Eluent-assisted Nonresonant Multiphoton Ionization of Polycyclic Aromatic Hydrocarbons in a Liquid Chromatograph-mass Spectrometer

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A sub-nanosecond visible laser coupled with a liquid chromatograph is utilized for atmospheric pressure laser ionization (APLI). We reveal that the range of applicable substances for nonresonant three-photon ionization is determined by the proton affinity and the ionization potential, the latter of which is lowered by solvation with eluents. APLI with a visible laser can be used for fragmentation- and background-free detection of analytes as well as to investigate the ionization threshold of solvated molecules.

Keywords: ionization potential, solvation, sub-nanosecond visible laser

Polycyclic aromatic hydrocarbons (PAHs) are regarded as environmental pollutants,[1] and some of them are mutagenic and carcinogenic.[2] A gas chromatograph[3] or liquid chromatograph,[4] coupled with an atmospheric pressure ionization mass spectrometer, in which mass spectrometry is performed by ionizing analytes in an atmospheric pressure environment, is commonly used to analyze PAHs. Several ionization methods that can be used under atmospheric pressure have been developed: atmospheric pressure chemical ionization (APCI),[5] atmospheric pressure photoionization (APPI),[6,7] and atmospheric pressure laser ionization (APLI).[8] APCI ionizes an eluent by corona discharges, and the proton transfer from eluent cations to analytes enables the detection of protonated analytes.[5] In the case of APPI, the VUV light (>10 eV) emitted from a rare gas lamp ionizes analytes by a single-photon absorption process.[6,7] APLI has made significant improvements in detection limits by utilizing the resonance-enhanced multiphoton ionization (REMPI) process, in which the two-photon ionization is enhanced when the first transition is resonant with an electronically excited state. In APLI, a UV photon emitted from a nanosecond excimer laser is commonly used. The typical wavelength, pulse duration, and repetition rate for an excimer laser used in APLI are 248 nm (4.99 eV), 5−10 ns, and 100−200 Hz, respectively. In some cases, a compact nanosecond UV laser[9] or picosecond UV laser[10] has been applied for APLI. APLI has been used successfully for the detection of a variety of molecules, but the reduction of ionization potential of analytes (<1 eV) in eluents cannot be investigated because sufficiently high energy for ionization is deposited by ultraviolet light. In order to investigate the ionization threshold of solvated molecules, a low-energy photon must be used for the ionization. As a consequence, short duration but narrow spectral width laser pulses are required for nonresonant multiphoton ionization.

In this study, we utilized a compact, high-repetition (1 kHz), visible (532 nm, 2.33 eV) laser with a 500 ps duration attached without modification to a conventional liquid chromatograph-mass spectrometer (LC-MS) for APLI. The experimental details are described in the Supporting Information. Anthracene was chosen as a typical PAH to investigate the ion signal variations depending on the parameters of the MS as well as the laser power. We also discussed the applicability for various PAHs. The results obtained by APLI were compared with those obtained by APCI. We reveal that the nonresonant multiphoton ionization of PAHs is possible due to the lowering of ionization potential by the solvation with eluents.

Figure 1 shows the selected-ion monitoring chromatogram of anthracene obtained by APLI. Anthracene methanol (MeOH) solution was directly injected into the MS (DI-MS) by a syringe pump. The results clearly showed that both the molecular cation radical ($M^{+\bullet}$) and protonated molecule (MH^+) appeared only when the laser beam was irradiated.

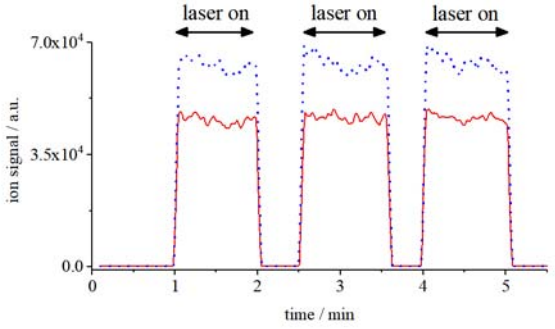

Figure 1. Selected-ion monitoring chromatogram of anthracene (300 ppm) measured by DI-MS (solid line, $M^{+\bullet}$, m/z =178, fragmentor voltage = 240 V; dotted line, MH^+, m/z = 179, fragmentor voltage = 120 V). Methanol was used as an eluent. Laser power was 42 mW. The arrows indicate the duration when the laser is irradiated.

