Ryuichi Kikkawa[1], Yoshiya Inokuchi[1], Takayuki Ebata[1], Hiroyuki Saigusa[2]
[1]Department of Chemistry, Graduate School of Science, Hiroshima University,
Higashi-Hiroshima 739-8586, Japan
[2]Graduate School of Bio – and Nano-System Science, Yokohama City University,
Yokohama 236-0027, Japan

MASS SPECTROMETRY FOR NONVOLATILE FUNCTIONAL MOLECULES BY LASER DESORPTION/VACUUM-ULTRAVIOLET PHOTOIONIZATION METHOD

Laser desorption (LD)/vacuum ultraviolet (VUV) photoionization (PI) mass spectrometry (LD/VUVPI MS) is presented for nonvolatile bio-related aromatic molecules (tyramine, *L*-phenylalanine and *L*-tyrosine) and functional molecules (dibenzo-18-crwon-6-ether, calix[4]arene, calix[8]arene and calix[4]resorcinarene). A pellet of the sample mixed with graphite matrix was irradiated by IR laser for the desorption and the vaporized sample was ionized by a vacuum ultraviolet light at 10.5 eV, which was obtained by the high harmonic generation of Nd:YAG laser light. The ionized sample was mass analyzed by a time-of-flight mass spectrometer. LD/VUVPI mass spectrometry has been successfully carried out for the molecules examined. It was found that the bio-related aromatic molecules exhibit considerable fragmentation by the ionization at 10.5 eV, while those of the functional molecules show strong peak at the parent mass with less fragmentation.

Introduction

Mass spectrometry has been widely used and important spectrometry in organic chemistry as well as biochemistry. It is a powerful tool for the identification of known material and the structure determination. In particular, matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS)[1-4] and electrospray ionization mass spectrometry (ESI-MS)[5] enable mass spectrometry for nonvolatile large size biomolecules. A common characteristic in the two methods is that ions are directly generated in the source. The MALDI method utilizes an ultraviolet or infrared laser light to ablate and ionize analytes simultaneously from the matrix. In the ESI method, liquid containing analytes is dispersed by electrospray though a needle loaded at high voltage and the ions are sampled into a mass spectrometer through a capillary. In both cases, the generated ions are normally protonated, deprotonated, or some metal cation is attached.

Another promising method for the mass spectrometry of nonvolatile molecules is the photoionization (PI) of neutral, gas-phase species combined with laser desorption. Especially, the PI method ionizes the neutral species at well-defined energies so that this method is important not only for general interest of analytic mass spectrometry but also for studying molecular orbitals of the species by the combination of photoelectron spectroscopy (PES). Most of molecules have the ionization potential between 7 and 12 eV, and molecules are ionized either by resonant two-photon ionization (R2PI) with a tunable UV laser at l = 250 – 300 nm if molecules have appropriate chromophores or by singe photon ionization with vacuum ultraviolet (VUV) light source. The VUV light source is obtained by excimer laser,[6-8] synchrotron radiation,[9] or by the high harmonic generation with strong laser light.[10-12]

In this study, we present our development of the laser desorption vacuum ultraviolet photoionization mass spectrometry (LD/VUVPIMS) for nonvolatile molecule such as bio-rerated molecules and functional molecules. Our group has been carrying out supersonic jet – laser spectroscopic study for functional molecules and bio-related molecules in the gas phase such as *L*-phenylalanine, *L*-tyrosine, calix[4]arene, and dibenzo-18-crown-6-ether.[13-17] In these studies, the molecules are vaporized by using heating pulsed nozzle. The difficulty in these studies is that the temperature to evaporate the sample is very close to that of thermal decomposition. For example, *L*-tyrosine easily decomposes to tyramine by the elimination of CO_2 upon the heating and careful temperature control is necessary for the vaporization. Furthermore, it is also difficult to evaporate functional molecules with large molecular weights. Thus, an evaporation method alternative to the heating is highly needed for the study of neutral functional molecules in the gas phase, and laser desorption is promising method.

