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Structural Characterization of Methylenedianiline Regioisomers by Ion Mobility-Mass Spectrometry, Tandem Mass Spectrometry, and Computational Strategies. 3. MALDI Spectra of 2-Ring Isomers

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Abstract

Characterization of methylenedianiline (MDA) 2-ring isomers (2,2'-, 2,4'- and 4,4'-MDA) is reported using matrix assisted laser desorption/ionization-mass spectrometry (MALDI-MS), a common technique used for characterizing synthetic polymers. MDA is a precursor to methylene diphenyl diisocyanate (MDI) a hard block component in polyurethane (PUR) synthesis. This manuscript focuses on comparing MALDI results to those of our previous electrospray ionization-mass spectrometry (ESI-MS) studies. In ESI, 2-ring MDA isomers formed single unique $[M+H]^+$ (199 Da) parent ions, whereas in MALDI each isomer shows significant formation of three precursor ions: $[M-H]^+ = 197$ Da, $[M]^+ = 198$ Da, and $[M+H]^+ = 199$ Da. Structures and schemes are proposed for the MALDI fragment ions associated with each precursor ion. Ion mobility-mass spectrometry (IM-MS), tandem mass spectrometry (MS/MS), and computational methods were all critical in determining the structures for both precursor and fragment ions as well as the fragmentation mechanisms. The present study indicates that the $[M-H]^+$ and $[M]^+$ ions are formed by the MALDI process, explaining why they were not observed with ESI.

TOC image

Keywords

Ion mobility-mass spectrometry; Positional isomers; Polyurethanes; Polymer hardblock precursors; Collision Cross Section; Fragmentation reactions

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Introduction

Previously, electrospray ionization-ion mobility-mass spectrometry (ESI-IM-MS) was used to characterize the MDA 2-ring regioisomers (i.e., 2,2'-MDA, 2,4'-MDA, and 4,4'-MDA) and larger multimeric species.^{1,2} These 2-ring MDA isomeric structures and their probable protonation sites are denoted with asterisks in Figure 1. The available protonation sites that played a critical role in our earlier manuscripts deserve repeating because they are critical for describing the gas-phase behavior of MDA. The 4,4'-MDA species can protonate only on *para*-amino groups (*p*-NH₂), whereas 2,2'-MDA can protonate at two locations: on *ortho*-amino groups (*o*-NH₂) or on the ring *para* to the *ortho*-amino group. The 2,4'-MDA species has all three types of protonation sites. The stability of these ions was monitored in ESI by measuring the degree of fragmentation of the parent ion ([M+H]⁺ = 199 Da) to form the characteristic 106 Da fragment ion. 4,4'-MDA (double *para*-substituted) proved to be much more stable than either 2,2'-MDA (double *ortho*-substituted) or 2,4'-MDA (combined *ortho*- and *para*-substituted) isomers. These findings were supported experimentally by MS, MS/MS, IM-MS, and computational modeling.

In the present study, 2-ring MDA isomers were characterized using matrix assisted laser desorption/ionization (MALDI), a technique commonly used in MS analysis of polymers^{3–6} including polyurethanes^{7,8}. The differences between ESI and MALDI are widely understood in the MS and analytical communities.^{9,10} Both are soft ionization sources (i.e., generally retaining structural integrity of the ion of interest), and frequently form the same parent ions. However, the laser-induced MALDI process can be more energetic, thus generating different precursor ions than those produced by ESI. We have observed the formation of two unique precursor ions [M–H]⁺ = 197 Da and [M·]⁺ = 198 Da, and suggest that they result from the MALDI process in addition to the [M+H]⁺ = 199 Da species observed alone in ESI. MS/MS, IM-MS, and computational strategies were utilized to characterize these new precursor ion species.

Experimental Section

Materials

Sample preparation and experimental parameters have been previously described in detail for ESI studies, but a few details will be mentioned here.¹ Methylenedianiline (MDA) 2-ring isomers (2,2'-MDA, 2,4'-MDA, and 4,4'-MDA) were provided by Dr. Stefan Wershofen (Bayer MaterialScience AG, 47812 Krefeld, Germany). Methanol and water with 0.1% formic acid were obtained from Fisher Scientific (Waltham, MA). Tetralkylammonium salts, α -cyano-4-hydroxycinnamic acid (CHCA), alkali salts, and 2,7-diaminofluorene (2,7-DAF) were obtained from Sigma-Aldrich (St. Louis, MO). Samples were dissolved at a concentration of 1 mg/mL in 9:1 methanol/water containing 0.1% formic acid (*v/v*). Each MDA isomer was combined in a 10:1 matrix-to-analyte ratio and 2 to 3 layers of 0.5 μ L of sample were spotted on a 100-well MALDI plate.

Traveling Wave MALDI-IM-TOF/MS

A traveling wave IM-MS (TWIM-MS, Synapt G2-S, Waters Corporation, Milford, MA) with interchangeable MALDI and ESI sources was used to acquire MS, IM-MS, and MS/MS data. Although collision cross section (CCS) values cannot be obtained directly from TWIM experimental drift time values, a relative CCS method that uses quaternary ammonium salts as CCS calibration standards was utilized to obtain CCS data from TWIM.^{12–14}

The MALDI source for the Synapt G2-S mass spectrometer features a frequency-tripled Nd:YAG laser which emits 355 nm at a 1 kHz pulse repetition rate. Laser attenuation ranged from 190 to 300 (arbitrary units), with 270 selected for all experiments unless otherwise noted. TOF calibration was performed using CHCA matrix cluster peaks. The TWIM drift cell settings were as follows: TWIM pressure 3 mbar nitrogen (2.25 Torr), electrodynamic wave height 35 V, wave velocity 700 m/s, TOF resolving power ca. 18,000 m/z at 200 m/z. Collision-induced dissociation (CID) experiments were performed using argon as the target gas. The mass resolving quadrupole was set to 18 resolving power for the MS/MS experiments. This provided the best separation of the three precursor ion species without significant signal loss. Experiments were conducted to evaluate the influence of the MALDI laser fluence and the resolving power of the MS/MS resolving quadrupole on the resulting intensities of the precursor ions and these results have been included in the supplemental materials, Figure S10. The results of these experiments demonstrate that the resolving quadrupole can efficiently separate the three precursor ions and that laser fluence does not affect the relative intensities of the three different ion forms.

