Photodissociation and photoionization of 2,5-dihydroxybenzoic acid at 193 and 355 nm

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Photodissociation and photoionization of 2,5-dihydroxybenzoic acid (25DHBA), at 193 and 355 nm were investigated separately in a molecular beam using multimass ion imaging techniques. Two channels competed after excitation by one 193 nm photon. One channel is dissociation from the repulsive excited state along O-H bond distance, resulting in H atom elimination from \textit{meta}-OH functional group. The other channel is internal conversion to the ground state, followed by H$_2$O elimination. Some of the fragments further proceeded to secondary dissociation. On the other hand, absorption of one 355 nm photon gave rise to H$_2$O elimination channel on the ground state. Absorption of more than one 355 nm photon resulted in the three-body dissociation which also occurs on the ground state. Dissociation on the excited state does not play a role at 355 nm. The large concentration ratio \((2 \times 10^5)\), between neutral fragments and cations produced from 355 nm multiphoton excitation indicates that internal conversion followed by dissociation, is the major channel for 355 nm multiphoton excitation. Multiphoton ionization is a minor channel. Multiphoton ionization of 25DHBA clusters only produces 25DHBA cations. Neither anion nor protonated 25DHBA cation were observed. It is very different from the ions produced from solid matrix-assisted laser desorption/ionization (MALDI), experiments. This suggests that protonated 25DHBA and negatively charged 25DHBA generated in MALDI experiments does not simply result from the ionization following proton transfer reactions or charge transfer reactions of the clusters in the gas phase. © 2010 American Institute of Physics [doi:10.1063/1.3518709]

I. INTRODUCTION

2,5-dihydroxybenzoic acid (25DHBA), is frequently used as a matrix compound in UV matrix-assisted laser desorption/ionization (MALDI).\textsuperscript{1-9} MALDI is regularly used for peptide and protein analysis, but the details of mechanism are not well understood. Several mechanisms have been proposed to explain how ionization occurs in MALDI.\textsuperscript{2-4} Photochemical ionization is one of the proposed MALDI mechanisms.\textsuperscript{5} In this mechanism, photoionization of matrix molecules is considered the primary process for subsequent ionization of the analyte in the gas phase. However, several processes can compete with photoionization after irradiation with UV photons. They include energy transfer and proton transfer between 25DHBA, and dissociation. Although 25DHBA is commonly used as the matrix compound for ultraviolet MALDI, the photochemistry of 25DHBA remains unclear.

In this work, we report the photodissociation of 25DHBA at 193 nm as well as the multiphoton dissociation and multiphoton ionization of 25DHBA at 355 nm in a molecular beam using multimass ion imaging. Comparison to the potential energy surface from \textit{ab initio} calculation was made.

II. EXPERIMENT

As the experimental techniques have been described in detail,\textsuperscript{10-12} only a brief description is given here. 25DHBA molecular beam was formed by flowing ultra pure Ne at a pressure of 250 Torr through a high temperature nozzle (400 K), contained 25DHBA. Molecules in the molecular beam were photodissociated with a pulsed UV (193 or 355 nm) laser, followed by ionization with a pulsed VUV laser at 118 nm. A pulsed electric field served to extract the ions into a mass spectrometer. At the exit port of the mass spectrometer, a two-dimensional ion detector was used to detect the ion position and intensity distributions. Photofragment masses were identified along with their translational energy distributions using multimass ion imaging techniques.

For the experiment concerning multiphoton ionization, the molecular beam and the 355 nm laser beam were operated under the same conditions as photodissociation experiment. Ions generated by 355 nm laser beam were detected by a linear time-of-flight mass spectrometer.

III. RESULTS

A. Photodissociation at 193 nm

Ions \(m/z = 153, 136, 135, 109, 108, 107, 80, 52, \) and \(51\) were obtained upon photodissociation of 25DHBA at 193 nm. The relative ion intensities are shown in Fig. 1. Figure 2 depicts the corresponding ion images. The photolysis laser fluence dependence study in the range of

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0.27–2.1 mJ cm$^{-2}$ shows that $m/z = 51$ results from two-photon dissociation, but all the other fragments were produced via one-photon dissociation. In this work, we will only discuss one-photon dissociation at 193 nm.

