Terahertz Imaging for Label-free Protein Detection

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***Abstract*— We demonstrate an imaging method combined a terahertz time-domain spectroscopy and an interference effect for label-free protein detection on a membrane filter. Biotin is linked to the membrane using poly ethylene glycol (PEG) or poly ethylene glycol methyl ether (MPEG) to prevent it from being washed off. Binding of the biotin with streptavidin is then observed by measuring the terahertz signal change due to the variation of the membrane refractive index. From result of competition binding experiments, it becomes clear that specific binding of antibody protein are detected by using this imaging method. This technique is used only to detect the existence of the binding. And the selectivity depends absolutely on specific ligand-protein interactions. This measurement principle is suitable to high throughput detection for drug discovery.**

I. Introduction And Method

Imaging technologies for biochips or microarrays allow fast, easy, and parallel detection of thousands of addressable elements in a single experiment. They can be expected to become a crucial tool for high-throughput drug discovery and life science. In these methods, label substrates, which might involve fluorescence, an enzyme reaction, or a radioisotope, are used for the detection of DNA and proteins. However, these procedures are complex and time consuming. Molecular recognition due to the differential activity of proteins is important in many biological processes. In recent years, small molecules have also received much attention from drug discovery scientists. Small molecules, from natural resources, are an important source of bioprobes, which are useful in the study of protein function or pharmacological effect. To screen those combinations of small molecules and proteins which have a biologically important function, microarrays sensors are studied or already commercialized. However, this method needs labeled substances to detect bound protein and an expensive arrayer system to prepare a sensor chip. In order to solve those problems, we attempted to apply the THz technology to label-free protein detection.

First, we report on an absorption change of graded reaction process on a polyvinylidene difluoride (PVDF) membrane. The PVDF membrane has been used as a support for sequence analysis of biopolymers. Such a filter membrane has a porous structure and the percentage of voids is over 80 %. Therefore, far infrared light such as terahertz waves can easily penetrate the membrane filter and its refractive index is small ($k \sim 0.05$, $n \sim 1.1$). As samples we chose IgG antibody bind to an antigen, and bovine serum albumin (BSA) were used as a negative control. The details of each sample preparation are shown in Figure 1. Absorption spectra of each sample in the 20-400 cm^{-1}

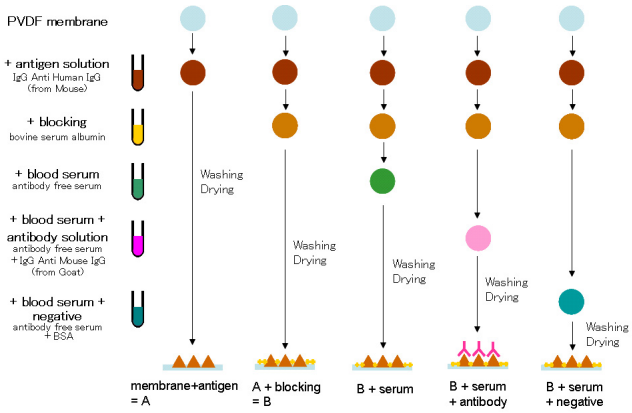

Figure 1. Schematic of process flow of sample preparation.

were obtained with FTIR using a silicon beam splitter and room-temperature detector. All measurements were performed in quadruplicate.

In addition, we demonstrate an interference terahertz label-free imaging technique to detect the interaction of protein and small molecules on a PVDF membrane using a terahertz imaging system based on THz-TDS (time-domain spectroscopy). In this experiment we acquired the images of the affinity binding between biotin fixed on PVDF membrane and the related bacterial protein streptavidin. The highly specific and strong binding of the biotin-streptavidin system has led to its wide usage in a variety of biotechnological applications.

Because the small molecular compound does not interact directly with the membrane, they flow off by washing after the reaction with the protein. To prevent flowing off of the biotin molecules from the membrane, we use a linking method, which consists in conjugating the biotin molecules with PEG or MPEG to the PVDF membrane. We used a multispectral reflection imaging system based on a THz-TDS to obtain an unlabeled image of biotin-streptavidin binding. The spectra were measured from 0.025 to 2 THz with 25 GHz resolution, resulting in 80 images. To yield high reflectivity of membrane surface, we constructed a high resistivity silicon plate (> 10 kΩ·cm, 9.2 mm thickness) as a sample stage. A membrane sample was sandwiched between the silicon plate and a mirror. We could use an interference effect such as in a Fabry-Perot etalon to achieve a highly sensitive measurement.

To confirm whether this imaging method detects a selective binding with protein, we performed a competition binding experiment. A series of solutions of digoxin and its analog molecule were dot-blotted on the PVDF membrane in doublet. The amount of digoxin molecules in reaction solution reduces the affinity of anti-digoxin antibody binds with molecules on

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membrane. We could expect the contrast of the THz image to lower according to the added amount of the digoxin in reaction solution.

II. RESULTS

Compared with absorption spectra of each sample this was processed gradually, it is clear that the binding protein increases the amount of absorption. Figure 2 shows a result of comparison of absorption at 265 cm^{-1}. This result indicates that the absorption of a membrane sample in THz region made it possible to detect a selective binding of protein on the PVDF membrane without label.