Computational Methods

To gain more insight into the experimental results, both a molecular dynamics conformational sampling calculation and quantum mechanical bond energy calculations were performed. A detailed description of the methods used can be found in the supplemental materials as well as in previous manuscripts, but briefly, a simulated annealing calculation implemented in AMBER¹⁵ was used to sample the conformational space of the [M–H]⁺, and [M+H]⁺ cations for the 2-ring MDA isomers. Corresponding theoretical CCS values were then obtained from MOBCAL^{16,17,18} and PSA^{19,20,21,22} to align the computationally generated structures with the experimental CCS measurements. Bond energy calculations were performed with Gaussian09²³ using density functional theory (DFT) to calculate the energy of the geometry optimized precursor and each of the two fragment structures resulting from breaking the bond of interest. The energies of the two fragments were summed and subtracted from the energy of the precursor structure resulting in the energy change of the reaction or relative bond energy of the broken bond. DFT/B3LYP/6-31G* level of theory was used for all calculations, which were performed *in vacuo* and in the ground state.

Results and Discussion

Differences Between ESI and MALDI Spectra for $[M+H]^+$

MS/MS spectra acquired at a fixed collision energy of 25 eV laboratory frame for $[M+H]^+$ MDA regioisomers are compared in Figure 2 for ESI and MALDI. The MS/MS spectra are shown for ESI on the left panels (Figure 2a–c) and for MALDI on the right panels (Figure 2d–f). The ESI and MALDI spectra for 4,4'-MDA (Figure 2a,d) are qualitatively identical in that 106 Da is the prominent fragment ion and additional low abundance peaks are observed at 182 and 165 Da in both spectra. In contrast, the ESI and MALDI spectra for 2,2'- and 2,4'-MDA exhibit significant differences in the fragment ions observed. For 2,2'- and 2,4'-MDA, the ESI MS/MS spectra (Figure 2b,c) exhibit a single major fragment ion peak at 106 Da and, unlike 4,4'-MDA, no ion signals are observed at 199 Da. The 2,2'- and 2,4'-MDA MALDI spectra (Figure 2e,f) are significantly more complex than ESI, with fragment ions observed at 198, 182, 181, 180, and 106 Da. These differences observed for the $[M+H]^+$ ion along with additional precursor ions indicate that these MDA regioisomers behave significantly differently when ionized by MALDI than with ESI.

Characterization of 2-Ring MDA by MS and MS/MS

Figure 3 shows the MALDI MS/MS spectra for the two additional precursor ions species ($[M-H]^+$ and $[M\cdot]^+$) for the MDA isomers and corresponding MS/MS spectra for a standard 2,7-diaminofluorene (2,7-DAF). Results for 2,7-DAF are shown in parallel with the MDA regioisomers due to its known fluorene backbone structure which we hypothesize to be the structure of the $[M-H]^+$ species (Figure 1). It is important to note that the 2,7-DAF species shown in Figure 3a is the $[M+H]^+$ species while the spectra shown in Figure 3b–d are the $[M-H]^+$ species. All species shown in Figure 3e–h correspond to the $[M\cdot]^+$ species, but the 2,7-DAF precursor in Figure 3e is 196 Da, whereas the MDA precursors in Figure 3f–h are 198 Da. Additional MS and MS/MS spectra can be found in the Supporting Information (Figures S1–S9) including MS/MS of fragments that result from operating with high (350 arbitrary units) laser fluence. The MS/MS spectra in Figure 3 were acquired at 25 eV and show fragments in many of the same regions that were observed in Figure 1 for the $[M+H]^+$ species. These fragment ions fall into four groups: 196–198 Da, 180–183 Da, 152–153 Da and 106 Da. The first group corresponds to a loss of one H-atom (1 Da) from the parent ion. The second fragment ion group represents nominal losses involving the loss of NH (15), NH₂ (16), NH₃ (17) or NH₄ (18). The third group involves nominal losses of both amino groups and an additional carbon atom. The fourth group of fragment ions corresponds to formation of a 106 Da fragment that has been previously discussed in earlier manuscripts. These four fragment ion groups are observed for the $[M+H]^+$, $[M-H]^+$, and the $[M\cdot]^+$ isomer species, but at very different intensities.