We then varied the fragmentor voltage to clarify whether the primary ion of anthracene is $M^{+\bullet}$ or MH^+. The formation of $M^{+\bullet}$ from MH^+ by the collisions with residual nitrogen gases in MS is enhanced when the fragmentor (acceleration) voltage increases. Thus, the primary ion is expected to be identified under low fragmentor voltage conditions. Anthracene solution was injected into the MS via the LC by using MeOH or acetonitrile (MeCN) as an eluent. Figures 2a and 2b clearly show that the primary ion of anthracene formed by APCI was MH^+ in both MeOH and MeCN. The same result was obtained for APLI when MeOH was used as an eluent (Figure 2c). In contrast, the abundant formation of $M^{+\bullet}$ was observed even at low fragmentor voltage when MeCN was used as an eluent in the case of APLI (Figure 2d). These differences are

attributed to the different primary ion formation processes. APCI mainly ionizes eluents by corona discharges followed by the proton transfer to the analyte. Protonation of anthracene is an exothermic process in both MeCN and MeOH because the proton affinity (PA) of anthracene (9.01 eV)[11] is higher than that of MeOH (7.82 eV) or MeCN (8.08 eV).[12] In contrast, anthracene is preferentially ionized to form $M^{+\bullet}$ by APLI, since the ionization potential (IP) of anthracene (7.43 eV) is much lower than those of the eluents: 10.85 eV for MeOH; 12.19 eV for MeCN.[13] In the case of APLI, the successive abstraction of hydrogen from the eluent by $M^{+\bullet}$ forms the secondary ion MH^+. It is also safe to say that the hydrogen abstraction is less efficient in MeCN than in MeOH.[14] Therefore, both $M^{+\bullet}$ and MH^+ are detected in MeCN, but only MH^+ is detected in MeOH at low fragmentor voltage in the cases of APLI. More importantly, the ion signal obtained by APLI in MeOH was about one order of magnitude larger than that in MeCN, while the ion signals obtained by APCI in both MeOH and MeCN were on the same order of magnitude. The ionization potential of anthracene in MeOH is lower than that in MeCN.[15] Therefore, the amount of $M^{+\bullet}$ produced by APLI in MeOH may be larger than that in MeCN as the efficiency of ion production by the multiphoton process is strongly dependent on the ionization potential. In the cases of APCI, the amount of MH^+ obtained in MeOH is expected to be similar to that obtained in MeCN because the transfer of proton that is produced by corona discharges of eluents is an exothermic process in both MeOH and MeCN.

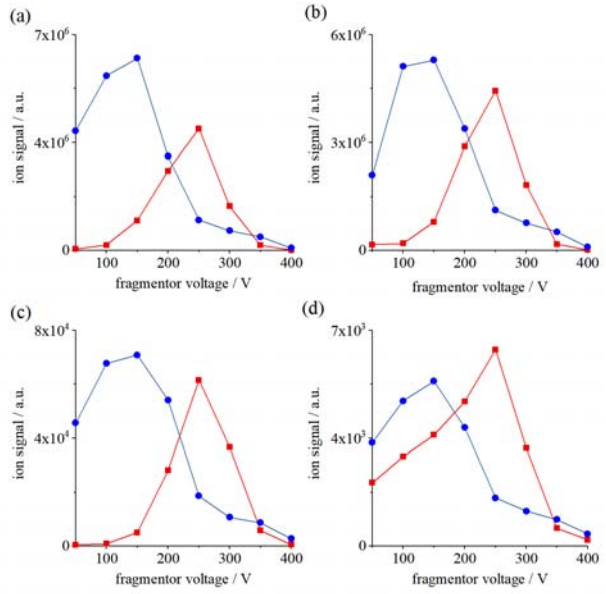

Figure 2. The correlations between ion signals of anthracene (triangles, $M^{+\bullet}$, m/z =178; circles, MH^+, m/z = 179; squares, the sum of $M^{+\bullet}$ and MH^+) and fragmentor voltages. Anthracene was analyzed by APCI (a, b) or APLI (c, d). MeOH (a, c) or MeCN (b, d) were used as eluents.

Having clarified the origin of $M^{+\bullet}$ and MH^+, we next focused on other ions produced by APCI and APLI. The mass spectra of anthracene obtained by APCI (Figure S2a) included ions that originated from anthracene, eluent, and presumably impurities, while that obtained by APLI (Figure S2b) included only the ions of anthracene. The fragmentation- and background-free features of APLI[8,16] were also confirmed with a sub-nanosecond visible laser.

