Photochemical Reactions of Aminonaphthols
Caused by Laser Desorption/Ionization

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The formation of monomeric and dimeric ions of seven different aminonaphthols (ANLs) has been studied by using laser desorption/ionization (LDI) with a nitrogen laser. The positive-ion data of all the ANLs merely showed molecular ion $M^{+}$ without protonated molecule $[M+H]^{+}$, while 1-amino-2-naphthol (1,2-ANL) and 2-amino-1-naphthol (2,1-ANL) showed an intense dimeric ion $[2M-2H_2O+H]^+$. The negative-ion data showed deprotonated molecule $[M-H]^{-}$ in common, while the spectra of 1,2-ANL, 2,1-ANL and 8-amino-2-naphthol (8,2-ANL) accompanied an intense peak corresponding to negative molecular ion $M^{-}$ and the 8,2-ANL and 4-amino-1-naphthol (4,1-ANL) accompanied dehydrogenated anion $[M-2H]^{-}$. The formation of monomeric ions was discussed from the standpoints of thermochemical properties such as ionization energy, gas-phase acidity, electron affinity, and bond dissociation energy. The formation of dimeric ions $[2M-2H_2O+H]^+$ observed in the 1,2-ANL and 2,1-ANL could be explained by the radical combination in the amino groups. An isomer 5-amino-1-naphthol (1-ANL) did not give any dimeric ions in the both positive- and negative-ion spectra. The influence of laser fluence upon the appearance of the monomeric ions such as $M^{+}$, $[M+H]^{+}$, $[M-H]^{-}$ and $[M-2H]^{-}$ of the 5,1-ANL has been examined.

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INTRODUCTION

Although matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS)¹⁻³ presents a practically useful analytical tool for detecting biological molecules, it is difficult to understand all the events of desorption and ionization processes in MALDI even at present. The mechanisms of MALDI have been studied by many researchers to date.¹⁻¹¹ It is important to recognize that the MALDI process is governed by ablation (or desorption) induced by photophysical or acoustical process being the major event, while the ionization is a minor event having the values of ion-to-neutral ratio (I/N) in the range of 10⁻⁵ to 10⁻³ for analyte with good ion intensity, and 10⁻⁹ to 10⁻⁷ for matrix or total species.¹⁰,¹¹ The most basic phenomenon in the MALDI events is the formation of matrix neutral/ionic species, such as $M^+$, $H^+$, $M^{+}+H^+$, $[M+H]^+$, $[M-H]^{-}$, $[M+2H]^+$, $[M+3H]^+$, $[M-2H]^{-}$ and $M^{-}$, caused by photochemical reactions.¹²,¹³ The formation of neutral matrix radicals $M^+$ and hydrogen atoms $H^+$ is a rather major event than ion formation.¹²,¹³ The hydrogen atoms can bring about reactive results in hydrogenated radical ions such as $[M+2H]^+$ of 2,5-dihydroxybenzoic acid (2,5-DHB) and 3-aminopyrrole-2-carboxylic acid.¹²,¹⁴ The product of $[M+2H]^+$ is dependent on materials, while the intensity for 2,5-DHB is very small. The hydrogen atoms can bind to carbonyl oxygen of organic molecules via photochemical reactions with $n-π^*$ transition of the carbonyl groups by ultraviolet (UV) photon absorption.¹⁵ In the case of MALDI MS of peptide and protein analytes (A), the hydrogen atoms are leading to the formation of hypervalent radical species $[A+H]^+$, and the following prompt and specific cleavage at N–Cα bond of the peptide backbone generates in-source decay (ISD) fragments c and z;¹⁶,¹⁷ and also generates $z' (z'-H^+)$ and $z-M (z'-M)$ due to radical combination reactions in MALDI- plume.¹⁸,¹⁹ Simultaneously, the neutral species $c$, $z'$ and $z-M$ are ionized by protonation/deprotonation depending upon the basic and acidic natures of the neutrals.¹⁸,²⁰