The MS/MS spectrum for 2,7-DAF in Figure 3a shows major fragment peaks at 196 Da, 180 Da and 181 Da. The $[M-H]^+$ MDA regioisomers all primarily form a 180 Da fragment in substantial amounts, but again the 4,4'-MDA is more stable in Figure 3b as significant depletion of the precursor ion occurs in Figure 3c,d at 25 eV for both the 2,2'-MDA and 2,4'-MDA. Both 2,2'-MDA and 2,4'-MDA $[M-H]^+$ ions exhibit far less of the 181 Da ion than that of 4,4'-MDA. The $[M\cdot]^+$ species form a combination of all the fragments

previously mentioned for $[M+H]^+$ and $[M-H]^+$. The MS/MS spectra of the 196 Da peak for 2,7-DAF in Figure 3e has fragment peaks at 195 and 180 Da. The MS/MS spectra for the MDA isomers (Figure 3f–h) show an additional peak at 106 Da, along with some minor peaks. This suggests that while the $[M-H]^+$ MDA ion behaves similarly to the 2,7-DAF $[M+H]^+$ ion, the $[M]^+$ species does not. As observed with the other ion forms, the $[M]^+$ of 4,4'-MDA exhibits greater stability than either 2,2'-MDA or 2,4'-MDA isomers as evidenced by a more prominent 198 Da precursor peak (Figure 3f). 2,2'-MDA forms a majority of a 180 Da fragment in Figure 3g and 2,4'-MDA forms primarily a 197 Da fragment and similar amounts of both the 180 and 181 Da fragments in Figure 3h. 4,4'-MDA produces significantly more of the 181 Da fragment than the other isomers, which suggests that preference for the 181 Da fragment arises from *para* amino substitution, and that the 180 Da fragment correlates predominantly with the *ortho*-amino group. The 106 Da fragment is also present in the $[M]^+$ MS/MS spectra, but not in the $[M-H]^+$ MS/MS spectra. Because there are numerous fragment ions which originate from three different precursor ions for each of the three different MDA regioisomers, the methods used to interpret and organize the data will be introduced briefly before detailed discussions of relative fragment ion intensities and fragmentation pathways resume.

While MS/MS spectra highlight the major fragment ions for each of these species, they offer only a snapshot of the numerous fragmentation experiments performed to better understand these different precursor ion species. MS/MS experiments were ramped from 0–50 eV for the three precursor ions for each of the MDA regioisomers in 5 eV increments and this energy correlation experiment (99 spectra) was performed in triplicate. Ion collision-energy curves corresponding to the change in percent relative ion current (%RIC) for the $[M+H]^+$, $[M-H]^+$, and $[M]^+$ ion forms of the MDA isomers are shown in Figures 4a–c for fragment ions exhibiting a %RIC of 20% or more along the 0–50 eV ramp. For clarity, the average %RIC of the three different isomers are shown here as solid traces, except the $[M+H]^+$ ion of 4,4'-MDA which exhibits different behavior (discussed later) and is represented by a dashed line in Figure 4a. Individual curves for each isomer, along with error bars, are provided as Supporting Information (Figure S10). Note that the percentage values projected on the y-axes of Figure 4 are relative to other ions which appear in the plot. Collision curves for all fragment ions present at %RIC values of 5% or more along the 0–50 eV ramp are included as Supporting Information (Figures S11–S14).

Figure 4d–f and Table 1 provide detailed %RIC data for the three precursor species, 4,4'-MDA (blue), 2,4'-MDA (green), and 2,2'-MDA (red), averaged over 0, 5, and 10 eV. At these low collision energies, the six fragment ions of interest are: 198 Da ($[M-1]^+$), 180–183 Da ($[M-18]^+$, $[M-17]^+$, $[M-16]^+$, and $[M-15]^+$) and 106 Da ($[M-93]^+$). These eight ions constitute greater than 95% of the total ion currents observed across all low energy spectra and thus participate in the primary fragmentation pathways for the MDA isomer system. A complete list of all fragment ions observed is provided as Supporting Information (Tables S7–S10), however, the fragment ions listed here will be the main focus of discussion. Statistical significance of each of these low energy measurements across the regioisomers was determined using an analysis of variance (ANOVA) and a t-test. Briefly, ANOVA allowed for comparison across the three regioisomers for each fragment to highlight when one regioisomer had a percent relative intensity that was significantly different from the

other two. A detailed summary of the ANOVA and t-test analysis is provided as Supporting Information (Tables S1–S6). When ANOVA testing indicates that the most abundant isomer in Table 1 behaves significantly differently than the other two isomers, yellow highlighting is used. Pink highlighting in Table 1 indicates that there is also a significant difference in fragment abundance between the other two isomers. For example, the mass 198 peak for $[M+H]^+$ is significantly more intense for 2,4'-MDA than for the other two isomers (which were not statistically different from one another), and 2,2'-MDA exhibits the most intense peak at 181 Da, but the other two isomers are significantly different.

$[M+H]^+$ Species—As observed in Figure 2, the MALDI MS/MS spectra are significantly more complex than the ESI MS/MS spectra for the $[M+H]^+$ ion of 2,2'-MDA and 2,4'-MDA. In contrast, the 4,4'-MDA isomer exhibits qualitatively similar spectra from both ion sources, which is due in part to the higher stability of the 4,4'-MDA isomer, that allows it to behave similarly despite a slightly more energetic ionization source (MALDI). Another reason for the similarities is that the 4,4'-MDA isomer can protonate only on *para* amino-groups, whereas the 2,2'-MDA and 2,4'-MDA species have additional protonation sites on the ring and on the *ortho* amino-groups (Figure 1), giving rise to the differences in MS/MS spectra seen for ESI and MALDI. The role of charge localization towards directing fragmentation processes of relatively small organic ions is well-established.²⁴

