Label-free detection of low-molecular-weight samples using a terahertz chemical microscope

Takuya Kuwana*, Masahiro Ogawa, Kenji Sakai, Toshihiko Kiwa, and Keiji Tsukada

Graduate School of Natural Science and Technology, Okayama University, Okayama 700-8530, Japan

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A terahertz chemical microscope (TCM) has been proposed and developed to visualize the distribution of biomolecular interactions on a sensing plate without any labels. In this study, the concanavalin A (Con A)–α-(+)-mannose (mannose) interaction was detected using the TCM with mannose applied as the analyte and Con A immobilized on the sensing plate. To demonstrate this interaction, the amplitude of terahertz pulses as a function of Con A–mannose interaction time, as well as the Con A–mannose coupling concentration, was evaluated. The results suggest that coupling kinetics may be evaluated using a TCM. © 2016 The Japan Society of Applied Physics

Lectin–sugar/sugar chain interactions play an important role in various bioactivities such as cell recognition, adhesion, blood typing, and ligand–receptor recognition.1) Accurate and efficient techniques for the screening of lectin–sugar/sugar chain interactions are therefore important for the elucidation of the mechanisms of complicated bio-reactions and for the development of novel drugs. Furthermore, the screening of immune reactions with low-molecular-weight biomolecules is important in the development of novel drugs.2)

The use of a surface plasmon resonance (SPR) system is recognized as a useful option for the screening of immunelike interactions.3) Typically, the SPR system uses a sensor chip comprising an optical prism coated with a gold film on which ligands are immobilized. The sensor chip is irradiated by an optical laser at an incident angle such that the laser beam is totally reflected. When the analytes are bonded to the immobilized ligands, the reflection angle of the laser beam shifts slightly, because the effective dielectric constant of the gold film surface is changed by the bonding. Thus, the SPR system can detect the interaction between the analyte and the sensor as the shift in the laser reflection angle. However, the sensitivity of the SPR system depends on the molecular weight of the analyte because the dielectric constant of an analyte is generally proportional to its molecular weight. Therefore, most SPR experiments are performed by immobilizing sugar/sugar chains on the sensor chips as the ligands.3) This implies that, while it is possible to screen lectins using the immobilized sugar/sugar chains in the SPR system, the screening of sugar/sugar chains using the immobilized lectin requires further development of highly sensitive measurement systems.

Terahertz (THz) spectroscopy4) with metal mesh filters is another useful option for the screening of immunelike interactions.5,6) In this technique, the transmission spectra of the metal mesh filters are measured with the filters typically designed to exhibit sharp absorption peaks in the THz frequency region. Since the positions of the sharp absorption peaks shift markedly upon the adsorption of molecules on the metal mesh filter surfaces, this technique enables the highly sensitive detection of immunelike interactions for biomolecules with low-molecular-weight. However, owing to the strong and broad-wavelength-range absorption of water molecules, drying of the surfaces of the metal mesh filters is generally required.

In our group, a THz chemical microscope (TCM) has been proposed and developed to visualize the interaction of various biomolecules.6,7) Although a THz system with metal mesh filters realizes highly sensitive detection, the TCM could be a compliment option, which can possibly measure the affinities or coupling kinetics of biomolecules. The key component of the TCM is the sensing plate that comprises SiO₂, Si, and sapphire substrates on which the ligands are immobilized. When femtosecond laser pulses hit the sensing plate, the carriers in the valence band are excited and the excited carriers are then accelerated by the electric field in the depletion region in the Si substrate.7,9) The accelerated carriers generate instantaneous current, radiating THz pulses that are proportional to the time derivative of the instantaneous current. The amplitude of the radiated THz pulses is given by

\[ E_{THz}(t) \propto \frac{\partial J(t)}{\partial t} \propto e \frac{\partial n(t)}{\partial t} v + e n(t) \frac{\partial v(t)}{\partial t}, \] (1)