5-Amino-1-naphthol (5,1-ANL) as a MALDI matrix has the capability of hydrogen releasing reagent to generate the ISD fragments.²²–²⁶ The 5,1-ANL has less volatility (more durability under vacuum condition) than another reductive matrix 1,5-diaminonaphtharene (1,5-DAN)²⁵ and without any sweet spots in the MALDI experiments. On the
other hand, the chemical 5,1-ANL has been noticed from the standpoint of the polymer-modified electrodes.\textsuperscript{26,27) Poly(5,1-ANL) products can be obtained from polymerization reactions with platinum electrodes as a catalyst in acidic solutions. It is believed that the polymerization mainly occurs via radical process of the amino group (\(-\text{NH}_2\)) at 5-position on 5,1-ANL molecules, while the hydroxyl group (\(-\text{OH}\)) does not affect the polymerization.\textsuperscript{28,29) It has also been reported that the amino group(s) on the naphthalene skeletal play the essential role of photochemical reactions for polymerization induced by UV-MALDI.\textsuperscript{28) Scheffler and Strupat have identified the polymer products, such as 2M−2H−\text{NH}+\text{H}\)⁺, [2M−2H]⁺ and [3M−4H]⁺, formed by UV-LDI of 1,5-DAN by using a Fourier-transform mass spectrometer.\textsuperscript{28) Studying the ionization behaviors of matrix chemicals caused by UV laser is of importance for understanding the MALDI events such as photochemical and radical processes.}

Here we examine the UV-LDI-induced photochemical reactions of seven different isomers of aminonaphthol (ANL). The paper describes about the formation of monomeric and dimeric ions of the ANLs on the basis of the positive- and negative-ion LDI data obtained at near the threshold laser fluences to generate ANL ions. The influence of the laser fluence upon the appearance of the monomeric ions of 5,1-ANL is also described.

MATERIALS AND METHODS

Materials and sample preparation

Aminonaphthols and acetonitrile were purchased from Tokyo Chemical Industry (Tokyo, Japan). Water used in all the experiments was purified using a MilliQ water purification system from Millipore (Billerica, MA, USA). The abbreviation and structure of the aminonaphthols used are summarized in Scheme 1. The each aminonaphthol was dissolved in water–acetonitrile (3 : 7, v/v) at a concentration of 10 mg/mL. A 1 µL of ANL solution was spotted onto a stainless steel sample plate, and then dried at room temperature in air.

Mass spectrometry

Mass spectra were acquired on an AXIMA-CFR MALDI-TOF mass spectrometer (Shimadzu Corp., Kyoto, Japan) equipped with a nitrogen laser (337 nm, 2–5 ns/pulse). Ions generated were accelerated at 20kV. Mass spectra were obtained in reflector mode with delayed extraction by accumulating 500 laser shots. The average of signal intensity was acquired. In order to regulate the laser fluence (LF, J/m²), a density wheel which is a filter was adjusted. The laser spot size was ca. 100 µm in diameter.

Ab initio calculations

All calculations were performed with a standard procedure according to the report of Bourcier et al.\textsuperscript{29) Thermochemical properties of 5,1-ANL were obtained with \textit{ab initio} methods with the DFT UB3LYP level of theory and 6–31+(d,p) basis set in the Gaussian 09 suite of programs. The initial structure of 5,1-ANL was made using CS Chem3D Ultra (Cambridge Soft, Cambridge, MA, USA). The input file was minimized under semi-empirical MO (PM7) run through a Winmoster interface using MOPAC2012.