The image for $m/z = 153$ corresponds to H atom elimination channel $C_6H_3(OH)COOH \rightarrow C_6H_3(OH)COOCH (m = 153) + H$. Image for $m/z = 135$ has the similar velocity profile to that of $m/z = 153$. However, the width of the image in vertical direction is larger than that of $m/z = 153$. It is likely a mixture of line-shape image and disk-like image. The line-shape image is from secondary dissociation, $C_6H_3(OH)COOCH (m = 153) \rightarrow C_6H_3O_2CO (m = 135) + H_2O$.

The image for $m/z = 136$ has very different velocity profile from that of $m/z = 153$ and 135, as shown in Fig. 2. It represents the $H_2O$ elimination channel $C_6H_3(OH)COOH \rightarrow C_6H_3(OH)COOCH (m = 136) + H_2O$. The corresponding photofragment translational energy distribution is shown in Fig. 3(b). Image for $m/z = 108$ is line shape. It is from three-body dissociation, $C_6H_3(OH)COOH \rightarrow C_6H_3(OH)O (m = 108) + H_2O + CO$.

Intensity of image $m/z = 109$ is small. A disk-like image superimposed on a line-shape image was observed. The disk-like image represents the dissociative ionization of heavy fragments, $C_6H_3(OH)COOCH (m = 153) + h\nu (118\ nm) \rightarrow C_6H_3O_2^+ (m/z = 109) + CO_2$. The line-shape image represents the secondary dissociation, $C_6H_3(OH)COOCH (m = 153) \rightarrow C_6H_3O_2 (m/z = 109) + CO_2$.

The rest of images, including $m/z = 107$, 80, and 52, are disk-like. The widths of the images increase as the delay time increases. They must result from the dissociative ionization of heavy fragments. Comparison of the widths of these disk-like images and the widths of heavy fragments, we can conclude that they are from the following dissociative ionization of heavy fragments, $C_6H_3(OH)COOCH (m = 153) + h\nu (118\ nm) \rightarrow C_6H_3O_2^+ (m/z = 107) + H_2O + CO$, $C_6H_3(OH)COOCH (m = 136) + h\nu (118\ nm) \rightarrow C_6H_3(OH)^+ (m/z = 80) + 2CO$, $C_6H_3(OH)COOCH (m = 136) + h\nu (118\ nm) \rightarrow C_6H_3(OH)^+ (m/z = 80) + 2CO$. 

FIG. 1. Photofragment ion intensities obtained from photodissociation of 25 DHBA at 193 nm. The ionization wavelength is 118 nm. Pump (193 nm) and probe (118 nm) laser pulse delay time is 1 μs.

FIG. 2. Photofragment ion images. The delay time between pump (193 nm) and probe (118 nm) laser pulses are (a) 13, (b) 13, (c) 29, and (d) 91 μs. Parent ions $m/z = 154$ are also shown in (d).

FIG. 3. Photofragment translational energy distributions. (a) $C_6H_3(OH)COOCH + h\nu (193\ nm) \rightarrow C_6H_3(OH)COOCH + H$. (b) $C_6H_3(OH)COOCH + h\nu (118\ nm) \rightarrow C_6H_3(OH)COOCH + H_2O$. 

