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Wavelength dependence of matrix-assisted laser desorption and ionization using a tunable mid-infrared laser

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Abstract

Matrix-assisted laser desorption and ionization by infrared laser (IR-MALDI) is expected to be an effective methods for soft-ionization of high-molecular weight proteins and intracellular proteins. IR-MALDI is not widely used because its low sensitivity, complexity, high cost, and as it does not work well on commercial MALDI time-of-flight mass spectrometers (TOFMSs). We employed a tunable mid-infrared (MIR) laser as a light source for MALDI to investigate the IR-MALDI. The laser wavelength can be tuned within a range from 5.5 to 10.0 μm, and included several biomaterial group vibration modes. We evaluated the wavelength dependence of ionization in IR-MALDI for four matrices: succinic acid, urea, 3,5-dimethoxy-4-hydroxycinnamic acid (sinapic acid) and 2,5-dihydroxybenzoic acid (DHB). These matrices contained various groups of vibration modes, and absorbed an infrared (IR) energy at a specific wavelength. The mass spectra of angiotensin II was obtained at a specific wavelength corresponding to the C=O stretching and benzene ring vibration mode. In IR-MALDI, we considered the strong molecular bond attracting an electron from a neighboring hydrogen atom, possibly protonating the hydrogen atom.

Keywords: IR-MALDI; Tunable mid-infrared laser; Group vibration mode; C=O stretching mode; IR absorption

1. Introduction

The possibility of matrix-assisted laser desorption/ionization (MALDI) [1] using infrared lasers was discovered in the early 1990s [2,3], and its fundamental concepts and applications have been studied. The generally accepted mechanism for MALDI is, the absorption of the laser energy by the matrix molecules, and proton transfer associated with vaporization of the matrix. IR-MALDI is a promising technique because most materials have a specific molecular vibration mode in the mid-infrared range; thus, the materials absorb an infrared light energy of a specific wavelength.

Recently, positive results have been reported for the IR-MALDI method, rather than the conventional MALDI method, using an ultraviolet (UV) laser for a few applications. Several proteins were directly analyzed with ice, water, and frozen alcohol, as a matrix [4–7] or from membranes after sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) separation and electroblotting or from the polyacrylamide gels themselves [8–11]. The analysis of large oligonucleotides, up to several hundred kilodaltons, was also demonstrated [12]. In addition, we successfully performed the difficult analysis of an insoluble protein contained in a high-concentration denaturant using an IR and UV laser [13–15].

The mechanism and fundamentals processes of IR-MALDI have been investigated with lasers of various wavelengths [16–19]. An Er:YAG laser, with a wavelength of 2.94 μm [5,20], and an optical parametric oscillator (OPO) with a wavelength ranging from 1.45 to 4.0 μm [4,6,21,22], can take advantage of the absorption bands of the O–H and N–H stretching vibrations of the IR matrix, such as in succinic acid, glycerol, and water or ice. Using a free electron laser (FEL) at Vanderbilt University, Cramer et al. have demonstrated the potential of the IR-MALDI at wavelengths near the C=O stretching vibration mode (5.7–6.0 μm) [23–25]. In these previous studies, the mechanism of the IR-MALDI was shown to depend on the IR absorption of the target molecule corresponding to the molecular vibration.

However, the process by which the vibration energy induces ionization has not been clarified, because the mechanism of the traditional electronic excitation process of UV-MALDI does not

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apply to IR-MALDI [26]. The FEL is suitable for explaining the IR-MALDI mechanism, because of the continuous wide tuning range of its high-power-pulsed laser, however it is too expensive and complicated to operate to be used in extensive experiments.

We used a MALDI time-of-flight mass spectrometer, combined with a tunable mid-infrared (MIR) laser, to investigate the IR-MALDI process. The MIR laser is a powerful tool to selectively excite a specific molecular vibration because its wavelengths can be tuned within a range of 5.5–10 μm. It is also more compact and easier to operate than the FEL. In this paper, we report the wavelength dependence of ionization in a wavelength range of 5.6–7.0 μm for four matrices: succinic acid, urea, 3,5-dimethoxy-4-hydroxycinnamic acid and 2,5-dihydroxybenzoic acid. These matrices contain various functional groups that exhibit strong absorption in this wavelength range. The wavelength dependence of ionization was compared with the IR spectra of these matrices to discuss the IR-MALDI mechanism.