We confirmed that the ion signal was linearly proportional to the concentration of anthracene in MeOH (Figure 3a, r^2 = 0.996, 1−300 ppm). Since the laser power was stable enough during the experiments, the fluctuation of the ion signal was satisfactorily small. However, the limit of detection was far from that achieved by the established APLI methods[8,16] applied for real environmental samples. For example, the limit of detection of PAHs achieved by APLI with a time-of-flight MS was on the order of tens of femtograms.[16] The unfavorable detection limit of the present results was attributed to the very small volume of primary ionization as well as the small cross section of multiphoton ionization. We must focus the laser beam to reach the high intensity required for induction of the multiphoton absorption process, whereas the sample and eluent from LC are nebulized in a heated tube and then spread out orders of magnitude wider than the focus of the laser beam. Therefore, only a small part of the sample vapor is exposed to a focused laser beam. Though the limit of detection is not within the scope of this study, we might suggest that this limitation would be improved by increasing the peak laser power and ionization volume as well as by using a time-of-flight MS instead of the quadrupole MS used in this study.

Ionization of anthracene by sub-nanosecond visible laser pulses occurred definitively by the multiphoton process because the signals of both $M^{+\bullet}$ and MH^+ were nearly proportional to the cube of the laser power (Figure 3b, MeOH). A least square fitting of data gives the slopes of 3.12 ($M^{+\bullet}$) and 3.19 (MH^+), respectively. However, the energy of three 532 nm photons (6.99 eV) is insufficient to ionize anthracene in the gas phase.

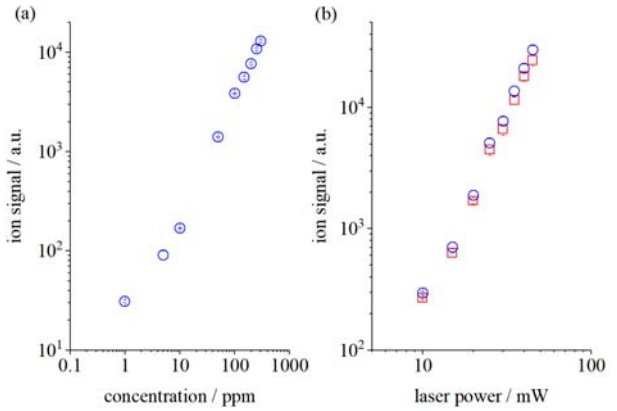

Figure 3. The correlation between ion signals (circles, MH^+, m/z = 179, fragmentor voltage = 120 V; squares, $M^{+\bullet}$, m/z =178, fragmentor voltage = 240 V) measured by APLI and (a) the concentration of anthracene or (b) laser power. The ions were measured by (a) LC-MS and (b) DI-MS. The vertical bars in (a) give the standard deviation obtained in the three measurements. MeOH was used as an eluent.

We can suggest three possible ionization mechanisms. First, the three-photon ionization process is possible if the

ionization potential of anthracene in the solvated form is sufficiently lower than that in the isolated form. The ionization potentials of anthracene solids have been reported to be 5.70 (bulk) and 6.4 eV (near to the surface), respectively.[17] It is known that the vertical ionization potential of metals and molecules decreases by clustering with polar molecules.[18] By analogy to those findings, a reduction in the ionization potential to 6.99 eV by polarization effects is expected for solvated anthracene.[15] The second possible explanation is that $M^{+\bullet}$ is formed by the 3+1 REMPI process, in which ionization occurs via the excited state of PAHs, which is reached by the three-photon absorption process. Due to the high density of states in such high-energy regions, the transition to a continuum level by an additional 532 nm photon absorption may be allowed. Assuming that this single-photon allowed transition occurs, the slope obtained by the power-dependence experiments would reflect the rate-limiting three-photon absorption process. However, the 3+1 REMPI process is unlikely, because the lifetime of highly excited states is extremely short (<0.1 ps).[19] Third, the absorption of four 532 nm photons, equivalent to 9.32 eV, which well exceeds the ionization threshold of anthracene in the gas phase, could be suggested. The discrepancy between the experimentally obtained slopes (3.1) and the number of photons required by the energy conservation law (4) is presumably explainable by the volume effect.[21] The expansion of ions that formed at the most tightly focused volume before detection by MS appears to be responsible for making the slope in Figure 3b less steep than expected.

Although we can suggest the three possible ionization mechanisms above, we cannot state which mechanism is operative based only on the results for anthracene. Therefore, we analyzed a variety of PAHs (50 ppm) by the scan mode (m/z = 50−300) of LC-MS to explore the applicability of APLI with a sub-nanosecond visible laser. Table S1 shows the properties of PAHs as well as the relative abundance of ions detected by APLI or APCI. We detected 7 out of the 13 PAHs by APLI. The detected PAHs were anthracene, pyrene, benzo[a]anthracene, benzo[a]pyrene, dibenz[a,h]anthracene, benzo[g,h,i]pelyrene, and indeno[1,2,3-c,d]pyrene. In the case of APCI, benzo[k]fluoranthene was also detected. This result indicates that the performance of APLI using a sub-nanosecond visible laser is similar to that of APCI, at least for the selected PAHs.