In Figure 4a, the depletion of the 199 Da parent ion (black, dashed) and the correlated increase in the 106 Da peak (grey, dashed) are consistent with the previously observed behavior of 4,4'-MDA and is due to stability and limited protonation on only the *para*-amino group. The average %RIC curves for 2,2'-MDA and 2,4'-MDA in Figure 4a clearly indicate that the 198 Da ion (purple) is derived directly from the 199 Da ion (black, solid) and that the 181 Da ion (teal) increases as both 199 and 198 Da ions decline. The 181 Da ion decreases in intensity as the 153 Da ion increases, indicating that the 153 Da ion (yellow) is derived from the 181 Da ion. On the basis of the dissociation curves in Figure 4a it appears that the 181 Da peak is generated from both the 198 and 199 Da ions. Because there are two possible protonation sites available for *ortho*-substituted MDAs, multiple pathways could occur. Based on the evidence in Figure 4d and Table 1, 2,2'-MDA (red) forms the majority of both the 181 and 182 Da fragments corresponding to mass losses of M-18 and M-17. 2,4'-MDA (green) forms the majority of both the 183 and 198 Da fragments corresponding to mass losses of M-16 and M-1. The t-test also indicates that 2,2'-MDA and 2,4'-MDA produce significantly different percentages of the 106 Da ion and that 2,4'-MDA and 4,4'-MDA produce significantly different percentages of the 181 Da ion.

$[M-H]^+$ Species—Collision energy curves in Figure 4b visualize the path that the $[M-H]^+$ isomers follow from precursor to major fragment ions. With increasing collision energy, the 197 Da precursor ion (black) depletes to form the 180 Da ion (teal) which in turn depletes to form the 152 Da ion (yellow) above 30 eV. This observed two-step depletion is similar to $[M+H]^+$ (Figure 4a), but here for $[M-H]^+$ the 4,4'-MDA isomer behaves similarly to the other two MDA isomers (Figure 4b averages the %RIC obtained from all three isomers).

Low energy pathways are also examined in Figure 4e for the MDA isomers and 2,7-DAF (orange). Somewhat parallel trends are observed for 2,7-DAF and 4,4'-MDA in that both

contribute to the majority of the 196 Da and 181 Da fragment ions. Figure 1 shows that these two species are more similar in structure, having both of their amino groups in either the *para* or *meta* positions (no *ortho*). The 2,2'-MDA and 2,4'-MDA isomers, which have similar protonation sites, namely an *ortho* amino group and a ring, both primarily form the 180 Da ion. In addition to the ANOVA testing, the t-test applied to the results in Table 1 indicate there are also significant differences between the two lesser peaks for the 196 Da, 182 Da, 181 Da, and 180 Da fragment ions. Based on the %RIC differences of the 196 Da fragment (2,7-DAF >> 4,4'-MDA > 2,4'-MDA > 2,2'-MDA), it is likely this fragmentation mechanism prefers the *para* and *meta* amino groups over the *ortho*. This trend which is repeated for the 181 Da fragment also indicates that the 181 Da fragment results from the 196 Da fragment rather than directly from the 197 Da precursor. In turn, the 180 Da fragment likely results directly from the 197 Da precursor, via neutral loss of NH₃.

[M·]⁺ Species—The [M·]⁺ radical cation peak (198 Da) appears prominently in the MALDI spectra of all MDA isomers. The question naturally arises whether it is formed by direct ionization of the parent MDA oligomer or by H-atom loss from [M+H]⁺, or possibly by both mechanisms. Direct ionization of aromatic molecules in MALDI, although not common, is well known.^{10,25} The 198 Da radical cation peak lies between the 197 Da ([M-H]⁺) and 199 Da ([M+H]⁺) peaks, both of which typically show greater intensity in the parent ion spectra. Although interference of the neighboring precursor peaks does occur, our experiments with the resolving quadrupole suggest (Figure S10) that the 198 Da peak can be mass-selected with at least 85% purity from the other two species.

Figure 4c displays the collision energy curves for [M·]⁺ which are qualitatively similar to those observed for the [M+H]⁺ precursor ions. The dotted lines indicate minor fragments not present at 20% but still worth noting for [M·]⁺ due to the more complex MS/MS spectra. Here, the [M·]⁺ precursor ion (198 Da, black) depletes to form directly the [M-1]⁺ (197 Da, purple) and [M-16]⁺ (182 Da, navy) fragment ion species. The [M-17]⁺ (181 Da, cyan) and [M-18]⁺ (180 Da, teal) species appear to be formed from the [M-1]⁺ species, as their appearance coincides with the depletion of the 197 Da ion. The 180 Da ion then appears to deplete to form the 152 Da ion (yellow), which was also observed for the [M-H]⁺ ion of all MDA isomers. An analysis of the low energy fragmentation pathways (Figure 4f) indicates the formation of fragment ions at 197, 180 to 183, and 106 Da. We also observe trends in both Figure 4f and Table 1 that those fragments favoring either 2,2'- or 4,4'-MDA also occur for 2,4'-MDA. This observation is consistent with the position of amino groups and availability of ring protonation playing a primary role in fragmentation preferences.

Characterization by Ion Mobility-Mass Spectrometry and Computational Modeling

IM-MS results also indicate that the [M-H]⁺ MDA species are similar to 2,7-DAF. The IM traces in Figure 5a–c are shown for the [M-H]⁺ (red) and [M+H]⁺ (black) MDA species as well as the 2,7-DAF [M+H]⁺ (blue). For 2,2'-MDA (Figure 5b) and 2,4'-MDA (Figure 5c), the [M-H]⁺ and [M+H]⁺ mobility traces overlap, but for 4,4'-MDA (Figure 5a) the [M-H]⁺ species has a smaller CCS than the [M+H]⁺ species. The smaller CCS observed for the 4,4'-MDA [M-H]⁺ species in Figure 5a also closely aligns with the mobility trace for 2,7-DAF, further evidence of the fluorene backbone for the [M-H]⁺ MDA species. Computational

