Chapter 17

High-Throughput SPR Biosensor

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Summary

Surface plasmon resonance (SPR) imaging technique is label free, real-time, and high-throughput analysis method for interaction studies with array format. The application of SPR imaging for the small molecule arrays, which were fabricated by photoaffinity crosslinking, can be the first screening step for reverse chemical genomics. The fabrication process of sugar array and sugar–lectin interaction study was demonstrated. The protocol of array fabrication did not require any chemical modifications of sugar chains for immobilizations. The biotinylated sugars were used to investigate signal ratios between lectin and antistreptavidin antibody binding. And it seemed that signal normalization could be achieved, even though the accurate densities of immobilized sugars were unclear.

Key words: Surface plasmon resonance imaging technique, Small molecule arrays, Photoaffinity crosslinking, High-throughput screening, Sugar array, Signal normalization

1. Introduction

Surface plasmon resonance (SPR) technique had become popular in interaction studies between biological molecules *(1)*. It is an optical biosensor, and the interactions can be detected by SPR angle shift or reflection light intensity. In typical SPR measurement, one of pair interacting biomolecules was immobilized on a gold chip, and another was flowed over the chip as its solution. There are two major advantages in SPR assay**; (a) real time evaluations on kinetics studies and (b) label-free measurements.

In last decade, SPR imaging technique, which can analyze biomolecules with array format, has been closed up as a high-throughput

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SPR biosensor *(2, 3)*. In SPR imaging measurement, a polarized parallel light and CCD camera were used as light source and detecting device, respectively *(4)*. Recently, several high-throughput analyses with SPR imaging had been reported on oligonucleotides–small molecules *(5, 6)*, oligonucleotides–proteins *(7–10)*, antibodies–proteins *(11, 12)*, peptides–proteins *(13, 14)* and proteins–proteins *(15, 16)*.

Most recently, we reported small molecule arrays on photoaffinity crosslinker coated gold surfaces *(17)*. The small molecule arrays were fabricated by photoreaction, and then analyzed by SPR imaging technique. The small molecules don't have to be modified chemically for immobilization. The small molecules, which can interact with a target protein, can be screened by this methodology. Therefore, the integration of photoaffinity small molecule array and SPR imaging technique can be the first step of reverse chemical genetics.

The integrated technology was applied for sugar array in this chapter. A sugar array was fabricated on gold with photoaffinity crosslinking, and the interaction between a lectin maackia amurensis hemagglutinin (MAH) and a sugar chain sialyl T antigen was analyzed by using SPR imaging technique. Signal normalization was also investigated by using biotinylated sugars.

2. Materials

2.1. Preparation of Sugar Solutions

1. Biotinylated sialyl T antigen with polyacrylamide (PAA) backbone (Sialyl T; Neu5Acα2–3Galβ1- 3GalNAcα-PAA-biotin, Glycotech, USA).
2. Biotinylated sialyl Tn antigen with PAA backbone (Sialyl Tn; Neu5Acα2–6GalNAcα-PAA-biotin, Glycotech).
3. Biotinylated sialyl LacNAc with PAA backbone (Sialyl LacNAc; Neu5Acα2–3Galβ1–4GlcNAcβ- PAA-biotin, Glycotech).
4. 96-well microplate with V-shaped bottoms (Toyobo, Japan).

2.2. Fabrication of Sugar Arrays by Photoaffinity Crosslinking

1. A photoaffinity crosslinker modified gold chip (Toyobo). Store at 6°C. Keep 10 min at room temperature before use.
2. A contact pin type automated spotter with Φ450 μm pin (Toyobo).
3. A UV transmission filter (Sigma-Koki, Tokyo), which can shield UV below 300 nm.
4. A UV crosslinking instrument with 365 nm lamps (Stratagene, USA).

2.3. SPR Imaging Analysis

1. *Tris-based Running buffer*. 50 mM Tris–HCl [pH 7.4], 0.1 mM $CaCl_2$, 0.1 mM $MnCl_2$.
2. Maackia amurensis hemagglutinin (MAH, Vector, CA).
3. Streptavidin (SA, Invitrogen, CA). antistreptavidin antibody (anti-SA, Vector).
4. SPR imaging instrument (Toyobo) with analysis program.

3. Methods

3.1. Fabrication of Sugar Arrays by Photoaffinity Crosslinking

1. Sialyl T was dissolved with distilled water to be 500, 250, 100, 50, and 25 μg/mL (*see* **Note 1**).
2. 500, 250, 100, 50, and 25 μg/mL of Sialyl Tn; Sialyl LacNAc were also prepared with distilled water.
3. 10 μL of 15 sugar solutions (three species, five concentrations) were poured into 96-well microplate with V-shaped bottoms.
4. A photoaffinity crosslinker modified gold chip and 96-well microplate with 15 sugar solutions was placed in a contact pin type automated spotter.
5. The spotting pattern was created according to the spotter program.
6. 10 nL drops of sugar aqueous solutions with 500, 250, 100, 50, and 25 μg/mL were delivered by φ150 μm pin of the automated spotter on the gold chip, which had photoreactive group (*see* **Note 2**).
7. The chip, on which aqueous drops were aligned, was placed into a vacuum chamber to evaporate water on the spots.
8. The chip with dried spots was covered with a UV transmission filter, and then placed into UV crosslinking instrument.
9. The chip was irradiated with 2.8 J/cm^2 with 365 nm lamps (*see* **Note 3**).
10. The chip surface was repeatedly rinsed with water and ethanol.