FIG. 4. Photofragment ion images. The delay time between pump (193 nm) and probe (118 nm) laser pulses are (a) 13, (b) 13, (c) 29, and (d) 91 μs. Parent ions $m/z = 154$ are also shown in (d).
FIG. 4. Photofragment ion intensities obtained from photodissociation of 25 DHBA at 355 nm. The ionization wavelength is 118 nm. Pump (355 nm) and probe (118 nm) laser pulse delay time is 1 \( \mu \)s.

\[ C_6H_3(OH)O (m = 108) + hv (118 \text{ nm}) \rightarrow C_5H_3(OH)^+ (m/z = 80) + CO, \]

\[ C_6H_3(OH)O (m = 108) + hv (118 \text{ nm}) \rightarrow C_4H_4^+ (m/z = 52) + 2\text{CO}. \]

In summary, we can divide the fragments into two groups. One includes fragments \( m/z = 153, 135, 109, \) and 107. They are generated from H atom elimination channel followed by secondary dissociation or dissociative ionization. The other group contains \( m/z = 136, 108, 80, \) and 52. They result from H\(_2\)O elimination channel followed by secondary dissociation or dissociative ionization.

B. Photodissociation at 355 nm

Ions \( m/z = 136, 108, 80, \) and 52 were obtained upon photodissociation of 25DHBA at 355 nm. The relative ion intensities are shown in Fig. 4. Figure 5 depicts the corresponding ion images. The slopes obtained from the plot of ln(ion intensity) as a function of ln(laser fluence) provide the information concerning absorption of photon number. The photolysis laser fluence dependence study in the range of 9.4–100 mJ cm\(^{-2}\) shows that the slopes of \( m/z = 136 \) and 108 are 0.9 and 1.0 respectively. They result from one-photon dissociation. The slopes are 1.45 and 1.5 for fragments \( m/e = 80 \) and 52. They are dissociation products from absorption of two photons.

The image for \( m/z = 136 \) has the largest intensity. It represents the H\(_2\)O elimination channel \( C_6H_3(OH)COOH + hv (355 \text{ nm}) \rightarrow C_6H_3(OH)^+ (m/z = 108) + H_2O. \) The corresponding photofragment translational energy distribution is shown in Fig. 6. Image for \( m/z = 108 \) is disklike, but the width of the image increases as the delay time increases. It is from the dissociative ionization of heavy fragment \( m/z = 136, \)

FIG. 5. Photofragment ion images. The delay time between pump (355 nm) and probe (118 nm) laser pulses is 26 \( \mu \)s.

\[ C_6H_3(OH)OCO (m = 136) + hv(118 \text{ nm}) \rightarrow C_6H_3(OH)O^+ (m/z = 108) + CO, \]

\[ C_6H_3(OH)OCO (m = 136) + hv(118 \text{ nm}) \rightarrow C_6H_3(OH)O^+ (m/z = 108) + CO. \]

C. Multiphoton ionization of 25DHBA at 355 nm

The time-of-flight (TOF) mass spectrum of the ions generated from 355 nm multiphoton ionization of 25DHBA in a molecular beam is shown in Fig. 7. Ions of \( m/z = 154, 136, 108, 82, 81, 80, 79, 75, 54, 53, 52, 51, 50, \) and 26 were observed. The ion intensities as a function of laser fluence in the region 9.4–160 mJ/cm\(^2\) were studied to investigate the photon number dependence of these ionization processes. The slopes obtained from the plot of ln(ion intensity), as a function of ln(laser fluence), provide the information

FIG. 7. Cations generated from multiphoton ionization at 355 nm. The laser fluence is 36 mJ/cm\(^2\).
ions from 118 nm photoionization of neutral fragments have a total intensity about two times larger than the ion intensity generated by 355 nm multiphoton ionization. The ratio between the number of ions generated from 355 nm ionization, N₁, and the number of neutral fragments produced by 355 nm dissociation, N₂, was calculated to be \( r = N_1/N_2 = 5 \times 10^{-6} \) from the estimated average photoionization cross section of fragments (10⁻¹⁸ cm²) and 118 nm photon number per pulse (10¹¹ photon/pulse). It shows that the number of neutral fragments generated from 355 nm multiphoton absorption is 2 × 10⁶ times larger than ionic fragments. In other words, the total branching ratio for the various multiphoton ionization channels is only about one part per million at laser fluence 36 mJ/cm². Most of the multiphoton excitation results in the dissociation into neutral fragments.