RESULTS AND DISCUSSION

Monomeric and dimeric ANL ions generated by photochemical reactions with nitrogen laser

Positive- and negative-ion LDI mass spectra of seven different isomers of the ANL obtained at near the threshold laser fluences are shown in Figs. 1 and 3, respectively. The positive-ion spectra merely showed molecular ions M⁺ at m/z 159 in monomeric region (Fig. 2a), while the spectra of 1,2-ANL and 2,1-ANL showed a strong peak at m/z 283 and 4,1-ANL gave a weak peak at m/z 301 in dimeric region (Fig. 2b). The molecular ion peak at m/z 159 did not accompany protonated peak [M+H]+, but an isotope peak (M+1)+ at m/z 160. On the other hand, the negative-ion LDI spectra showed deprotonated peak [M−H]− at m/z 158, while the 1,2-ANL, 2,1-ANL and 8,2-ANL gave intense negative molecular ion M− at m/z 159 (Fig. 4a). The negative-ion data of 4,1-ANL and 8,2-ANL remarkably showed dehydrogenated/deprotonated peak [M−2H]− at m/z 157, although the 4,1-ANL gave other hydrogen-abundant peaks at m/z 161, 163 and 165 (Fig. 4a). From the monomeric ions observed in positive- and negative-ion data of the ANLs described above, the photochemical reactions by UV-LDI can be presented as follows:

\[
\begin{align*}
R_1 + n\nu & \rightarrow [M−H]− + H^+ \quad (1) \\
M + n\nu & \rightarrow M^− + e− \quad (2) \\
M + M + n\nu & \rightarrow [M−H]− + [M + H]^+ \quad (3) \\
M + e− & \rightarrow M^− \quad (4) \\
[M−H]− & \rightarrow [M−2H]− + H^+ \quad (5) \\
M^− + H & \rightarrow [M + H]^+ \quad (6)
\end{align*}
\]
According to the reports of Ehring et al.\textsuperscript{(12)} and Scott et al.,\textsuperscript{(13)} energetically most favorable unimolecular process of naphthol (NaphOH) and phenolic (PhOH) compounds under UV-LDI (nitrogen laser, 337 nm, 3.7 eV) conditions is radical formation (1), as seen in the bond dissociation energy ($DE$) for 5,1-ANL (NaphO-H, 3.63 eV) and for 2,5-DHB (PhO-H, 3.74 eV)\textsuperscript{(29)} (see Table 1). The formation of positive molecular ion $M^+$ in (2) can be explained by two-photon absorption with nitrogen laser (337 nm, 3.7 eV) under LDI conditions\textsuperscript{(12)}, because the ionization energy ($IE$) of 5,1-ANL (6.78 eV in Table 1) is lower than that of two-photon energy (7.40 eV). The generation of deprotonated matrix [M–H$^-$] is an energetically favorable if bimolecular proton transfer reaction (3) is considered. Considering the knowledge that naphthol and phenolic compounds have strong acidic property via excited states M* with UV-photon absorption\textsuperscript{(30)}, the multi-photon irradiation to the condensed ANL phase may result in deprotonated matrix as described in the reaction (3). Regarding the formation of negative molecular ion M$^-$ in (4) observed in the isomers of 1,2-ANL, 2,1-ANL and 8,2-ANL, it should be noticed that electrons generated in (2) can be resonantly trapped by the electron clouds of the ANL molecules via an electron-withdrawing effect with both hydroxyl and amino groups of these isomers. Although the electron affinity (EA) of naphthalene has negative value, the derivatizations of the naphthalene skeleton by polar or electron-withdrawing functional groups change from negative to positive values in EA\textsuperscript{(31,32)} In the case of 1,2-ANL, 2,1-ANL and 8,2-ANL, furthermore, a cooperative-effect owing to the presence of relatively adjacent functional groups of –OH and –NH$_3$ having non-bonding electrons may increase in the electron affinity due to the electron-withdrawing effect. The formation of a hydrogen-deficient radical anion [M–2H]$^-$ in (5) can be explained by the combination of the radical formation (1) and deprotonation (3). Regarding this, the observation of monomeric ions such as hydrogen-defi-
cient ion \([M-2H]\cdot -\) and extraordinary hydrogen-abundant ions \([M+nH]\cdot -\) \((n=2, 4, 6)\) in the 4,1-ANL isomer is strange in the hydrogen releasing and accepting properties. This may be due to the excess release of hydrogen atoms by the formation of \(p\)-quinone structure (Scheme 2a) and the attack of hydrogen atoms to the 4,1-ANL backbone pi-electron systems without change a resonantly electron capture bay region consisting of carbonyl at 1-position and conjugated pi-electron at 8-position (Scheme 2b).

