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Label-free reading of microarray-based proteins with high throughput surface plasmon resonance imaging

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Abstract

A simple method is presented discriminating proteins at a gold surface by using an emerging technology, surface plasmon resonance (SPR) imaging. As a high throughput method, the protein array of bovine serum albumin (BSA), poly-L-lysine (PL), casein and lactate dehydrogenase (LDG) was fabricated and SPR imaging enables detection from different kinds of proteins immobilized on the sensor surface. These proteins can be discriminated directly by various reflected intensity or changing the incident angular position of light. Denaturation of these immobilized proteins on SPR sensor by interacting with denaturant 6 M GdnHCl solution was also performed and obvious changes in reflected intensity were occurred after denaturation. The observation of denaturation of these proteins further supported the fact that different proteins could be discriminated on protein array before denaturation. On the other hand, the procedure of denaturation provided useful information that any change of molecular structure with the progress of denaturation would result in change of SPR signal. Excellent reproducibility with a chip-to-chip for label-free discriminating various proteins was achieved.

Keywords: Label-free; Protein array; Surface plasmon resonance imaging; Denaturation

1. Introduction

Protein arrays can be used to identify and quantify bioaffinity interactions of proteins, DNA, RNA, and peptides in a multiplexed format. To date, protein arrays have been used to detect clinically interesting proteins in human serum at concentrations relevant to the analysis of blood samples (Pavlickova et al., 2002; Haab et al., 2001). Protein arrays have also been used as a proteomics screening tool to characterize biochemical processes by identifying novel protein–protein and protein–DNA binding interactions (Zhu et al., 2000, 2001). These results suggest that protein arrays will become essential tools in both clinical and research settings to identify the presence or absence of multiple proteins in a sample, to characterize protein function, and to design pharmaceutical agents that disrupt or stimulate specific bioaffinity interactions.

Microarrays of immunoreagents and other proteins continue to be developed largely by extending technologies used for gene chips (MacBeath and Schreiber, 2000) but remain much more problematic. For example, the fluorescence detection methods are so successful for gene chips, but much less convenient with protein chips due to synthetic challenges, multiple label issues, and the potential for interfering with the binding site. Also, signal-producing reactions catalyzed by commonly used enzyme-linked antibodies are difficult to implement in immunoassay microarray format. Strategies for the immobilization of proteins in the most efficient orientation and conformation for interactions with binding partners are much more complex (Wilchek and Miron, 2003). Nonspecific adsorption is also a much greater problem for proteins than for DNA. Finally, it is not likely that a standard set of proteins will be made readily available in soon future that has a large enough scope to meet all needs, so that custom-made protein chips that are easy to fabricate will be needed.

Most protein arrays currently developed rely on detection technologies that apply enzymatic or fluorescent tags. In contrast, SPR imaging is a label-free, surface-sensitive spectro-

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scopic technique used to study bioaffinity interactions at thin gold films. SPR detects changes in refractive index within a short distance from the surface of a thin metal film as variations in light intensity reflected from the back of the film and, thus, does not require labeling. SPR imaging has been successfully applied to the screening of bioaffinity interactions using DNA (Guedon et al., 2000; Tawa and Knoll, 2004; Shumaker-Parry and Campbell, 2004), peptide (Wegner et al., 2002,2004), carbohydrate arrays (Smith et al., 2003) and proteins (Kanda et al., 2004; Pyo et al., 2005; Jung et al., 2004). In this study, a facile method for label-free reading of proteins on gold is demonstrated using SPR imaging. Four kinds of proteins were immobilized on SPR sensor chip and could be directly distinguished from the distinct reflected light intensity resulting from the various molecular size and molecular structure of these proteins.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA, MW 66,200), poly-L-lysine (average molecular weight 14,000), casein (MW 24,000), and lactate dehydrogenase (LDG, 140,000) were received from Sigma. *N*-hydroxysuccinimide (NHS) was obtained from ACROS (New Jersey, USA), *N*-ethyl-*N*-[(dimethylamino) propyl]carbodiimide (EDC) from Avocado Research Chemicals Ltd. (Lancashire, UK), mercaptoundecanoic acid (MUA) from Aldrich (Milwaukee, USA). All of the chemicals, unless mentioned otherwise, were all of analytical reagent grade and used as received. Aqueous solutions were prepared in doubly distilled water.