3.2. SPR Imaging Analysis

1. The SPR analysis procedures were indicated in **Fig. 1**. The binding of MAH on sialyl T, regeneration with NaOH and the binding of SA and anti-SA were observed.
2. 300 μL of 25 μg/mL MAH, 2.5 μg/mL SA and 2.5 μg/mL anti-SA were prepared with the tris-based running buffer (*see* **Note 4**).
3. The fabricated sugar array was placed into an SPR imaging instrument.

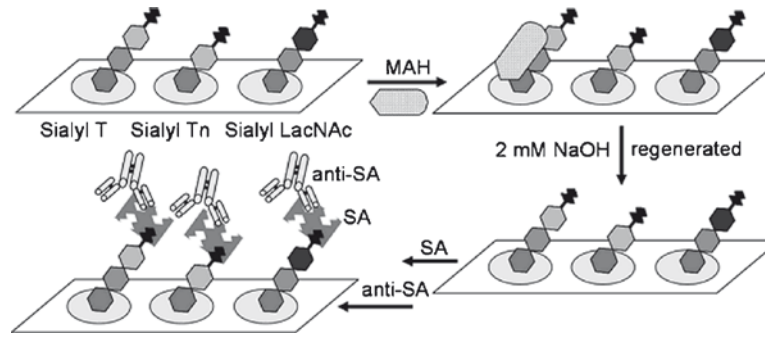

Fig. 1. An analysis procedure of a photocrosslinked sugar array by SPR imaging. A fabricated sugar array with sialyl T, sialyl Tn and sialyl LacNAc were exposed with MAH, regenerated with NaOH and then, exposed with SA and anti-SA.

4. Tris-based running buffer was flowed through SPR instrument with 100 μL/min. SPR experiment was performed at 30°C. The SPR measurement was performed at the angle, which the reflectivities on the sugar spots were approximately 10%.
5. The SPR measuring program was started, and stable base lines were established.
6. 25 μg/mL MAH solution was injected for 10 min with recycling mode, subsequently the running buffer was flowed for 5 min.
7. The chip was regenerated with 2 mM NaOH for 5 min. then the running buffer was flowed for 5 min (*see* **Note 5**).
8. 2.5 μg/mL SA solution was injected for 10 min with recycling mode, subsequently the running buffer was flowed for 5 min.
9. 2.5 μg/mL anti-SA solution was injected for 10 min with recycling mode, subsequently the running buffer was flowed for 5 min.
10. The signal data and images were collected and analyzed with an analysis program.
11. The signal data and image data on the sugars spots, which were prepared with 500 μg/mL sugar solutions, were described in **Fig. 2a**, **b**, respectively (*see* **Note 6**).
12. The image data on Sialyl T spots, which were prepared with 500, 250, 100, 50, and 25 μg/mL sugar solutions, were shown in **Fig. 3** (*see* **Note 7**).
13. The signals by MAH (S_1) and by anti-SA (S_2) on Sialyl T spots (500, 250, 100, 50, and 25 μg/mL) were summarized in **Table 1**. The signal ratios (S_1/S_2) were calculated (*see* **Note 8**).

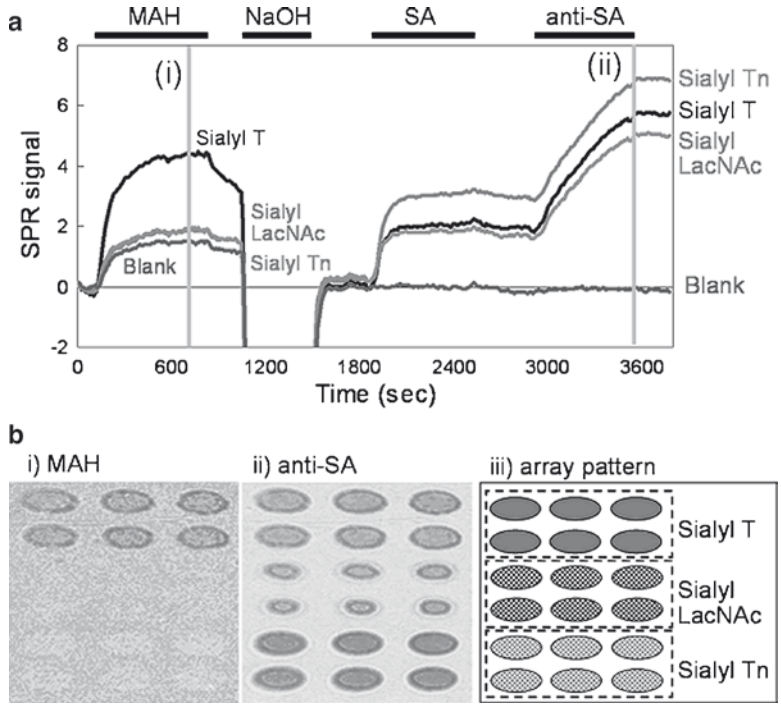

Fig. 2. The interaction analysis of the photocrosslinked sugar array by SPR imaging. (**a**) SPR signal changes on sialyl T, sialyl Tn, sialyl LacNAc and blank spots. (**b**) SPR difference images by exposures of MAH and anti-SA.