In positive-ion LDI mass spectra of the seven ANL isomers (Fig. 1), the 1,2-ANL and 2,1-ANL gave strong peak at \(m/z\) 283 and the isomer 4,1-ANL gave a peak at \(m/z\) 301 in dimeric region (Fig. 2b). Considering the polymerization reactions of 5,1-ANL without the affect of hydroxyl group,\(^26,27\) it is likely that the dimeric ions at \(m/z\) 283 and 301 are produced by photochemically radical combinations with the loss of water molecule(s) as shown in Schemes 3 and 4, respectively. The other peaks at \(m/z\) 279, 300, 314, 315, 316, 317, 330, 331, and 332 with very low abundance in dimeric region were observed, while only the 5,1-ANL did not give any dimeric ions at the threshold fluence (Fig. 2b). The monomeric and dimeric ions of the ANLs observed here are summarized in Table 2.

![Fig. 3. Negative-ion LDI mass spectra of seven different aminonaphthol isomers.](image1)

**Table 1. Thermochemical data of matrices 5,1-ANL and 2,5-DHB.**

| Matrix     | IE (eV) | PA (eV) | GA (eV) | DE (eV) |
|------------|---------|---------|---------|---------|
| 5,1-ANL Calcd | 6.78    | 9.27    | 14.73   | 3.63    |
| 2,5-DHB Calcd\(^26\) | 7.86    | 8.77    | 14.05   | 3.74    |
| Exp1       | 8.05\(^a\) | 8.86\(^b\) | 13.78\(^c\) |        |

IE: ionization energy, PA: proton affinity, GA: gas-phase acidity, DE: bond dissociation energy.

\(^a\)See ref. 33. \(^b\)See ref. 34. \(^c\)See ref. 35.
Influence of the laser fluence upon monomeric ion formation of 5,1-ANL

Figure 5 shows positive and negative monomeric ions of the 5,1-ANL obtained at several laser fluences. Positive-ion data obtained with higher laser fluence showed the peaks of intense molecular ion $M^+$ at $m/z$ 159 and protonated molecule $[M+H]^+$ at $m/z$ 160 (Fig. 5a). Interestingly, the 5,1-ANL did not show hydrogenated ion $[M+2H]^+$, while the 2,5-DHB gave $[M+2H]^+$. This may be due to that 5,1-ANL is lacking in any sites such as carbonyl groups for hydrogen atom attachment. Negative-ion data showed the peaks of preferential deprotonated molecule $[M−H]−$ at $m/z$ 158 and $[M−2H]−$.
dehydrogenated radical ion \([\text{M}^{-2\text{H}}]^−\) at \(m/z\) 157 (Fig. 5b).