In the present work, the desorption of the samples was achieved by irradiating IR laser light to a pellet of samples mixed with graphite, where the latter works as a matrix. Vaporized samples are ionized efficiently by single photon ionization with VUV light generated by high harmonic generation

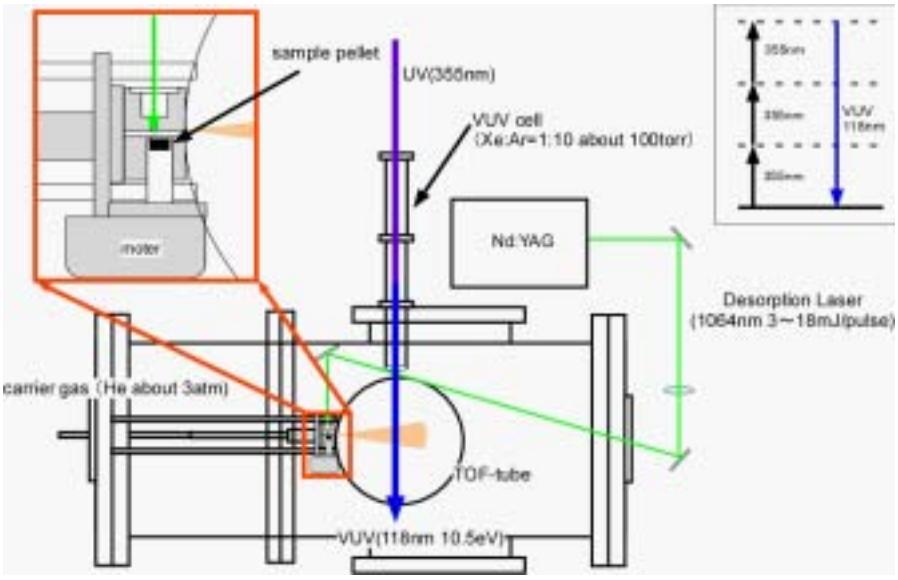

Fig. 1. Experimental setup of laser desorption / vacuum-ultraviolet photoionization mass spectrometry (LD/VUVPI MS), and the schematic diagram of VUV generation.

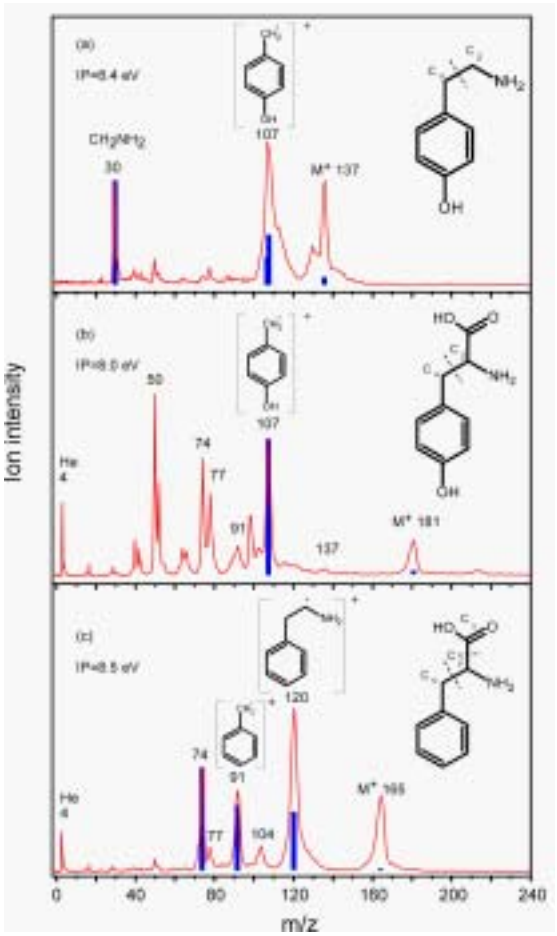

Fig. 2. LD / VUVPI mass spectra of (a) tyramine, (b) L-Tyr, (c) L-Phe. Ionization potential (IP), (a) 8.4 eV (b) 8.0 eV (c) 8.5eV. The blue-colored stick diagrams are the mass spectra obtained by EI (ref. 25), where only major mass peaks are shown

of a Nd:YAG laser. This spectrometry method is called LD/VUVPI mass spectrometry (LD/VUVPIMS). [10,11] We show results of the application to bio-related biomolecules, tyramine, *L*-phenylalanine (*L*-Phe) and *L*-tyrosine (*L*-Tyr), and functional molecules, dibenzo-18-crwon-6-ether (DB18C6), calix[4]arene (C4A), calix[8]arene (C8A) and calix[4]resorcinarene (C4RA).