D. Multiphoton ionization of 25DHBA clusters in a molecular beam at 355 nm

In changing the carrier gas from He (250 Torr), to Ar (300 Torr), various clusters formed. Dimers and trimers of 25DHBA clusters as well as the complexes of (25DHBA)ₘ(H₂O)ₙ, (25DHBA)ₘ(Ar), were observed from the mass spectrum obtained by 118 nm photoionization, as shown in Fig. 8. Water molecules are likely from the residual gas in the sample gas line. However, the mass spectrum obtained from 355 nm multiphoton ionization under the same molecular beam conditions gives different results. The ratios of various clusters to monomer decreases as the ionization laser beam changes from 118 to 355 nm. Most of these clusters tend to dissociate into monomer when they are ionized by 355 nm laser beam.

Protonated monomer and negatively charged ions of the matrix compounds are frequently observed in MALDI
experiments. Proton transfer has been suggested as one of the important steps in MALDI. We also searched for protonated 25DHBA and negatively charged ions from the 355 nm multiphoton ionization of clusters in the molecular beam. We did not find the corresponding ions. This suggests that protonated 25DHBA and negatively charged 25DHBA generated in MALDI experiments does not simply result, for example, from the proton transfer reactions of the clusters in the gas phase such as the reaction described below.

\[(25\text{DHBA})_2 + n\text{hv (355 nm)} \rightarrow (25\text{DHBA})^+ + (25\text{DHBA} - 1)^-\]

Complicated reactions must occur to generate these ions in MALDI experiments.

IV. DISCUSSION

25DHBA contains both hydroxy and carboxyl functional groups. We have investigated the photodissociation of phenol, benzolic acid, and hydroxybenzoic acid at 193 nm. The comparison of these molecules to 25DHBA reveals the competition of these two functional groups in 25DHBA.

The absorption of UV photons of phenol corresponds to excitation to the $\pi\pi^*$ excited state. The excited $\pi\pi^*$ state is bound with respect to OH bond distance. The dark state $1\pi\sigma^*$, which is repulsive along the OH bond distance, crosses the $\pi\pi^*$ state at energy level about 120 kcal/mol. Following UV excitation, some of the population in the $\pi\pi^*$ state can be transferred to the $1\pi\sigma^*$ state through the crossing point. Molecules in the $1\pi\sigma^*$ state result in H atom elimination, with a lot of energy released in fragment translational degrees of freedom. On the other hand, some excited phenol molecules in the $\pi\pi^*$ state become highly vibrationally excited in the ground state after internal conversion. Dissociation occurs for the channels with low barrier heights, including H atom elimination (89.1 kcal/mol), CO elimination (84.0 kcal/mol), and H$_2$O elimination (86.4 kcal/mol). The photofragment translational energy released in these channels is small.

Three dissociation channels were observed from the photodissociation of benzoic acid at 193 nm. They include (1) C$_6$H$_5$COOH $\rightarrow$ C$_6$H$_5$ + COOH, (2) C$_6$H$_5$COOH $\rightarrow$ C$_6$H$_5$CO + OH, and (3) C$_6$H$_5$COOH $\rightarrow$ C$_6$H$_5$ + CO$_2$.

The first two dissociation channels occur on the electronic excited states and the third channel occurs on the ground state. CO$_2$ elimination is the only dissociation pathway that has low barrier height (71.7 kcal/mol) on the ground state. The dissociation barriers for the other channels on the ground state, like CO elimination (87 kcal/mol), H$_2$O elimination (81 kcal/mol), H atom elimination (107 kcal/mol), OH elimination (107 kcal/mol), and COOH elimination (108 kcal/mol), are relatively large and they do not occur.