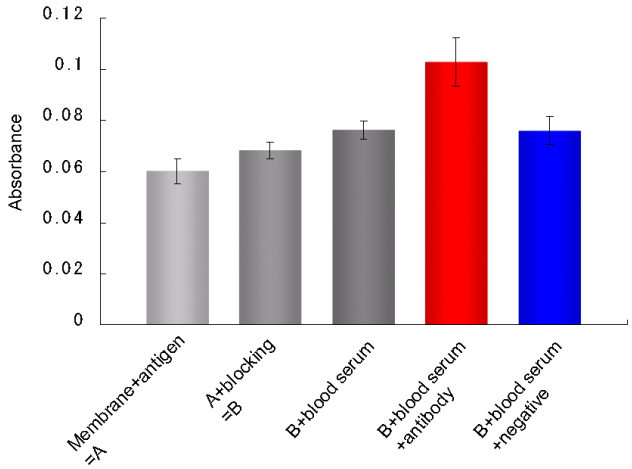

Figure 2. A comparison of absorption of each treated sample at 265 cm^{-1}. In this experiment, antibody free serum is used for control.

Figure 3 shows a schematic of the biotin array membrane and terahertz image at 1.5 THz. This result indicates that PEG and MPEG were effective for the immobilization of biotin molecules on the PVDF membrane. The image of the binding between biotin immobilized on the membrane using PEG or MPEG and the non-labeled streptavidin was obtained. In this experiment, we could not confirm less than 8 $\times 10^{-5}$ M streptavidin from fluorescent labeling image (not shown), however, using the label-free THz imaging we succeed in detection of 1.6 $\times$ 10^{-5} M (27 ng mm^{-2}) streptavidin at the two top lines. We will present another label-free imaging results which are the competition binding experiment of digoxin antibody and the sugar-lectin binding experiment.

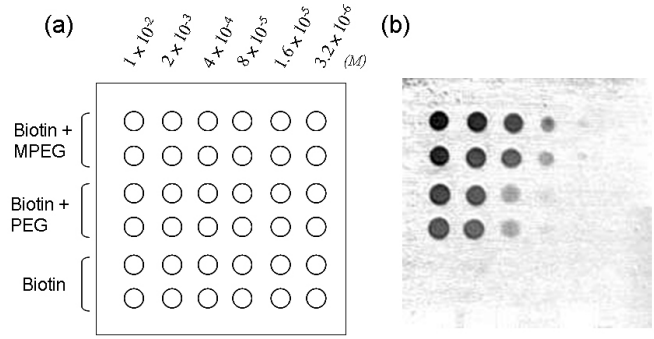

Figure 3. (a) Schematic of the membrane with the array of samples and (b) label-free THz image of the membrane at 1.5 THz. [1]

The result of competition binding experiment is shown in figure 4. The image of the binding between digoxin and its analog molecule immobilized on the membrane and the non-labeled anti-digoxin antibody was obtained. The THz images of non-labeled anti-digoxin antibody showed similar findings to the images of fluorescently-labeled it. These results indicate that our THz imaging method allows a label-free detection of the selective binding with anti-digoxin antibody and molecule on membrane. However, we also confirmed some noise in THz image due to a ruck or a density inhomogeneity of the PVDF membrane. In order to reduce such noise, it is necessary to improve an installation configuration of membrane sample and search an optimum material of membrane for this imaging method.

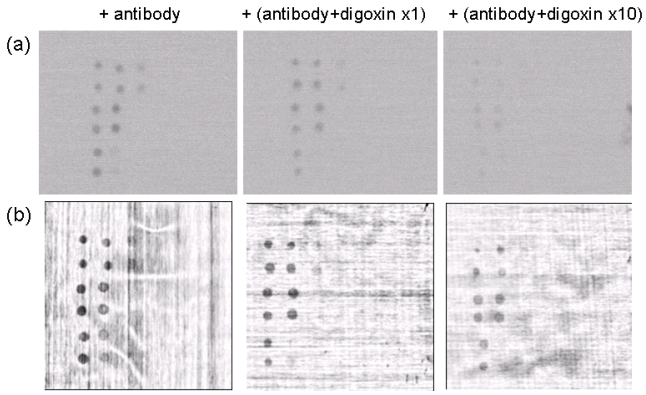

Figure 4. A result of competition binding experiment. (a) Fluorescently-labelled protein images and (b) label-free THz images of the membrane at 1.5 THz (processed image). As the amount of combinable antibody decreased, a contrast change in image is observed.

The binding specificity of the membrane, that is, the amount of biotin that adheres to it, depends on the membrane material and linkers. THz imaging is used only to detect the existence of the binding. As linkers, PEG and MPEG have proved to be useful not only for the biotin molecule but also for other low-molecular compounds. This property is suitable to a high throughput detection for drug discovery. We expect that our method can be used in a variety of applications such medical diagnosis and allergy testing based on an antigenantibody reaction, and as a sensor for industrial use.

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REFERENCES

[1] Y. Ogawa *et al.*, "Interference terahertz label-free imaging for protein detection on a membrane," Opt. Express, 16(26), 22083-22089, 2008.