where \( E_{THz}(t) \) is the electric field of the radiated THz pulses, \( J(t) \) is the instantaneous current, \( e \) is the elementary electrical charge, \( n(t) \) is the density of excited carriers, and \( v(t) \) is the velocity of the excited carriers. Equation (1) shows that the amplitude of the radiated THz pulses is proportional to the electric field in the depletion region. The interaction of the molecules on the sensing plate surface changes the electric field in the depletion region with the changes related to the chemical or electrical potential on the sensing plate surface. In this study, the positive or negative charge of sugars affects the chemical or electrical potential on the sensing plate surface. Hence, the electric field in the depletion region is changed proportionally to the charge of sugars. Because the electrical potential changes of the immune reaction are independent of the molecular weights of the samples, this type of system can realize a higher sensitivity for immune reactions with low-molecular-weight biomolecules. Thus, the TCM can detect the chemical or electrical potential at the laser-illuminated area on the sensing plate surface by detecting the amplitude of the radiated THz pulses as the signal. In our previous study, we showed that the TCM can be used to measure biomolecular interactions such as that of biotin and avidin bindings and IgG and anti-IgG bindings in...
In this study, the interaction between lectins and sugars was demonstrated in the case where the lectins were applied as ligands, and the possibility of the evaluation of kinetics was also examined by measuring the TCM signals for various reaction times. We also evaluated the standard deviation of the TCM signals and found that the evaluated values could be useful for the investigation of kinetics.

Figure 1 shows a schematic of the sensing plate. Femtosecond laser pulses were irradiated onto the sensing plate from the substrate side with an incident angle of 45°. When the femtosecond laser pulses hit the sensing plate, the THz pulses were radiated from the sensing plate into free space with a reflection angle of 45°. The center wavelength of the femtosecond laser was 790 nm, the average laser power was approximately 130 mW with a repetition frequency of 82 MHz, and the pulse full-width at half-maximum (FWHM) was 100 fs. The laser spot size was approximately 300 µm on the sensing plate, limiting the spatial resolution of the TCM to approximately 300 µm because the THz pulses are radiated only at the locations illuminated by the laser. The radiated THz pulses were focused onto a detector by parabolic mirrors, and a conventional bowtie-type photoconductive antenna made from a low-temperature-grown GaAs film was used as a THz pulse detector. The intensity of the laser pulses was modulated by an optical chopper in order to amplify the detected THz pulse signals using a lock-in amplifier. The arrival timing of the trigger laser pulses that drive the detector was fixed at times at which the detected THz signals show peak amplitudes. This optical system was described in detail in Ref. 10.

To measure the lectin–sugar/sugar chain interactions using the TCM, concanavalin A [Con A, purified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), Vector Lab. Inc., CA] was used as a lectin sample and immobilized on the sensing plate as a ligand. D- (+)-Mannose (Wako special grade, Wako Pure Chemical Industries) in 4-(2-hydroxyethyl)-1-piperazineethananesulfonic acid (HEPES) solution was used as a sugar sample and as an analyte. A small amount of calcium was added to the solution to produce the proper conformation for carbohydrate bindings.11) The pH of the HEPES solution was adjusted to 7.4 because Con A exists as a single dimer in solutions with pH 4.5–5.6, and it is predominantly found as a tetramer when the pH of the solution is above 7. Therefore, Con A shows optimal activation near pH 7.12,13) We used an amine coupling reaction14,15) for immobilizing Con A on the sensing plate as follows. First, the sensing plate was washed with acetone (Wako) and ethanol (Wako). After washing the sensing plate, the SiO2 surface was modified with ester groups using 2-(carbomethoxy)ethyltri-chlorosilane (Wako CMETS). 35% HCl (Wako) was used to form the COOH-modified SiO2 surface, and the latter was activated by the amine coupling reaction using N-hydroxy-succinimide (NHS) (Wako) and 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide hydrochloride (EDC; Wako). The sensing plate was then soaked in a solution of 1 µM Con A to immobilize Con A on the sensing plate surface. To prevent the mannose from binding to the SiO2 surface nonspecifically, the sensing plate was dipped in a HEPES solution containing 2-aminoethanol (Tokyo Kasei) at pH 8.5 to block nonspecific interactions. Following the immobilization and blocking, the sensing plate was mounted on a remote-controlled X–Y stage of the TCM system, and the THz signals were measured by changing the illumination position of the laser pulses across the sensing plate surface.16) Thus, the signal map across the sensing plate (TCM image) was constructed.