2. Experiments

2.1. Mass spectrometer

The experimental setup used for this study is schematically reported in Fig. 1. A mass spectrometer (MS-Z040301TOFBU) with a 1.3 m equivalent flight length was developed in partnership with Japan Electron Optics Laboratory Co. Ltd. (Tokyo, Japan). All experiments were performed using the mass spectrometer, combined with a tunable MIR laser [27–30]. The laser, developed by the Institute of Physical and Chemical Research (RIKEN, Wako, Japan) and Kawasaki Heavy Industries Ltd. (KHI, Tokyo, Japan) can be tuned within a range of 5.5–10 μm, which includes several group vibration modes. The maximum-pulsed energy was approximately 1.4 mJ at 5.5 μm, and decreased to a minimum of 0.2 mJ at 10 μm. The laser has a pulse width of 5 ns, and repetition rate of 10 Hz. The laser beam was focused using a gold-coated parabolic mirror (f = 254 mm) and coupled to the mass spectrometer via zinc selenium window

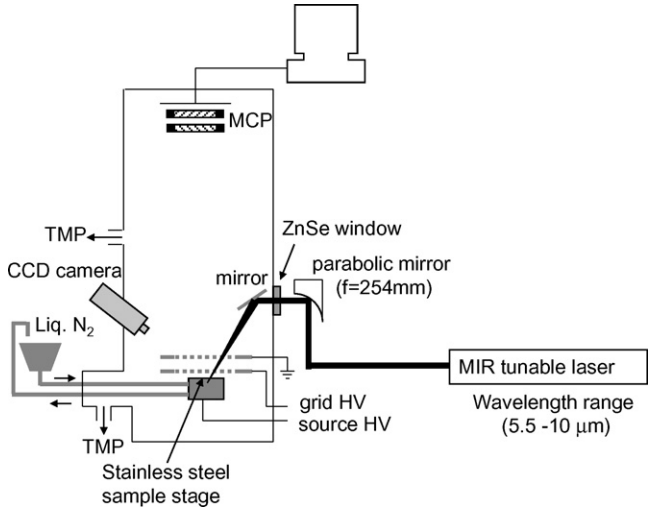

Fig. 1. Experimental setup for IR-MALDI experiments.

at an angle of incidence of 20° to the sample plate. The spot size of the laser beam was approximately 300 μm in diameter. A charge-coupled device (CCD) camera and a macro-zoom lens were used to monitor the laser alignment on the sample.

All experiments were performed in the positive ion mode. Ions were accelerated through a potential difference of 20 kV in a two-stage grid Wiley/McLaren ion extraction source, with the distance between the sample plate and the two grids set at 2.8 and 15 mm, respectively. Delayed extraction could be employed with a delay time of 650 ns and switched voltage of 0.75 kV. Although the stainless steel sample plate can be cooled with liquid nitrogen, the cooling function was not employed for these experiments. A secondary electron multiplier (AF880, SGE International Pty. Ltd., Australia) was used for ion detection, and detection voltage was set to −2.8 kV. The system was equipped with a turbomolecular pump and rotary pump, and was kept under vacuum at 10^{-7} Torr.

2.2. Sample preparation

Angiotensin II was purchased from Sigma–Aldrich (St. Louis, MO, USA) dissolved in pure water (99.9%, Milli-Q), and adjusted to a concentration of 10 pmol/μl. Urea (Nacalai Tesque, Kyoto, Japan), succinic acid (Wako Pure Chemical Industries Ltd., Osaka, Japan), 3,5-dimethoxy-4-hydroxycinnamic acid (Sigma–Aldrich), and 2,5-dihydroxybenzoic acid (Sigma–Aldrich) were used as matrices. Urea was dissolved in pure water and adjusted to concentration of 8 M. The other matrix solutions were prepared by dissolving 10 mg of each compound in 1 ml of acetonitrile/water (1:1). The angiotensin II solutions were mixed with each matrix solution in equal volume. A drop of this matrix solution (1 μl) was applied on the sample plate and allowed to dry under atmospheric conditions. The sample plate was introduced into the sample chamber, which was then vacuumed to below 10^{-5} Torr. Finally, the stage was set in the ionization source of the mass spectrometer.

2.3. IR absorption of matrix

The IR absorption spectrum of each matrix was measured using a Fourier transform infrared spectroscope (FT-IR) to compare their wavelength dependences for ionization. A drop of each matrix solution (1 μl) was placed on an infrared optical crystal made of barium fluoride (BaF_2), with 13 mm in diameter and 1-mm thick (GL Science Inc., Tokyo, Japan), and allowed to dry under atmospheric conditions. FT-IR measurements were performed with the infrared microscope (Nicplan, Thermo Fisher scientific Co., USA) with a mercury–cadmium–telluride type A (MCT-A) detector coupled to the FT-IR spectrometer (Magna750, Thermo Fisher Scientific Co., USA). The measurement conditions were as follows: a measured area, in wavenumbers, from 800 to 4000 cm^{-1}, a gain of 1, an integrated time of 200, and a resolution of 2 cm^{-1}.