2.2. Surface attachment chemistry

The SPR experiments utilized thin gold films (47 nm) that had been vapor deposited onto BK7 glass slides. MUA monolayers were formed on gold films by immersing vapor deposited gold surfaces into a 5 mM ethanolic solution for at least 18 h followed by thorough rinsing with ethanol and water. The modified gold film was bond to NHS by immersing it in an aqueous solution of 75 mM EDC and 15 mM NHS for 30 min (Patel et al., 1997). The same concentration of BSA, poly-L-lysine, casein, and lactate dehydrogenase (1 mg/mL) dissolved in phosphate buffer saline (pH 7.4) were spotted on the NHS-terminated gold surface with a spot diameter of about 120 μm, respectively, using a homemade x-y micro spotting device. After reaction for about 1 h, the surface was rinsed with phosphate buffer saline and then blocked by 1 M ethanolamine at pH 8.5 for 10 min. The unreactive ethanolamine was removed and the surface was rinsed exhaustively with water and dried under a stream of nitrogen gas.

2.3. SPR imaging setup and procedure of imaging

A two-dimensional surface plasmon resonance imaging system was constructed to obtain microarray images of the protein spots. The schematic of the SPR imaging setup is shown elsewhere (Huang et al., in press). The incident light from a light emission diode was polarized through a polarizer and then expanded by a beam expander. After hitting and being reflected from the gold film via a 90° glass prism Kretschmann's all total reflection (ATR) coupler (Kretschmann, 1971), it was focused by optical lens and collected by CCD camera (MTV-1881EX, Taiwan). The collected image was finally treated and saved by software developed in this laboratory. The polarizer was so adjusted that, for excitation, only p-polarized light was produced, and for reference, only s-polarized wave came out. At the beginning of experiment, a image obtained from s-polarized light was collected and stored as background, then the SPR images were obtained automatically subtracting the background by soft. The glass prism was made of BK7 with $n = 1.517$. The slide with gold film was attached to the prism through an index matching fluid ($n = 1.51$), with the gold film facing the sample solution, which filled in a Teflon flow cell and was sealed (60 μL volume). The exposing area was 1 cm^2. In the process of experiment SPRI setup was in a box to avoid ambient light, which provided superior contrast images in aqueous solution.

2.4. GdnHCl-induced denaturation

GdnHCl solution was prepared by dissolving in PBS. Denaturation treatment was started by injection of GdnHCl solution onto the sensor surface in the flow cell, which was equilibrated with running buffer (10 μL/min), 10 mM Tris–HCl at pH 7.4. The injection was terminated by replacement with running buffer. The value of the equilibrium resonance signal of the acid treatment was therefore obtained.

3. Results and discussion

3.1. Reading of various proteins

As one of the most sensitive detection methods for monitoring variations in the thickness and refractive index in ultra-thin films, SPR is very sensitive to detect the change of refractive index as small as 10^{-6} order of magnitude (Zybin et al., 2005) and particularly suitable for the detection of biological macromolecules. Slight changes of molecular structure of biological macromolecules would lead to changes of refractive index, which results in changes of SPR signal. At the same time, light intensity reflected from the back of gold film varies with the change of incident angle, and a SPR curve can be obtained from a plot of reflected intensity against incident angle. Fig. 1 shows the calculated SPR reflectance curves for different proteins, which elucidates various proteins relevant to SPR curves and resonant angle (unicorn.ps.uci.edu/calculations). In contrast to SPR where either the angle or the wavelength is fixed and the reflected light is detected by a single-element detector, SPR imaging is performed with both fixed, measuring the change in reflectance across the sample. The distinct areas are present for the real-time detection of biopolymer that bind to the gold surface, as this will result in a change of the surface plasmon resonance condition or a change of the reflected light intensity. A CCD camera is used to capture the reflected light. The principle of SPR imaging and the relationship between SPR imaging and