Experimental

The experimental setup is shown in Fig. 1. A cylinder-shaped sample pellet with a diameter of 6 mm and a height of 5 mm is prepared by pressing a powder mixture of sample and graphite matrix at 1000 kg/cm^2, and the pellet is set into a channel-type nozzle, which is similar to the one reported by Saigusa and coworkers.[20] The sample pellet is rotated with a rotational speed of 5 rpm to change the ablation position, so that we can obtain better shot-to-shot stability of the ion intensity. The fundamental output (1064nm) of a Nd:YAG laser (Continuum Minilite, 2-18 mJ/pulse), is focused on the surface of the sample pellet with a spot diameter of 2 mm. The sample vapor generated by the laser desorption is mixed with Ar carrier gas at 3 atm and is expanded into vacuum through the nozzle to obtain a supersonically cooled jet. Finally, a supersonic beam is formed by skimming the jet by a 1 mm skimmer located at 50 mm downstream of the nozzle. A vacuum-ultraviolet light (VUV) at a wavelength of 118 nm crosses the supersonic beam at 50 mm downstream of the skimmer

and ionizes the sample. The VUV light is generated by tripling the third harmonic (355 nm) of a Nd:YAG laser (Continuum Surelite II); the 355 nm output is focused into a cell containing a Xe:Ar (mix ratio = 1:10) gas mixture at the total pressure of 100 Torr, and the third harmonic light (118 nm) is obtained.[21,22] The ions are mass-selected with a time-of-flight (TOF) mass spectrometer, and detected by an electron multiplier (Burle, Model 4821G).

Results and discussion

1. LD/VUVPI TOF mass spectra of bio-relevant molecules

Fig. 2 shows LD/VUVPI TOF mass spectra of (a) tyramine, (b) *L*-Tyr and (c) *L*-Phe. We do not find any ion signals without the Xe:Ar gas mixture for the VUV light, indicating that these ions are generated only with the VUV light. In the mass spectrum of tyramine (Fig. 2(a)), the peak at m/z = 137 is attributed to tyramine$^+$. The peaks at m/z = 107 and 30 are assigned $C_7H_7O^+$ and $CH_2NH_2^+$ ions, respectively. These fragment ions are produced via the C_a-C_b bond cleavage after the ionization. The ionization potential of tyramine is reported to be 8.4 eV[23]. Therefore, tyramine is excited ~2.1 eV above the ionization threshold with the 118 nm light (10.5 eV), leading to the dissociation.[24] Fig. 2a also shows the reported mass spectrum of tyramine ionized by electron impact (EI) for comparison.[25] The features of the mass spectra are similar between PI and EI ionization, except that the PI mass spectrum exhibits a stronger parent mass signal.

Fig. 2(b) shows the LD/VUVPI TOF mass spectrum of *L*-Tyr. Since the ionization potential of *L*-Tyr is ~0.5 eV lower than those of tyramine and *L*-Phe, an extensive fragmentation is seen in the mass spectrum of L-Tyr. The ion signal at m/z = 181 is L-Tyr$^+$. The peak at m/z = 107 corresponds to $C_7H_7O^+$ generated by the C_a-C_b bond cleavage, which is similar to the case of Tyramine (Fig. 2(a)). The signals at m/z = 74 and 77 may be attributed to counter part ions of $C_7H_7O^+$ in the C_a-C_b bond cleavage. The origin of the signal at m/z = 50 is not clear. In Fig. 2(b) is also shown the mass spectrum obtained by EI for comparison.[25] It is clear that the PI mass spectrum exhibits a clear parent mass signal, which is very weak in the EI mass spectrum.

Fig. 2(c) shows the LD/VUVPI TOF mass spectrum of *L*-Phe. Besides the parent ion peak (*L*-Phe$^+$) at m/z = 165, the mass spectrum exhibits three intense peaks at m/z = 120, 91, and 74 and two weaker ones at m/z = 104 and 77. The signal at m/z = 91 is assigned to $C_7H_7^+$ fragment, which is generated through the C_a-C_b bond cleavage.