3. Results

Fig. 2 shows five mass spectra of angiotensin II (M = 1046.18) with succinic acid taken in 100 nm increments at wavelengths

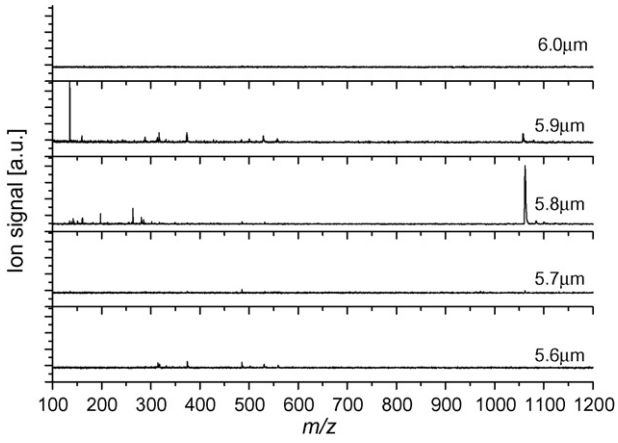

Fig. 2. Mass spectra of angiotensin II ($M = 1046.18$) with succinic acid taken in 100 nm increments at wavelengths between 5.6 and 6.0 μm.

between 5.6 and 6.0 μm. Each mass spectrum was obtained by the integration of 10 instances of an average of 20 laser shots. Using the same energy, 150 μJ, the analyte signal intensity reaches a maximum 5.8 μm. The peak sequences in the low mass range from 100 to 600 *m/z* are assigned as analyte fragments and matrix molecules. At a wavelength of 5.8 μm, the peak intensity of the analyte signal is much stronger than the peaks of the low mass range. Fig. 3 indicates a plot of the inverse of the threshold fluence, $1/H_0$, as a function of the wavelength and IR absorption spectrum of succinic acid (solid line). The succinic acid matrix has two IR absorption bands, i.e., the C=O stretching vibration mode (approximately 5.9 μm) of the carboxyl group, and the C–O stretching or OH bending vibration mode (approximately 7.0 μm). The $1/H_0$ data plotted in a wavelength range of 5.6–6.0 μm tracks closely with the IR absorption spectrum. However, the maximum wavelength 5.75 μm is shifted by approximately 150 nm with respect to maximum IR absorption. In contrast, the $1/H_0$ plots in a wavelength range of 6.8–7.2 μm disagree with the IR absorption spectrum, and the ionization of analyte molecule in the wavelength range is required if the threshold fluence exceeds 4000 J/m^2, which is higher than the threshold fluence of 1500 J/m^2 at a wavelength of 5.8 μm. In addition, the analyte ions were generated by high

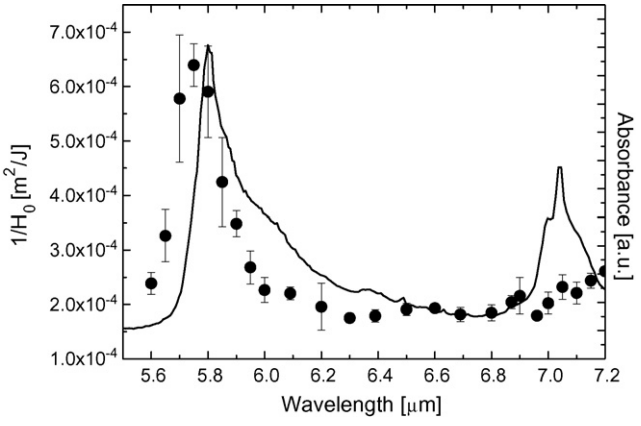

Fig. 3. Plot of the inverse of the threshold fluence, $1/H_0$, as a function of the wavelength and IR absorption spectrum of succinic acid (solid line).