Figure 2(a) shows an image of the sensing plate. As shown in the figure, the area on the sensing plate surface was divided into four regions using an engineering plastic. (b) Differential TCM image of the sensing plate before and after the interaction.
a conventional rotation shaker. The rotation speed of the shaker was 300 rpm, and the interaction time was 90 min. These conditions of the shaker were an example. As shown in Refs. 7 and 8, biomolecular interaction could be detected without using a shaker. In this study, the shaker was used to accelerate the interaction. After the interaction, the sensing plate was washed with the HEPES solution and dried.

Figure 2(b) shows the differential TCM image of the sensing plate before and after the interaction with a mapping resolution of approximately 300 µm. To make the image clearer, an offset value was added to the data so that the signal from the area without the mannose was almost zero. The examination of the image shows that the THz amplitudes increased in the areas where the Con A–mannose interaction occurred.

The distribution of the Con A–mannose interaction was then visualized using the TCM. The THz amplitude change was estimated by averaging the values obtained at the area surrounded by a dashed line in Fig. 2(b), which corresponds to a well. The THz amplitude change is then plotted in Fig. 3 as a function of mannose concentration with the error bars showing the calculated standard deviations at each concentration. These error bars were estimated from the spatial deviation of a single image. Figure 3 shows that the mannose concentration and THz amplitude changes are clearly related, suggesting that the quantitative evaluation of Con A–mannose interactions is possible. To estimate the detection limit of this system, the data was fitted using linear regression, as shown by the solid line in Fig. 3, and the detection limit was obtained by the extrapolation of the linear fit. Since the typical noise level of the obtained THz amplitude was approximately 0.2 mV, the detection limit of the current system was 0.3 mM. Such a detection limit for mannose is already comparable to those of typical SPR systems. It is important to note that, while Con A is an analyte for the SPR system, mannose is an analyte for the TCM.

To evaluate the effect of the Con A–mannose interaction time, the interaction time was varied in the wells. Figure 4(a) shows the sensing plate divided into four regions and the interaction time for each well. The mannose concentration was 100 mM for all the wells and the interaction was promoted by the shaker. Figure 4(b) shows the differential image of the TCM signals before and after the interactions with the offset added so that the signal at the reference area was almost zero. Similarly to the plot in Fig. 3, the obtained data were averaged at each area indicated by the dashed lines and are then plotted in Fig. 5 with the error bar showing the standard deviation of the data in the area. Figure 5 shows that the THz amplitude changes first increased and then saturated with increasing interaction time, implying that the mannose molecules were gradually coupled with Con A until almost all the mannose molecules were coupled after 60 min of interaction. Moreover, although the standard deviation of THz amplitude was approximately 0.39 mV before the interaction, the standard deviations were 1.3 mV for 1 min of interaction.
of interaction and 0.93 mV for 5 and 60 min of interaction. This may be due to the nonuniform coupling of the mannose molecules to Con A at the beginning of the interaction, which became more uniform after a sufficiently long interaction time. The standard deviation for 60 min was still large compared with that at the initial state; this may be because Con A was not uniformly immobilized, and further immobilization was not carried out. However, this result suggests that the evaluation of the TCM signal standard deviation could be useful for the investigation of kinetics. Although further experiment is required for the precise evaluation of kinetics, this result indicates that a TCM can be used for studies of the kinetics of low-molecular-weight molecules.

In conclusion, the use of a TCM for the evaluation of immune reactions of low-molecular-weight molecules was demonstrated. A clear relationship could be observed between the THz amplitude change and the mannose concentration. The relationship between the THz amplitude change and the interaction time indicates that a TCM can be used for the evaluation of the kinetics of the lectin–sugar/sugar chain interaction. The analysis of the standard deviation of TCM images can be a useful option in studies of kinetics.

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