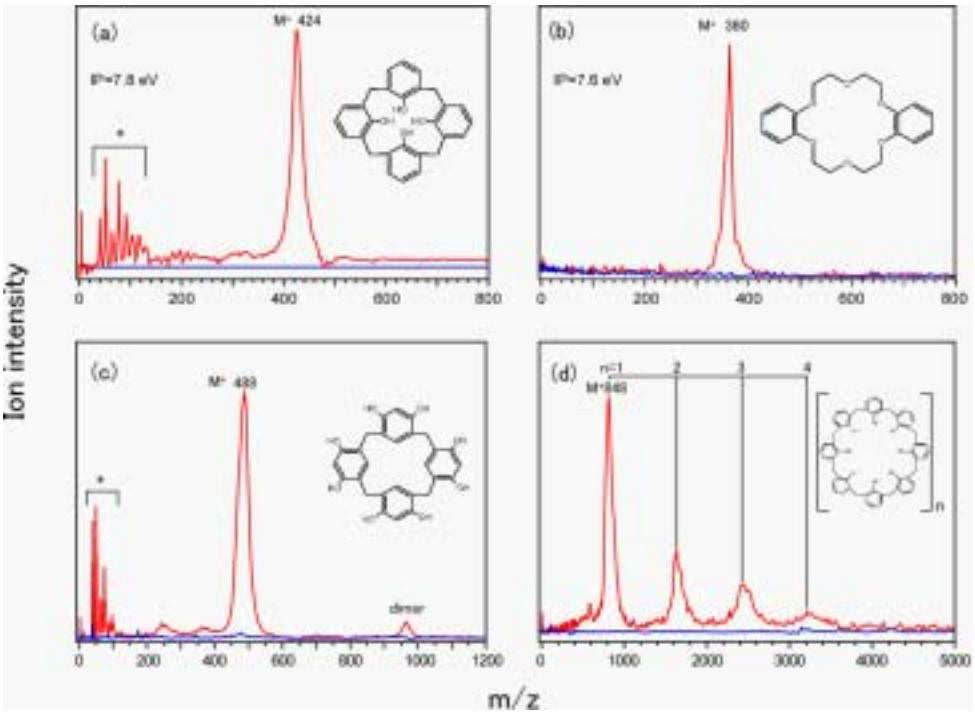

Fig.3 LD/VUVPI mass spectra of functional molecules, (a) C4A, (b) DB18C6, (c) C4RA and (d) C8A.

The species at m/z = 120 is $C_8H_{10}N^+$ fragment generated by the C_b-C_g bond cleavage. This fragmentation pattern is not seen in the case of *L*-Tyr. The species at m/z = 77 and 74 are thought to be counterpart ions of C_a-C_b bond cleavage, similar to the case of *L*-Tyr. A reported mass spectrum with EI ionization[25] is also shown for comparison. Though the mass spectra show very similar feature between EI and PI methods, it is again clear that the PI mass spectrum exhibits a much stronger parent mass signal, indicating the advantage of using PI compared to EI.

2. LD/VUVPI TOF mass spectra of functional molecules

Fig. 3 shows LD/VUVPI mass spectra of (a) calix[4]arene (C4A, m/z = 424), (b) dibenzo-18-crown-6-ether (DB18C6, m/z = 360), (c) calix[4]resorcinarene (C4RA, m/z = 488) and (d) calix[8]arene(C8A, m/z = 848). Though several weaker mass peaks are seen at masses lower than m/z ~ 100, the signals corresponding to the parent ion appear strongly in all the mass spectra. This feature is quite different from the cases of tyramine, *L*-Tyr, and *L*-Phe despite that the excess energy of these functional molecules after the ionization is 0.5–1 eV higher than the bio-related molecules.[26,27] The results show that the functional molecules are hardly decomposed by PI, indicating that they are suitable for this spectrometry and also for spectroscopic investigation. In the mass spectra of C4RA and C8A, signals assignable to cluster ions are seen clearly. Thus, this method is suited for the studies of larger size of clusters of functional molecules.

Conclusion

In this study, we demonstrated the usefulness of laser desorption /vacuum UV photo-ionization mass spectrometry (LD/VUVPI MS) for nonvolatile molecules. The method is successfully applied for the bio-related molecules, such as tyramine, *L*-Tyr and *L*-Phe, and functional molecules, such as C4A, DB18C6, C4RA and C8A. Though the PI mass spectra by 118 nm VUV light show considerable fragmentation for bio-related molecules, the parent ion signals are still strongly observed. The fragmentation can be reduced by using longer wavelength VUV light, by using an excimer laser[28], tunable synchrotron VUV light source[10,29] or tunable coherent laser light source[30,31]. The LD/VUVPI mass spectra of the functional molecules exhibit strong parent ion signals with less fragmentation, suggesting they are robust against the photodecomposition. Furthermore, the PI mass spectra show appearances of cluster ion signals, especially for C4RA and C8A. Thus, an extension to the larger aggregates will be interesting for future work.

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