modeling can provide some structural meaning to these observations. Theoretical results are shown in Figure 5d–f for the three 2-ring isomers and 2,7-DAF. The conformations resulting from simulated annealing calculations are shown for the $[M-H]^+$ (red) and $[M+H]^+$ (black) MDA species as well as 2,7-DAF (blue). Figure 1 is a helpful reference for the number of protonation sites for each regioisomer since each of these is modeled separately: one for 4,4'-MDA, two for 2,2'-MDA, and three for 2,4'-MDA. Each simulated annealing calculations generate clusters of 3,000 unique structures, which in many cases overlap with one another. To avoid complete disappearance of these data points, shading has been utilized in Figures 5d–f for the various $[M+H]^+$ protonation sites including the *para* (black), *ortho* (light grey), and ring (dark grey), as well as corresponding $[M-H]^+$ charge sites for *para* (red), *ortho* (yellow), and ring (orange) locations. A separate conformational space plot is shown for each protonation site for each precursor species in the Supporting Information (Figures S17–S18). It is also important to note that while the magnitude of the predicted CCS is larger here than what is measured experimentally, the relative ordering of theoretical CCS values is in qualitative agreement with the experimental trends observed for IM-MS. The $[M-H]^+$ conformations (red) of 4,4'-MDA, which has only a single protonation site at the *para* amine, are separated in CCS both theoretically (Figure 5d) and experimentally (Figure 5a) from the $[M+H]^+$ ions (black). The 2,7-DAF and 4,4'-MDA $[M-H]^+$ conformational space plots and mobility traces also overlap. In contrast, two protonation sites are available for the 2,2'-MDA $[M-H]^+$ and $[M+H]^+$ on both the *ortho* amine and the ring. These protonation sites not only overlap with each other, but the $[M-H]^+$ and $[M+H]^+$ also overlap for both experimental (Figure 5b) and theoretical results (Figure 5e). The reduced conformational space of the $[M-H]^+$ species becomes especially apparent in Figure 5e as the two protonation sites result in a very narrow CCS space (146.5–148.3 Å²) versus the $[M+H]^+$ species which occupies a much wider CCS space (145.7–153.3 Å²). The distribution width in Figure 5b also suggests the slightly larger conformational flexibility of the $[M+H]^+$ species. The 2,4'-MDA results are somewhat more complicated as there are now three possible protonation sites, but qualitatively, the deprotonated conformers (red, orange, and yellow) are all shifted to lower CCS than the protonated conformers (black, dark grey, and light grey) in Figure 5f. The mobility traces in Figure 5c do show a very slight shift to a smaller CCS for the $[M-H]^+$ species. These protonation site assignments from computational results are supported by observations from the MS/MS experiments and will be further analyzed in the mechanistic discussions.

The theoretical conformations (RMSD clustering) in Figure 5d–f provide a visual representation of the reduced flexibility for $[M-H]^+$ regioisomers. While the $[M-H]^+$ conformation for 2,2'-MDA and 2,4'-MDA would not be expected to decrease in CCS compared to their $[M+H]^+$ forms, it would for the 4,4'-MDA because the external amines become more restricted with the central fluorene motif. The fluorene ring structure for the $[M-H]^+$ ion creates a much more compact structure for the 4,4'-MDA (Figure 5d) and slightly more compact structure for the 2,4'-MDA (Figure 5f) due to the middle fused ring restricting the flexibility of the central carbon bond, whereas the $[M+H]^+$ ion has a more elongated structure and greater flexibility. The overall conformation of the 2,2'-MDA (Figure 5e) does not change significantly from the $[M+H]^+$ ion due to the amine groups being centrally located on the structure. While the conformations of the 2,7-DAF and 4,4'-

MDA $[M-H]^+$ clearly reflect similarities (Figure 5d), the second, smaller mobility peak for the 2,7-DAF (Figure 5a) is difficult to assign to a second conformation as flexibility is clearly restricted due to the fluorene motif, but rather likely represents an impurity, possibly a positional isomer present in the analytical standard. Additional IM-MS, including ESI results for 2,7-DAF are provided as Supporting Information (Figures S15–S16), but an in-depth discussion on 2,7-DAF is outside the scope of the current manuscript.

Mechanisms for Fragmentation of MDA Isomers

In addition to the MS/MS and IM-MS data described earlier, bond energy calculations were performed for all the precursor ions listed in Table 1. The complete results are provided as Supporting Information (Figures S19–S22), but bond energies for the weakest bonds are included in Scheme 1. In general, the weakest bond (highlighted in green) in a molecule is shown to be either the protonated N-H or the protonated ring C-H, where appropriate. Typically, the methylene bridge C-H is the second weakest bond for all cases. The remaining fragmentation mechanism discussions will focus on the MDA species that produces the largest %RIC for each representative fragmentation ion.

M-1 Fragmentation—Scheme 1 summarizes M-1 fragmentation for the three MDA isomers and 2,7-DAF. Scheme 1a describes H-atom loss from species having a protonated amine group that is the weakest bond in all $[M+H]^+$, $[M-H]^+$ and 2,7-DAF species. The dashed lines in the structures indicate that the reaction occurs for both $[M+H]^+$ and $[M-H]^+$ precursor species. Scheme 1b shows similar reactions for $[M+H]^+$ and $[M-H]^+$ precursor species having ring protonation. Fragmentation of the protonated ring C-H bond is the lowest energy pathway. Scheme 1c describes H-atom loss from the $[M\cdot]^+$ parent ion that does not have a protonated amine, the bridge C-H bond is the weakest bond for the three MDA regioisomers. Loss of the bridge hydrogen has different consequences than loss from an amine group in that the remaining charge is delocalized over the entire aromatic system. The two resulting structures shown in the scheme are Pauling-type resonance structures for the 197 Da fragment ion. It is important to note that this 197 Da fragment ion, derived from the $[M\cdot]^+$ parent, is different from the 197 Da $[M-H]^+$ parent ion which cannot be produced by a M-1 reaction.