Photodissociation of hydroxybenzoic acid shows that it depends not only on the relative positions between COOH and OH functional groups, but also depends on the conformers. For 3-hydroxybenzoic acid and 4-hydroxybenzoic acid, no intramolecular hydrogen bonding is formed. Only H atom elimination channel from the $1\pi\sigma^*$ state was observed for 3-hydroxybenzoic acid. This channel remains as the major channel for 4-hydroxybenzoic acid. A small amount of OH elimination, analogous to the OH elimination from benzoic acid, was also observed for 4-hydroxybenzoic acid. On the other hand, 2-hydroxybenzoic acid shows totally different dissociation properties. Unlike 3-hydroxybenzoic acid and 4-hydroxybenzoic acid which do not have intramolecular hydrogen bonding, the OH and COOH functional groups in 2-hydroxybenzoic acid are close enough that if OH functional group points to the COOH functional group, an intramolecular hydrogen bond can be formed. It can be OH–O(H)C = O, or OH–O = COH. The ab initio calculations show that the crossing point between $1\pi\pi^*$ and $1\pi\sigma^*$ change from 120 kcal/mol for the conformers of 2-hydroxybenzoic acid without intramolecular hydrogen bonding to 140 kcal/mol for the conformers of 2-hydroxybenzoic acid with intramolecular hydrogen bonding. As a result, H atom elimination becomes the minor channel for the conformers with intramolecular hydrogen bonding. This was confirmed in molecular beam experiment. For the 2-hydroxybenzoic acid generated in the molecular beam, the temperature is low enough that most of the molecules have the structure with intramolecular hydrogen bonding. Experimental results show that H atom elimination channel is almost completely quenched for 2-hydroxybenzoic acid. In addition, the carboxyl functional group does not play a role on the excited state dissociation of 2-hydroxybenzoic acids. H$_2$O elimination (and H$_2$O elimination followed by CO elimination), following internal conversion to the ground state is the major dissociation channels. The dissociation barriers for the other channels on the ground state, like CO, CO$_2$, H, OH, and COOH eliminations are very high and they do not compete with H$_2$O elimination.

The UV absorption spectrum of 25DHBA has been measured in water/ethanol solution and solid powder. The spectrum obtained from solution is slightly blue shift compared to the spectrum from solid power. However, they share the similar feature. A weak absorption band is located between 270 nm and 390 nm, corresponding to the n$\pi^*$ state excitation. The second band is located around 240 nm, corresponding to the $\pi\pi^*$ state excitation. It partially overlaps with the third band which only part of the band was measured. Absorption of 355 nm photon corresponds to excitation to the n$\pi^*$ state. Absorption of 193 nm photon corresponds to the excitation to the S$_3$ or the S$_4$.

Comparison of phenol, benzoic acid, hydroxybenzoic acids, and 25DHBA shows that the carboxyl functional group still remains as a spectator and does not play a role in the excited state dissociation dynamics of 25DHBA. H atom elimination from the repulsive state at 193 nm is the only dissociation channel occurred on the excited state. No other excited state dissociation analogous to that benzoic acid, like OH and COOH elimination, was observed. According to the previous study of hydroxybenzoic acid, an intramolecular hydrogen bond must be formed in 25DHBA between carboxyl group and ortho-OH group. No intramolecular hydrogen bond is formed for meta-OH group. Therefore, H atom elimination from the $1\pi\sigma^*$ state of 25DHBA must mainly occur from the meta-OH functional group. Indeed, this can be confirmed.
by the experimental results. Experimental results show that the fragments generated from H atom elimination channel, C₆H₃(OH)COOH, can easily eliminate H₂O molecule by secondary dissociation, or by VUV dissociative ionization, C₆H₃(OH)COOH → C₆H₂O₂CO⁺ + H₂O. In order to eliminate H₂O, the remaining OH group needs to be close to the carboxyl group, indicating H atom elimination from the meta-OH group. In addition to the H atom elimination, the other major channel for 25DHBA is H₂O elimination. The dissociation mechanism must be similar to the conformer of 2-hydroxybenzoic acid with intramolecular hydrogen bonding, that is, dissociation occurs on the ground state after internal conversion. The potential energies for various dissociation channels on the ground state obtained by ab initio calculation are shown in Fig. 9. 25DHBA have many conformers, depending on the orientation of carboxyl and hydroxyl groups. The most stable conformer has the structure that hydroxyl group points to the carboxyl group to form intramolecular hydrogen bonding. For H₂O elimination, the dissociation starts from the rotation of carboxyl group, followed by the movement of hydrogen atom from ortho-OH group toward the OH portion in carboxyl group. The dissociation barrier is only 45 kcal/mol. It has the smallest dissociation barrier among various dissociation channels. Once H₂O elimination takes place, the secondary dissociation C₇O₃H₄ → C₆O₂H₄ +CO with a low barrier can easily occur. According to the calculation, it is a stepwise three-body dissociation. CO₂ elimination has very small heat of reaction, ΔH = 0.5 kcal/mol. However, the energy barrier is quite large (~62 kcal/mol). The heats of reaction for H atom elimination are 85.5 and 87.6 kcal/mol from meta-OH and ortho-OH functional groups, respectively. For H atom eliminated from meta-OH group, the final products can easily undergo secondary dissociation, C₆H₃(OH)OOCOOH → C₆H₂O₂CO (m = 135) + H₂O. The total energy requires is only 123.3 kcal/mol. On the other hand, fragment that is generated from H atom eliminated of ortho-OH group can undergo the other secondary dissociation, C₆H₁₁(OH)OOCOOH → C₆H₃(OH)₂ (m = 109) + CO₂. The barrier height is 129 kcal/mol. The calculations suggest H₂O elimination must be the major dissociation channel on the ground state, and fragment m = 135 must result from the secondary dissociation after H atom elimination from the repulsive excited state along meta-OH bond distance. They are consistent with our experimental observation.