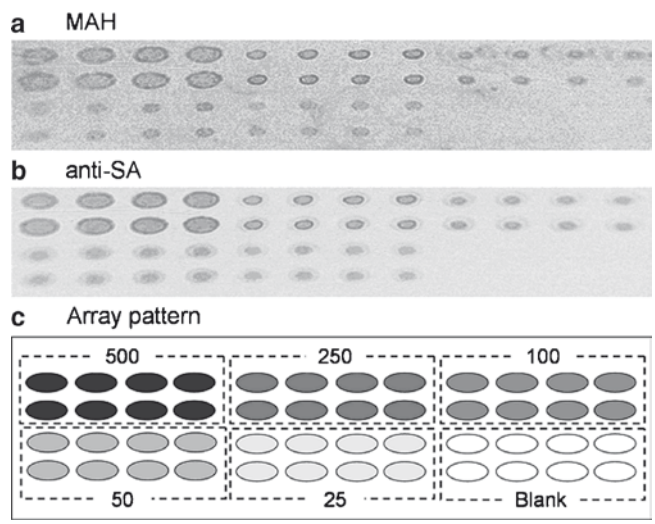

Fig. 3. SPR difference images of the photocrosslinked sugar array fabricated with 25–500 mg/mL sugar concentrations by exposure of (**a**) MAH and (**b**) anti-SA. The array pattern was shown in (**c**).

Table 1
The obtained SPR signals and signal ratios by MAH and anti-SA exposure on sialyl T spots, which were formed with 25–500 μg/mL sugar concentrations

Concentration of spotted sugar solutions (μg/mL)	Signals by MAH (S_1)	Signals by anti-SA (S_2)	Signal ratios (S_1/S_2)
500	3.68 ± 0.68	3.56 ± 0.25	1.03 ± 0.13
250	3.27 ± 0.31	3.11 ± 0.27	1.05 ± 0.05
100	3.01 ± 0.32	2.95 ± 0.19	1.02 ± 0.07
50	2.95 ± 1.06	2.39 ± 0.90	1.25 ± 0.08
25	2.23 ± 0.21	1.99 ± 0.25	1.13 ± 0.05

The average and standard deviation values were calculated from 8 spots (n = 8)

4. Notes

1. Distilled water or organic solvents should be used in sample preparations for photoaffinity crosslinking. When detergents or organic salts were contained in diluting solution, the detergents or organic salts molecules might be immobilized preferentially by photocrosslinking. Buffer solutions should not be used for dilution, because precipitation of inorganic salts on spots may weaken UV irradiation.
2. A cylindrical pin with 450 μm diameter was used for spotting. Sharp-edged pins are not preferable, because they may damage gold thin layer on chips. The distance between spots centers were 1 mm.
3. Surface-introduced photoreactive group, aryl diazirine, can generate carbenes by 365 nm UV exposure. However, gold–sulfur bonds, which support photolinker on gold, can be easily broken by UV light below 300 nm. Therefore, UV transmission filter is essential in UV irradiation.
4. The dilution buffer solutions and SPR running buffer must be the same. The lot of buffer should not be changed during the single experiment. Slight component difference of the buffers affect SPR signals, it is so-called "bulk effect."
5. The sugar array could be regenerated with 2 mM NaOH treatment. 0.2 mM was too low to regenerate (not returned to the base lines), and 20 mM might cause hydrolysis of sugars in this experiment (became below zero).
6. The stronger binding of MAH on sialyl T was observed, when MAH was exposed on the array. We thought that the signals

on sialyl Tn and sialyl LacNAc were induced by nonspecific bindings, because the signals were almost same as that of blank spot. SPR signal increases were observed in all sugar spots by exposure of SA and anti-SA. Immobilizations of sugars could be verified by SA and anti-SA.

7. Smaller spots were observed with lower sugar concentrations, even though the same spotting pin was used for all spots. It seemed that the spots of sugar solutions were shrunk and concentrated, during the drying of spots.
8. Higher signals were obtained at the sugar spots, which were prepared with higher concentration solutions. Importantly, the signal ratios (S_1/S_2) were almost constant (around 1.00), and this suggested that lectin binding affinities could be evaluated properly by signal normalization with anti-SA, even though the accurate densities of sugar spots were unclear.

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