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Study of ionization process of matrix molecules in matrix-assisted laser desorption ionization

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ABSTRACT

Proton transfer and adduction reaction of matrix molecules in matrix-assisted laser desorption ionization were studied. By using 2,4,6-trihydroxyacetophenone (THAP), 2,5-dihydroxybenzoic acid (DHBA), and their related compounds in which the position of a hydroxyl group is different, it was clarified that a hydroxyl group forming an intramolecular hydrogen bond is related to the ionization of matrix molecules. Intramolecular proton transfer in the electronic excited state of the matrix and subsequent proton adduction from a surrounding solvent to the charge-separated matrix are the initial steps for the ionization of matrix molecules. Nanosecond pump–probe NIR–UV mass spectrometry confirmed that the existence of analyte molecules having large dipole moment in their structures is necessary for the stabilization of $[matrix + H]^+$ in the electronic ground state.

1. Introduction

Matrix-assisted laser desorption ionization (MALDI) is one of the useful "soft ionization" methods as it does not decompose analyte molecules during the ionization process. MALDI combined with time-of-flight (TOF) mass spectrometry is widely used as a powerful tool to study biological macromolecules, such as DNA, proteins, and peptides, because it enables observation of analyte ions in terms of their molecular weights [1–3]. MALDI consists of two important processes: ionization and desorption. Since the discovery of MALDI, several studies aimed at fully understanding the ionization process have been carried out. In the photochemical ionization model proposed by Ehring et al., the multi-photon ionization of matrix molecules and the production of $[m]^+$ are considered to be the initial process [4]. Karas et al. proposed a cluster ionization mechanism in which higher clusters of analyte are desorbed during laser irradiation. The higher clusters are decomposed in the gas phase to produce analyte ions [5–7]. This model is similar to the mechanism in electrospray ionization (ESI). Meanwhile, Chang et al. reported a pseudo proton transfer process that occurred between a matrix and an analyte during crystallization. In the pseudo proton transfer process, a proton in the matrix is dominantly shared with the basic site of the analyte [8]. Although the three models offered new knowledge of the ionization process in MALDI, they did not explain why typical MALDI matrices, such as CHCA (α-cyano-4-hydroxycinnamic acid), THAP (2,4,6-trihydroxyacetophenone), and DHBA (2,5-dihydroxybenzoic acid), are suitable for many measurements and widely used. It is necessary therefore to understand the reaction mechanism after the electronic excitation of matrix molecules. Recently, Wan and co-workers argued initial reactions of typical MALDI matrices based on their chemical properties. They concluded that the ionization of matrix molecules may occur via multiple pathways including photoionization ($[m]^+$) in the electronic excited state and thermal ionization in the ground state, depending on their chemical properties such as absorption cross section and excited state lifetime [9]. In addition to the ionization process, the mechanism of the desorption process has remained unclear. In this regard, we recently proposed a possible desorption process based on exciton migration in organic crystals [10].

In this work, we tried to clarify the ionization process in MALDI, focusing on the reaction mechanism after the electronic excitation and their molecular structures of matrix molecules. By using THAP, DHBA, and their related compounds as MALDI matrices, we found that the existence of a hydroxyl group (ortho-OH) in the matrix structure, which typically formed an intramolecular hydrogen bond with a carboxyl group, was important to produce ions of both protonated matrix and protonated analyte. The intramolecular proton transfer from ortho-OH to carboxyl group (CO) in the electronic excited state and the subsequent proton adduction to ortho-O^- (C–O^-) from a solvent were the initial process of protonation in MALDI. The protonated matrix was stabilized in the electronic ground state by nonvolatile organic compounds having large dipole moment, and this stabilization was confirmed by nanosecond time-resolved mass spectrometry with an NIR pump and a UV probe.

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2. Experimental

THAP (m/z = 168) and other compounds used as the matrix were purchased (Wako Chemical). Model peptides, substance P (SubP) and angiotensin II (Ang), were used as analyte molecules. To measure mass spectra of SubP with THAP, for example, THAP was dissolved in a mixture of acetonitrile and water (7:3 in volume), and a 59.5 mM solution was obtained. SubP was dissolved in a mixture of acetonitrile and water (7:3 in volume), and a 74.2 μM solution was obtained. One microliter each of the matrix solution and the analyte solution was pipetted onto a stainless steel plate (matrix 59.5 nmol, analyte 74.2 pmol), left in air for a few minutes to evaporate the solvent, and then analyzed with a commercial (MALDI micro MX, Waters; 337 nm, 2.5 ns) or home-made mass spectrometer as described previously[11]. Briefly, matrix-analyte crystals on the stainless steel plate were put inside a vacuum chamber set at 5×10^{-5} Pa. The fundamental laser output from a Nd:YAG laser (Rayture Systems, 1064 nm, 10 Hz, 1 ns) and the fourth harmonics generation (266 nm) were used as excitation light. For nanosecond pump–probe experiments, NIR pump (1064 nm) and time-delayed UV probe (266 nm) pulses were collinearly focused onto the sample. The produced ions were accelerated with an electric field of 1.7 kV/cm and detected with a linear TOF tube and an MCP detector.