M-16 Fragmentation—Scheme 2 summarizes the reactions involved in loss of 16 Da from the MDA and 2,7-DAF parent ions. The most likely loss is NH_2 from a charge-site reaction involving the protonated amine group. Scheme 2a indicates transfer of a protonated amine H-atom onto the aromatic ring along with charge transfer to the same ring. The same reaction occurs for 2,7-DAF. The driving force is fracture of an N-H bond and subsequent formation of an aromatic C-H bond. It costs about 60 kcal/mol to break the N-H bond and 140 kcal/mol to break the C-N bond for a protonated amine. About 120 kcal/mol is reclaimed by aromatic C-H formation, giving a net cost of about 80 kcal/mol for the reaction; this is very similar to the energy associated with M-1 loss. Loss of NH_2 is not likely when protonation occurs on the ring, although it could shift into an amine protonated species by a tautomeric equilibrium reaction. Direct loss of an amine group from a $[M\cdot]^+$ parent ion via a simple C-N bond fracture is also not likely, given that the C-N bond energies for unprotonated amines are ~110 kcal/mol. Figure 4c indicates that the 182 Da fragment ion

of $[M\cdot]^+$ is formed directly from the parent ion, in parallel with the 197 Da fragment ion. Also, Table 1 indicates it is most prominent for the 4,4'- and 2,4'-MDA parent ions. Scheme 2b indicates this could be accomplished by a concerted reaction involving N-H transfer to the aromatic ring and bridge C-H loss. The 182 Da fragment has charge delocalization over the whole aromatic system, helping to drive the reaction. The distributions of %RIC intensities are quite similar for the 197 Da and 182 Da fragment ions of $[M\cdot]^+$ (Figure 4f). Another reaction must also be considered for M-16, namely, loss of 16 Da from the 197 Da fragment of $[M\cdot]^+$ to form the 181 Da fragment, nominally this would be M-17 from the parent ion. The proposed M-16 reaction for 2,2'-MDA is shown in Scheme 2c. This is based on collective evidence from Figure 4c which indicates that formation of the 181 Da fragment ion occurs from the 197 Da ion for $[M\cdot]^+$, and evidence from Table 1 which indicates that 181 Da formation is most prominent for the *ortho*-substituted oligomers.

M-17 Fragmentation—Scheme 3 summarizes fragmentation reactions involving loss of 17 Da, presumably as ammonia (NH_3). For compounds containing a protonated *ortho*-amino group, this corresponds to transfer of a bridge H-atom to displace NH_3 as shown in Scheme 3a for both $[M+\text{H}]^+$ and $[M-\text{H}]^+$ parent ions. The specific reaction is shown for 2,2'-MDA. Lack of proximity of the protonated amino and bridge C-H groups in the 4,4'-isomers and 2,7-DAF, requires a different mechanism shown in Scheme 3b. The data in Table 1 for $[M-\text{H}]^+$ indicate that this is the case based on the decreases of %RIC seen for the M-17 fragment ions from 2,2'-MDA > 2,4'-MDA > 4,4'-MDA. However, both reactions (Scheme 3a,b) must be significant. This is the major fragmentation reaction for all $[M-\text{H}]^+$ MDA oligomers, a 89 %RIC is seen for 2,2'-MDA. However, the 4,4'-oligomer shows a 51 %RIC, indicating a very significant influence of the more rigid $[M-\text{H}]^+$ structure. The M-17 fragments corresponding to the three $[M+\text{H}]^+$ oligomers are much less intense, averaging about 12 %RIC. The $[M\cdot]^+$ parent has no protonated amino group but still shows a significant M-17 loss, with a 20 %RIC for the 2,2'-oligomer as discussed above. However, the $[M\cdot]^+$ parent ion does show a nominal M-18 loss at 180 Da. Figure 4c indicates that the 197 Da peak is the precursor for the 180 Da ion of $[M\cdot]^+$, as was the case for the 181 Da ion in Scheme 2. Scheme 3c shows such a reaction for the 2,4'- oligomer, which forms the same mass as the $[M-\text{H}]^+$ parent (180 Da). Another reaction is also possible for the $[M\cdot]^+$ ion. Figure 4a indicates that the M-18 ion (181 Da) of $[M+\text{H}]^+$ can be formed from the $[M\cdot]^+$ ion produced by the 199 Da parent ion. Also, Table 1 indicates that the 181 Da ion is most prominent for 2,2'-MDA. Based on this evidence, the 181 Da fragment ion is probably produced by a concerted reaction involving loss of an amino group and an amine H-atom as shown in Scheme 3d.