As the photolysis wavelength changes from 193 to 355 nm, one photon energy of 355 nm only reaches the nπ* state. It is not enough to reach the energy level of the crossing point between 1ππ* and 1πσ* states. Therefore, dissociation from 1πσ* does not occur. Only H₂O elimination on the ground state after internal conversion occurs. It is interesting to note that the energy from two-photon absorption at 355 nm is large enough to reach crossing point between 1ππ* and 1πσ*. However, two-photon absorption can be a sequential absorption of two photons before any internal conversion or intersystem crossing occurs. Molecules are excited to a highly electronically excited state. If this excited state is not strongly coupled to the 1πσ* state, then the highly excited electronically molecules can transfer the electronic energy to vibrational energy quickly through internal conversion and dissociate through H₂O and CO elimination channels. Alternatively, two-photon absorption can be such a process that internal conversion to the ground state occurs before the excitation to the nπ* state again by the second photon. Molecules remain in the nπ* state with a lot of vibrational energy. If there is no crossing between nπ* and πσ* at low enough energy, H atom elimination will not occur. Since no H atom elimination from 25DHBA was observed after 355 nm excitation, one of these two situations must occur.

In summary, two channels compete after excitation by 193 nm. One is dissociation from the repulsive excited state along the meta-O–H bond distance, resulting in H atom elimination from meta-OH functional group. The other is internal conversion to the ground state, followed by the H₂O elimination. Some of these fragments further proceed secondary dissociation. Absorption of one or two photons of 355 nm only results in the dissociation channels that occur on the ground state. Dissociation on the excited state does not play a role at this wavelength. The large ratio between the neutral fragments to cations produced from 355 nm excitation indicates that internal conversion following absorption of 355 nm photons dominates the multiphoton absorption processes. Multiphoton ionization of 25DHBA clusters only produces 25DHBA cations. Neither anion nor protonated 25DHBA cation were observed. Proton transfer reaction does not occur in clusters. The results of multiphoton dissociation and multiphoton ionization are very similar to 25-dihydroxyacetophenone which is another compound frequently used in UV MALDI.\textsuperscript{18} It is very different from the ions produced from solid matrix-assisted laser desorption/ionization (MALDI) experiments. This suggests that protonated 25DHBA and negatively charged 25DHBA generated in MALDI experiments do not simply result from the ionization following proton transfer reactions of the clusters in the gas phase.