In order to confirm the reactions (1)–(6) in UV-LDI described above, furthermore, the influence of the laser fluence upon the appearance of positive and negative monomeric ions \([\text{M}^+]^\oplus\), \([\text{M}+\text{H}]^\oplus\), \([\text{M}−\text{H}]^−\) and \([\text{M}−2\text{H}]^−\) at \(m/z\) 157, 158, 159 and 160, respectively, was examined (Fig. 6). The positive molecular ion \([\text{M}^+]^\oplus\) was appeared at lowest fluence of 120 J/m² and rapidly increased at 140 J/m². The protonated molecule \([\text{M}+\text{H}]^\oplus\) was markedly observed at the fluence over 160 J/m², as shown in Fig. 6a. The formation of positive molecular ion \([\text{M}^+]^\oplus\) caused at the lowest fluence can be rationalized by the effects of two-photon absorption and volatile nature of naphthalene compounds. These effects can be considered as a direct influence of the irradiation of laser photons. The formation of \([\text{M}+\text{H}]^\oplus\) in the second stage event by means of the UV-LDI can be explained by several models such as the direct ion-pair formation or excited-state proton transfer (ESPT) accompanied ablation (3) and the consecutive photoionization/photochemical reactions (6) constituting of (1) and (2). In the case of 5,1-ANL, the consecutive reaction (6) is likely to take place because the negative-ion data did not show the peak of deprotonated molecule \([\text{M}−\text{H}]^−\) at the laser fluence for \([\text{M}+\text{H}]^\oplus\) formation.

On the other hand, the dehydrogenated radical ion \([\text{M}−2\text{H}]^−\) was observed at higher fluence than the deprotonated molecule \([\text{M}−\text{H}]^−\), as shown in Fig. 6b. The ion \([\text{M}−2\text{H}]^−\) should be generated via at least two-steps such as dehydrogenation in (1) and deprotonation in (3), which need the energies for \(DE\) (3.63 eV) and \(GA\) (14.73 eV), respectively, as shown in Table 1. Although it is not likely that the total energy 18.36 eV is supplied to an ANL molecule from the irradiation of nitrogen laser photons (337 nm, 3.7 eV), the early stage of photochemical processes in the LDI-plume as a transition-plasma state, which consists of the abundant electronic and vibronic excited matrix \(\text{M}^*\), the radical species \(\text{M}^+\) and \(\text{H}^−\), and the ionic species \(\text{M}^+\) and \(\text{H}^−\), make it possible to form a wide variety of ionic species including \([\text{M}−2\text{H}]^−\) through collisional interactions in the plume.12) Although it is difficult to explain the mechanisms and structures of all the ionic species observed here, the possible ion structures of the 5,1-ANL are shown in Fig. 6. Many of the results obtained here can be explained by the coupled photophysical and chemical dynamics (CPCD) model.36,37

CONCLUSION

The formation of monomeric and dimeric ions of seven different aminonaphthols (ANLs) has been studied by using UV-LDI with a nitrogen laser (337 nm, 3.7 eV). The negative-ion data showed dehydrogenated anion \([\text{M}−2\text{H}]^−\), deprotonated molecule \([\text{M}−\text{H}]^−\), molecular ion \([\text{M}^+]^\oplus\) and hydrogen-abundant anions \([\text{M}+\text{H}]^\oplus\), while the positive-ion data merely showed molecular ion \([\text{M}^+]^\oplus\), \([2\text{M}−2\text{H}_2\text{O}+\text{H}]^+\) and/or \([2\text{M}−\text{H}_2\text{O}+\text{H}]^+\), according to the structure of ANLs. The formation of each ion was discussed from the standpoints of thermochemical properties such as ionization energy (\(IE\)), gas-phase acidity (\(GA\)), electron affinity (\(EA\)) and bond dissociation energy (\(DE\)). The formation of dimeric ions \([2\text{M}−2\text{H}_2\text{O}+\text{H}]^+\) at \(m/z\) 283 observed in the 1,2-ANL and 2,1-ANL could be explained by the radical combination in the amino groups with the loss of water molecule. The 5,1-ANL isomer did not give any dimeric and more cluster ions.
at the threshold laser fluence, while other isomers showed wide variety of dimeric and/or trimeric ions. Although the influence of laser fluence upon the appearance and abundance of monomeric ions such as $M^+$, $[M+H]^+$, $[M-H]^-$ and $[M-2H]^-$ of the 5,1-ANL was examined, the detailed mechanisms including energetics remain unknown. In order to discuss the MALDI mechanisms in detail, further MALDI experiments added analytes (proteins or peptides) to the ANLs as matrices would be conducted in the future works.

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