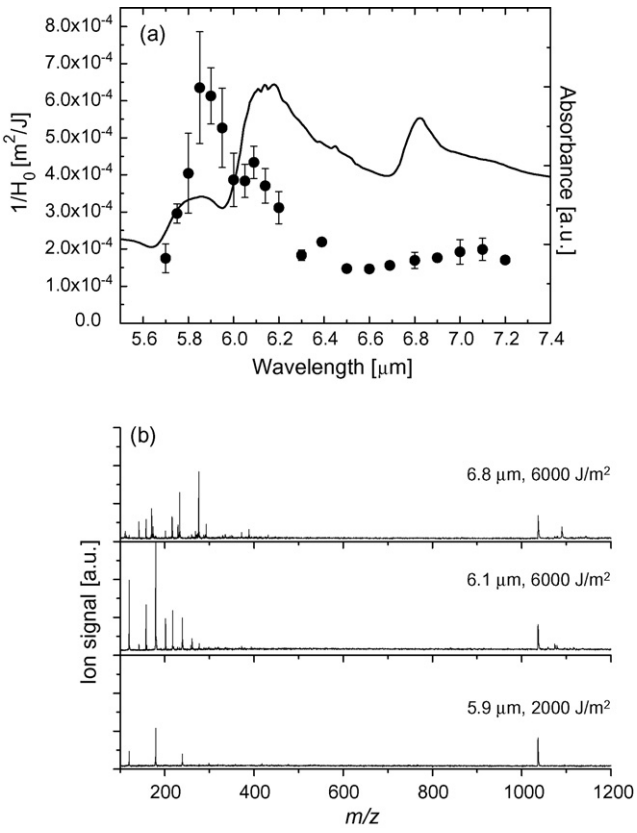

Fig. 4. (a) A plot of $1/H_0$ with urea matrices at wavelengths between 5.7 and 7.2 μm and the IR absorption spectrum of urea (solid line), and (b) mass spectra measured at 5.9, 6.1, and 6.8 μm. Molecular structure of the matrices used in this study: succinic acid, urea, sinapic acid and CHCA.

fluence in a wavelength range of 6.0–6.8 μm because peptide absorbs the IR laser energy for the amide bands I and II [31]. In a wavelength range of 6.0–7.2 μm, the analyte signal obtained is of a low resolution, and the signal intensity is lower than that for the analyte fragment or matrix signal.

A plot of $1/H_0$ with urea matrices at wavelengths between 5.7 and 7.2 μm and the IR absorption spectrum of urea (solid line), and three mass spectra measured at 5.9, 6.1, and 6.8 μm are shown in Fig. 4. Urea was observed to have a wide ranging absorption band of approximately 6.0 μm because of the C=O bond. The higher $1/H_0$ plot, corresponding to the C=O stretching vibration mode, shifted into a wavelength lower than that of the IR absorption peak, similar to that observed for succinic acid. While the threshold fluence at 5.85 μm was 1650 J/m^2, the ion production at 6.8 μm, corresponding to the NH bending vibration mode, required a laser fluence of 6000 J/m^2. In Fig. 4(b), three mass spectra were measured at each threshold fluence—2000, 6000 and 6000 J/m^2, respectively. At 6.1 and 6.8 μm, a large portion of sample was ablated from the sample plate by a laser shot, and subsequently analyte fragment and matrix ion were produced.

Fig. 5(a) and (b) shows a plot of the inverse of the threshold fluence $1/H_0$ as a function of the wavelength, and the IR absorption spectrum (solid line) of DHB and sinapic acid, respectively. These matrices include various molecular bonds, and the IR

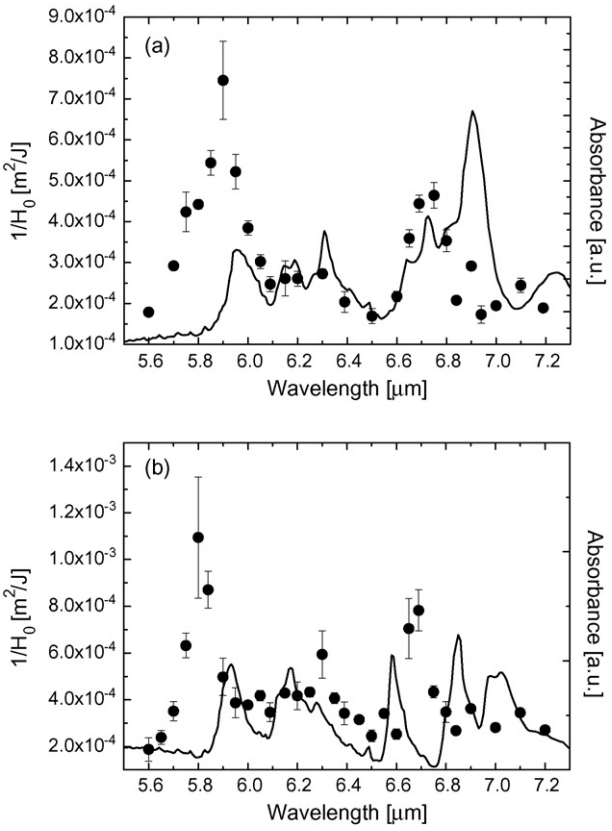

Fig. 5. Plot of the inverse of the threshold fluence $1/H_0$ as a function of the wavelength, and the IR absorption spectrum (solid line) of (a) DHB and (b) sinapic acid.

