and destroyed by sonication. The soluble and insoluble fractions of the sonicated solution were obtained by centrifugation (16,600 × g, 15 min; Micro 17R; Hanil, Incheon, Korea). The insoluble fraction was dissolved in 50 mM Tris-HCl (pH 8.0) buffer containing 8 M urea. Ten microliters of each soluble or insoluble sample was mixed with an equal volume of 2× sodium dodecyl sulfate (SDS) sample buffer and boiled for 5 min. Proteins were analyzed by SDS–12% polyacrylamide gel electrophoresis (PAGE) and visualized by Coomassie brilliant blue R-250 staining.

Ion-exchange column chromatography. Crude cell extract was loaded onto an anion-exchange column (Uno Q1; Bio-Rad). The column was washed and equilibrated with 50 mM Tris-HCl (pH 8.0). The bound proteins were eluted with a linear gradient of 0 to 1 M NaCl in the equilibration buffer. Fractions were collected in a 0.5-ml volume throughout the purification procedure.

Analysis of six-His-tagged proteins spotted onto an Ni2+-IDA gold chip with the SPRI system. The Ni2+-iminodiacetic acid (IDA) gold chip used was prepared as previously reported (5). Sample solutions containing six-His-tagged proteins were spotted onto the modified gold chip with a microarrayer (Proteogen, Seoul, Korea). SPRI measurement was conducted by capturing the image reflected by the gold chip with a charge-coupled device camera as described previously (5).

Monitoring of the expression of six-His-tagged esterase S21. The expression of esterase S21 in Escherichia coli cells was measured with the SPRI protein chip system, as well as SDS-PAGE. At the initial stage of E. coli cell culture, there was no detectable esterase expression until the inducer was added. Addition of isopropyl-β-D-thiogalactopyranoside (IPTG) immediately induced the expression of the protein, as shown in Fig. 1. Further analysis of the sonicated samples revealed that most of the expressed protein was produced as an insoluble form (Fig. 1A). In order to investigate whether the SPRI protein chip could correctly reflect the SDS-PAGE data, we spotted the samples used in the SDS-PAGE analysis onto the Ni2+-IDA gold chip. The SPRI measurement showed that the SPR signal intensity increased as the protein expression level increased (Fig. 1B). The signal intensity-versus-time plot re-
revealed that the result of the SPRI analysis was comparable to that of the SDS-PAGE band intensity analysis (Fig. 1C).

**Monitoring of six-His-tagged esterase S21 in the purification process.** To show the effectiveness of the SPRI protein chip in identifying the fractions containing a target protein in the chromatography process, we traced the six-His-tagged esterase S21 by SDS-PAGE analysis and SPRI measurement. The chromatogram indicated that the proteins started to elute at 0.25 M NaCl, which corresponded to the 10th fraction (Fig. 2A). Subsequent SDS-PAGE analysis (Fig. 2B) and assay of the activity of the fractions (data not shown) revealed that the target protein eluted in a broad range of NaCl concentrations with the peak density of the protein with a molecular mass of 31 kDa, which corresponds to esterase S21, in fraction 20 (Fig. 2B). The same samples were subjected to SPRI analysis. All of the fractionated samples were spotted onto a single Ni²⁺-IDA gold chip. The chip was washed with washing buffer, and the bound, six-His-tagged protein was detected by the SPRI system (Fig. 2C). The spot intensity corresponded well to the amount of the target protein in the fractions judged by SDS-PAGE analysis (Fig. 2D). The densest spot, which corresponded to the thickest protein band in the SDS-PAGE analysis, was found in fraction 20.

**Effect of protein size on signal intensity.** SPRI signal intensity was measured with proteins with different molecular weights. The proteins used in the experiment were CAC95742 (P1, 22 kDa), ZP_00266588 (P2, 41 kDa), and CAB15152 (P3, 53 kDa). The purified, six-His-tagged proteins (Fig. 3A) were spotted onto the chip, and the resulting SPRI image is shown in Fig. 3B. SPRI signal intensity increased linearly as the protein concentration increased. Further analysis of the data revealed that the rate of the increase in signal intensity was independent of the protein molecular weight, indicating that the signal intensity reflects not the size of the protein but its concentration (Fig. 3C).

**Rapid detection of multiple microbial esterases.** To demonstrate the high-throughput nature of the SPRI system, we spotted crude cell extracts expressing 30 six-His-tagged microbial esterases from our previous report (10) onto the Ni²⁺-IDA gold chip. SPRI protein chip analysis revealed that the esterases were expressed at various concentrations, while the control experiment with bovine serum albumin resulted in virtually no signal (Fig. 4). In addition, the concentration of the expressed proteins in the cell lysates could be directly calculated by using the standard curve shown in Fig. 3C. For example, esterases S1 to S7 were expressed within a concentration range of 0.5 to 0.8 mg/ml. The S8, S22, S24, and S29 concentrations were more than 3 mg/ml, while the S16, S28, and S30 concentrations were less than 0.05 mg/ml.

In conclusion, we have demonstrated that the SPRI protein chip system can be used for fast monitoring of a six-His-tagged protein in the expression and purification processes. The major advantages of the SPRI protein chip system are as follows: first, it requires a sample amount as small as several nanoliters per spot; second, since it is an array-type assay, a large number of samples can be processed with a single chip; third, it directly detects a protein without the use of labels; and fourth, since
FIG. 2. Ion-exchange column chromatography of esterase S21. A chromatogram (A) and SDS-PAGE analysis of the column fractions (B) are shown. The letters T and U represent the total cell lysate and the unbound fraction, respectively. The number on each column is the fraction number. The arrows indicate the target protein. (C) SPRI analysis of fractionated samples. (D) Digitized SPRI signal intensity of each sample. Three sets of duplicate readings were performed and analyzed.
SPR signal intensity is proportional to the protein concentration, it enables quantitative analysis on a single chip. These features of the SPRI protein chip system are especially beneficial when rapid and high-throughput analysis of expressed proteins is required. Lastly, we stress that application of the SPRI protein chip system is not limited to high-throughput protein analysis. For example, arrayed antibodies specific for pathogenic or environmentally important microorganisms on the chip will enable rapid detection and classification of contaminants.

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REFERENCES