3. Results and discussion

3.1. Excitation conditions

We first examined the excitation conditions to clarify whether the cationic or the charge-neutral matrix was the reactant. An excitation laser pulse was typically focused to ~0.2 mm diameter. In the case of 266 nm excitation, photon numbers of the excitation pulse were estimated to be 1.07×10^{20}, 2.14×10^{20}, 3.00×10^{20}, and 4.28×10^{20} photons/m^2 when pulse energies of 2.5, 5, 7, and 10 μJ were used, respectively. For the calculation of excitation rate, we used the absorption coefficient of matrix in solution (THAP in acetonitrile, ~6120 mol^{-1} dm^3 cm^{-1}) at 266 nm because we could not determine the absorption coefficient of matrix in the crystals prepared for MALDI-MS measurements. Considering the time profile of the used laser, the excitation rate was estimated to be 2.5×10^8, 5.0×10^8, 7.0×10^8, and 9.3×10^8 s^{-1} for the 2.5, 5, 7, and 10 μJ excitation, respectively. Therefore, it was calculated that THAP could be photoexcited every 4, 2, 1.4, and 1.1 ns. However, as the pulse width of the used laser was ~1 ns, the multi-photon excitation of THAP during one pulse was unlikely at these pulse energies. The absorption coefficient of THAP at 337 nm was less than that at 266 nm, and was ~ 2130 mol^{-1} dm^3 cm^{-1}. The pulse width of N_2 laser in a commercial MALDI system was ~2.5 ns. Therefore, the excitation velocities were lower than those in the case of 266 nm excitation, indicating that the multi-photon excitation of THAP was more unlikely than the 266 nm excitation. In the mass spectrometry in the following sections, we treated only the results measured in the above-mentioned one-photon excitation condition. We did not argue the results obtainable in the high-power excitation condition by which multi-photon excitation of THAP could occur. Therefore, we considered that the ionization process in MALDI was a chemical process rather than ionization by multi-photon excitation (photoinization) in the low-power excitation condition.

3.2. Mass spectra measured in positive and negative ion modes

We carried out mass spectrometry of matrix and analyte/matrix systems in the negative and positive ion modes. Mass spectra were measured with the commercial MALDI system with the excitation power slightly above the threshold for observing ions (337 nm,5 μJ). Fig. 1(a) shows the mass spectrum of matrix (THAP only) in the negative ion mode; a prominent peak of [THAP-H]$^-$ was clearly observed at m/z = 167. Strong peaks of the THAP dimer, [2THAP-H]$^-$, and the THAP trimer, [3THAP-H]$^-$, were observed as well. In the positive ion mode shown in Fig. 1(b), the peak of such dimers as [2THAP + Na]$^+$ was also observed. However, peaks from the THAP higher clusters almost disappeared when the analyte molecules were mixed. Fig. 1(c) and (d) show the mass spectra of SubP/THAP obtained in the negative and positive ion modes, respectively. In both spectra, peaks from the THAP higher clusters were not observed. In addition, the decrease of the peak intensity of the THAP monomer was clearly observed in both spectra. Particularly for the mass spectrum observed in the negative ion mode, the peak intensity of [THAP-H]$^-$ was remarkably decreased and no longer comparable to that observed for the [THAP + H]$^+$ monomer in the positive ion mode. In this series of experiments, the conditions, such as the amounts of analyte and THAP and the excitation power, were the same. Therefore, it could be easily understood that the difference in the mass spectra observed without and with an analyte (SubP) was a result of the difference in the ionization mechanism. From the spectra obtained without the analyte molecules in which (1) intense peaks of the matrix higher clusters were observed, and (2) peak intensities in negative and positive ion modes were almost comparable, it became clear that the ionization in this case was completed only in THAPs; THAP solid dissociated into [THAP + H]$^+$ and [THAP-H]$^-$ ions with the irradiation of excitation laser pulses. We named this ionization process the "*dimer mechanism*" for further discussion. This result is practically the same as that reported in the recent paper [9], although we

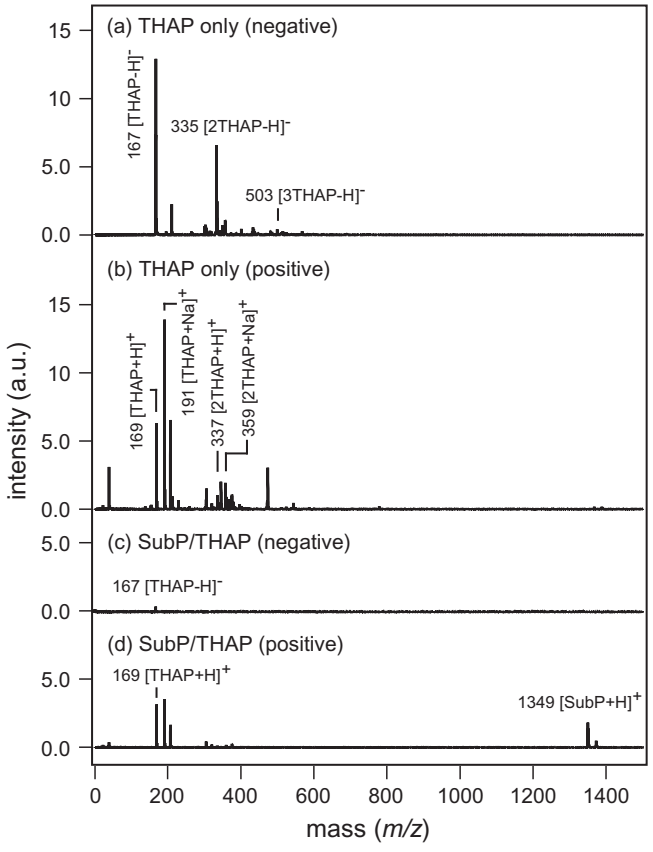

Fig. 1. Mass spectra of THAP solid in (a) negative and (b) positive ion modes. MALDI mass spectra of SubP/THAP in (c) negative and (d) positive ion modes.