M-15 and Higher Energy Fragmentation—Two MDA species show M-15 losses, $[M-\text{H}]^+$ (182 Da) and $[M\cdot]^+$ (183 Da), which corresponds to a loss of NH. This can occur in a charge remote reaction involving transfer of an amine H-atom to the ring, as shown in Scheme 4a,b. The difference in N-H bond energies for protonated and unprotonated amine groups, 62.1 and 97.3 kcal/mol respectively, coupled with lack of charge transfer, make these H-atom reactions less likely than their protonated-amine counterparts. The 152 and 153 Da ions assume prominence in Figure 4a–c as the fragmentation energy is increased. The 152 Da ion is related to the $[M-\text{H}]^+$ and $[M\cdot]^+$ parent ions and is formed from the 180

Da fragment, whereas the 153 Da ion is related to the $[M+H]^+$ and originates from the 181 Da fragment. Both the 152 Da and 153 Da fragmentation reactions involve loss of the remaining amine nitrogen atom, a ring carbon atom, and hydrogens. Scheme 4c shows the reaction for the 180 Da fragment ion formed from $[M-H]^+$. The net effect is converting a six-member ring into a five-member fused ring, by separate loss of HCN and a H-atom. Another high energy fragmentation process, loss of 28 Da from the 181 Da fragment ion, is more challenging to assign a mechanism. While a structure equaling 153 Da could be imagined by replacing the bridge C-H group in the 152 Da structure with a nitrogen atom, how this might happen mechanistically is hard to reconcile. The structure shown in Scheme 4d for formation of the 153 Da ion probably involves loss of HCN and a H-atom, similarly to the 152 Da mechanism (Scheme 4c). One hydrogen loss must be remote from the NH group. Bridge H-atom loss is shown in Scheme 4d because it is the weakest C-H bond in the molecule. However, it is noted here that other 153 Da structures are also possible.

Conclusions

In this study, 2-ring MDA isomers (2,2'-, 2,4'-, and 4,4'-MDA) have been characterized using a MALDI-TOF MS platform and a variety of other MS techniques. In previous work, we concluded that $[M+H]^+$ is the only parent ion formed in ESI, whereas in this current study, MALDI was observed to form three unique parent ions for all MDA isomers: $[M+H]^+$, and two other ions formed by laser irradiation, $[M\cdot]^+$ and $[M-H]^+$. Unique fragment ions observed for each of the respective precursor ions were explored and their origins were determined using energy-resolved MS/MS studies. IM-MS provided further conformational distinctions between parent ions observed in the precursor region. 2,7-DAF confirmed the $[M-H]^+$ proposed fluorene motif structure. Characterizing fragment ions associated with 2,7-DAF allowed for a greater understanding of how the $[M-H]^+$ fragment ion forms from each of the three MDA regioisomers. Analytically, these findings provide important insight into the complex ion formation and associated fragmentation behavior for polymer precursors investigated using MALDI ionization methods. It is anticipated that this work will help further the interpretation and development of MALDI-based polymer studies by MS, which remains a key MS technology for polymer characterization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Forsythe JG, Stow SM, Nefzger H, Kwiecien NW, May JC, McLean JA, Hercules DM. *Anal Chem.* 2014; 86:4362–4370. [PubMed: 24678803]
2. Stow SM, Onifer TM, Forsythe JG, Nefzger H, Kwiecien NW, May JC, McLean JA, Hercules DM. *Anal Chem.* 2015; 87:6288–6296. [PubMed: 25971782]
3. Liu J, Loewe RS, McCullough RD. *Macromolecules.* 1999; 32:5777–5785.
4. Nielen MWF. *Mass Spectrom Rev.* 1999; 18:309–344.
5. Belu AM, Desimone JM, Linton RW, Lange GW, Friedman RM. *J Am Soc Mass Spectrom.* 1996; 7:11–24. [PubMed: 24202790]
6. Montaudo G, Samperi F, Montaudo M. *S. Prog Polym Sci.* 2006; 31:277–357.
7. Bletsos IV, Hercules DM, VanLeyen D, Benninghoven A, Karakatsanis CG, Rieck JN. *Macromolecules.* 1990; 23:4157–4163.
8. Gies AP, Hercules DM. *Anal Chim Acta.* 2014; 808:199–219. [PubMed: 24370105]
9. Gaskell SJ. *J Mass Spectrom.* 1997; 32:677–688.
10. Zenobi R, Knochenmuss R. *Mass Spectrom Rev.* 1998; 17:337–366.
11. May, JC., Goodwin, CR., McLean, JA. *Encyclopedia of Drug Metabolism and Interactions.* Lyubimov, AV., editor. John Wiley & Sons, Inc; 2011.
12. Ruotolo BT, Benesch JLP, Sandercock AM, Hyung SJ, Robinson CV. *Nat Protoc.* 2008; 3:1139–1152. [PubMed: 18600219]
13. Hines KM, May JC, McLean JA, Xu L. *Anal Chem.* 2016; 88:7329–7336. [PubMed: 27321977]
14. Harper B, Neumann EK, Stow SM, May JC, McLean JA, Solouki T. *Anal Chim Acta.* 2016; 939:64–72. [PubMed: 27639144]
15. Case, DA., Babin, V., Berryman, J., Betz, RM., Cai, Q., Cerutti, DS., Cheatham, TE., III, Darden, TA., Duke, RE., Gohlke, H., et al. *AMBER 14.* University of California; San Francisco, CA: 2014.
16. Mesleh MF, Hunter JM, Shvartsburg AA, Schatz GC, Jarrold MF. *J Phys Chem.* 1996; 100:16082–16086.
17. Shvartsburg AA, Jarrold MF. *Chemical physics letters.* 1996; 261:86–91.
18. Campuzano I, Bush MF, Robinson CV, Beaumont C, Richardson K, Kim H, Kim HI. *Anal Chem.* 2011; 84:1026–1033. [PubMed: 22141445]
19. Bleiholder C, Wyttenbach T, Bowers MT. *Int J Mass Spectrom.* 2011; 308:1–10.
20. Bleiholder C, Contreras S, Do TD, Bowers MT. *Int J Mass Spectrom.* 2013; 345:89–96.
21. Anderson SE, Bleiholder C, Brocker ER, Stang PJ, Bowers MT. *Int J Mass Spectrom.* 2012; 330–332:78–84.
22. Bleiholder C, Contreras S, Bowers MT. *Int J of Mass Spectrom.* 2013; 354:275–280.
23. Frisch, MJ., Trucks, GW., Schlegel, HB., Scuseria, GE., Robb, MA., Cheeseman, JR., Scalmani, G., Barone, V., Mennucci, B., Petersson, GA., et al. *Gaussian09.* Gaussian Inc; Wallingford, CT: 2009. Revision A.02
24. McLafferty, FW., Ture ek, F. *Interpretation of Mass Spectra.* 4th. University Science Books; Sausalito, CA: 1993.
25. Knochenmuss R, Zenobi R. *Chem Rev.* 2003; 103:441–452. [PubMed: 12580638]