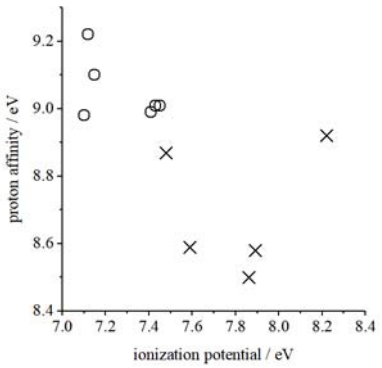

Figure 4. Correlation between the proton affinity and ionization potential of PAHs in the gas phase. Circles and crosses indicate that PAHs were detected and not detected by APLI, respectively. MeOH was used as an eluent.

Figure 4 shows the correlation between PAs and IPs of 11 PAHs whose IPs and PAs are known or estimated. The PAHs detected by APLI are indicated by circles. Those not detected by APLI are indicated by crosses. The largest signal obtained by both APCI and APLI was that for benzo[a]pyrene, which had the second smallest IP and the largest PA among the 11 PAHs. The quantitative arguments about the amount of ion cannot be made on the basis of the present experiments because the ion production is influenced by many factors, such as the order of elution. In any case, it is evident that PAHs having IP lower than or equal to 7.45 eV were detected by APLI. In contrast, PAHs having IP higher than or equal to 7.48 eV were not detected by APLI under our experimental conditions. Based on this fact, we can conclude that a nonresonant three-photon (6.99 eV) rather than a nonresonant four-photon (9.32 eV) ionization process is more likely to be operative in the cases of anthracene and other PAHs. Consequently, the actual ionization potentials of the detected PAHs were lower than or equal to 6.99 eV. The maximum ionization potential lowering was estimated to be 0.46 eV.

Benzo[k]fluoranthene, whose IP (7.48 eV) is slightly higher than the above-mentioned IP threshold, was not detected by APLI but was detected by APCI. It should be mentioned that the PAHs detected by APLI have PAs higher than 8.98 eV, while the PA of benzo[k]fluoranthene is 8.87 eV. Therefore, we conclude that there are additional thresholds for APLI detection under our experimental conditions. The first is the IP of PAHs, which determines whether the primary ion $M^{+\bullet}$ is formed. The second threshold is the PA of PAHs, which regulates the hydrogen abstraction of $M^{+\bullet}$ to form the secondary ion MH^+. As the efficiency of hydrogen abstraction is determined by both IP and PA,[14] the threshold of APLI may be more severe than that of APCI.

We reveal that the fragmentation- and background-free detections by APLI[8,16] are also possible with a sub-nanosecond visible laser. Ionization of an eluent requiring at least five (MeOH) or six (MeCN) 532 nm photons is almost impossible, because the order of the absorption cross section decreases by about 10^{33} as the order of the multiphoton process increases.[21] We suggest that the success of APLI with a visible laser extends the possibility of APLI, since only several ionization wavelengths in the UV region have been examined by APLI thus far. Because there is a degree of freedom to choose the optimal excitation and fluorescence wavelengths in the case of a fluorescence detector coupled with LC, our results show that APLI with a wavelength-tunable visible laser will provide an approach that is sensitive to ionization potential, i.e., a solvation-sensitive ionization method.

Nonresonant multiphoton ionization processes have been extensively used in studies for isolated molecules in gas phase. During the history of nonresonant multiphoton

ionization studies, the ionization potentials measured in a vacuum have been referred to discuss the ionization behavior. The present work shows a possible method for investigating the actual threshold of the ionization potential of solvated molecules, since the degree of solvation can be controlled by changing the condition of LC-MS. More accurate determination of the ionization potential of solvated molecules is possible by tuning the ionization wavelength in the visible region. Because a more powerful, wavelength-tunable and short-duration picosecond laser is expected to appear in the near future,[22] we can expand the applicable substances and eluents.

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Supporting Information is available on http://dx.doi.org/10.1246/cl.******.

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Graphical Abstract

Textual Information

A brief abstract	Atmospheric pressure laser ionization using a compact sub-nanosecond visible laser attached without modification to a conventional liquid chromatograph-mass spectrometer is utilized to detect polycyclic aromatic hydrocarbons. The nonresonant multiphoton process achieved by a visible laser is useful to investigate the ionization potential of solvated anlaytes compared with the use of the resonant enhanced two-photon process by using an ultraviolet laser.
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