absorption spectrum is overly complicated. Both matrices produced a amount of analyte ion at the same wavelength ranges (approximately 5.8 and 6.7 μm) corresponding to the C=O stretching vibration and the C=C in-plane framework vibration of the aromatic ring. In the wavelength range of 6.2–6.7 μm, the ionization threshold is lower than that for succinic acid or urea. However, we cannot obtain an excellent mass spectrum for high sample consumption per laser shot and several peaks at low mass range by matrix molecule and fragment ions because these matrices contain various absorption bands in addition to that of protein.

4. Discussion

We investigated four matrices, succinic acid, urea, sinapic acid and DHB, to investigate the relationship between ionization and absorption bands. The different matrix compounds have a variety of IR absorbing moieties, each with a characteristic group frequency. The mass spectrum was observed at the infrared absorption wavelength of a characteristic group bond that includes double bond. In particular, an excellent mass spectrum of angiotensin II was found at a wavelength near the C=O stretching vibration (5.7–6.0 μm). Similar observations that indicate the correlation between mass spectrum and IR absorption were reported using an OPO [3,5,15,16,20,21] or FEL [22–24].

The desorption/ionization process for a standard UV-MALDI method is described by the intermolecular proton transfer resulting from the electronic excitation of the aromatic matrix. The process does not apply to IR-MALDI because the photon energies of IR light are an order of magnitude lower than those of the UV lasers commonly used for UV-MALDI.

In previous studies, the desorption/ionization using IR-MALDI have been reported to occur because of the vibration excitation of functional groups of the matrices. Many of these reports discuss the ionization process of IR-MALDI referring to the measurement results at wavelengths of approximately 3 μm corresponding to OH stretching vibration mode. Cremer et al. have shown that one of the possible models for the IR-MALDI process is desorption or ablation, induced by a thermal or structure phase charge.

Laser irradiation at 3 μm O–H impurity band induces surface melting followed by explosive vaporization deep inside the surface [18]. Murray et al. [32] reported that in the ionization mechanism the analyte absorbs sufficient laser energy to melt and is ejected into a dense plume, and ionization occurs through the reactions of individual ions and molecules in the plume.

This study produced interesting results. We observed the melting of the sample and plume at all MIR absorption wavelengths of a matrix; however, an excellent mass spectrum was observed only at a specific wavelength region corresponding to the C=O and benzene ring stretching vibration bands. These results cannot explain the use of the IR absorption of the IR matrix and vaporization of the sample. In the sinapic acid matrix, the absorbance is approximately constant at wavelengths between 5.9 and 7.0 μm. In the case of succinic acid matrix, although the absorbance at a wavelength of 7.0 μm is stronger than at 5.8 μm, the ionization at the wavelength of 5.8 μm occurs because of the lower threshold fluence.

Thus, the suggested mechanism of IR-MALDI is as follows: The C=O bond strongly resonates in response to the MIR laser irradiation. The oxygen atom attracts an electron from a neighboring hydrogen atom, because the electronegativity of the oxygen atom is higher than that of hydrogen. As a result, the hydrogen atom is possibly protonated, and the analyte is ionized through proton transfer. However, the IR-MALDI mechanism is not yet clean and requires more information, such as the intermolecular bond energy and vibration energy. Most biomolecules contain carboxyl groups that include C=O bonds. If IR-MALDI technique using a matrix containing C=O bonds is established, it may rapidly be adopted as a novel method for analyzing intracellular proteins. The tunable MIR laser is also promising as a powerful tool for IR-MALDI.

5. Conclusions

We evaluated the wavelength dependence of ionization in IR-MALDI using a tunable MIR laser. Four different matrices were employed to confirm the wavelength dependency of ionization. With the use of urea and succinic acid matrices, strong mass spectra of angiotensin II were obtained at wavelength of 5.8 and 5.9 μm, which correspond to the C=O stretching vibration mode, despite the fact that these matrices have several regions

of absorption wavelengths. In sinapic acid and DHB matrices, which contain benzene rings, the analyte signal was observed in the ring vibration mode (6.6 μm). The desorption/ionization of IR-MALDI is associated with specific molecular bonds. Because the carboxyl group is commonly found in most biomolecules, IR-MALDI that uses matrices that containing C=O bonds has a strong potential for the direct analysis of intracellular molecules.

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