Figure 1.

MDA 2-Ring isomers and 2,7-DAF. Neutral molecular masses (M) are shown along with those for $[M+H]^+$ (2,7-DAF) and $[M-H]^+$ (MDA) ions. Possible protonation sites are noted with an asterisk (*).

Figure 2. MS/MS spectra for the $[M+H]^+$ isomer species for both (left) ESI and (right) MALDI. Results for 4,4'-MDA are shown in a) and d), for 2,2'-MDA in b) and e) and for 2,4'-MDA in c) and f). These spectra were obtained at 25 eV lab frame energy.

Figure 3. MS/MS spectra for the (left) 197 Da precursor and (right) $[M]^+$ isomer species for MALDI. Results for 2,7-DAF are shown in a) and e), for 4,4'-MDA in b) and f), for 2,2'-MDA in c) and g) and for 2,4'-MDA in d) and h). These spectra were obtained at 25 eV lab frame energy.

Figure 4.

Collision induced dissociation curves monitoring the transition of precursor to fragment ions for a) $[M+H]^+$ ions, b) $[M-H]^+$ ions, and c) $[M]^\cdot$ ions. The different line weights represent the average of all three isomers (solid); the 4,4'-MDA isomer for the protonated species (dashed); and secondary fragments for the radical cation species (dotted). Color coding is for precursor ions (black); the M-1 species (purple); the M-17 and M-18 species (teal); the M-45 and M-46 species (yellow); the M-93 species (gray); and the M-15 and M-16 species (cyan and navy). Bar graphs of %RIC at low collision energies are shown for d) $[M+H]^+$, e) $[M-H]^+$, and f) $[M]^\cdot$ ion species. The bar colors correspond to 4,4'-MDA (blue); 2,2'-MDA (red); 2,4'-MDA (green); and 2,7-DAF (orange).

Figure 5. Ion mobility traces and computational modeling results for 2-Ring MDA. [M+H]⁺ results are shown in black, [M-H]⁺ results are shown in red, and 2,7-DAF results in blue.

Scheme 1.

Fragmentation mechanisms for the M-1 mass loss when a) an N-H bond from a protonated amine is broken, b) a C-H bond from a protonated ring is broken, and c) when a bridge C-H bond is broken. Selected bond energies are included for the MDA isomers. Green highlighting specifies the weakest bond.

Scheme 2.

Fragmentation mechanisms for the M-16 mass loss when a) an NH_2 fragment is lost from a protonated amine group, b) when an NH fragment and a bridge hydrogen are lost, and c) when an *ortho* NH_2 group is lost by hydrogen rearrangement.

Scheme 3.

Fragmentation mechanisms for the M-17 mass loss for a) when an NH_3 group is lost b) when an NH_2 group and bridge hydrogen are lost, c) an NH group and two hydrogens are lost from a 197 fragment ion, and d) loss of NH_2 and an amine hydrogen from $[\text{M}\cdot]^+$.

Scheme 4.

Fragmentation mechanisms for the M-15 mass loss for a) loss of NH from $[M-H]^+$ and, b) loss of NH from $[M]^+$; Higher energy fragmentation for c) loss of nominal H-C=N-H from a 180 fragment ion and d) loss of nominal H-C=N-H from a 181 fragment ion.

Table 1

Percent relative ion current data for the major fragments from the $[M+H]^+$, $[M-H]^+$, and $[M]^{+}$ for the three 2-ring MDA isomers and 2,7-DAF. See text for details.

Isomer	106	180	Fragment Ions for $[M+H]^+$			196	197	198
			181 M-18	182 M-17	183 M-16			
2,2'-MDA	7.1 ± 1.5%	1.4 ± 0.6%	21.7 ± 1.4%	16.2 ± 1.4%	10.4 ± 3.7%	0.2 ± 0.1%	1.4 ± 1.0%	41.5 ± 3.1%
2,4'-MDA	5.3 ± 0.9%	0.5 ± 0.3%	6.7 ± 0.6%	9.3 ± 1.2%	15.4 ± 1.2%	0.2 ± 0.1%	2.0 ± 1.1%	60.7 ± 2.6%
4,4'-MDA	27.1 ± 11.6%	1.4 ± 0.9%	3.9 ± 1.1%	11.4 ± 3.1%	10.9 ± 2.2%	0.2 ± 0.2%	2.8 ± 2.9%	42.3 ± 11.9%
Isomer	106	180	Fragment Ions for $[M-H]^+$			196	197	198
			181 M-16	182 M-15	183 M-14			
2,2'-MDA	1.6 ± 0.3%	89.3 ± 2.2%	4.3 ± 1.2%	1.6 ± 0.2%	1.8 ± 0.7%	1.4 ± 0.7%		
2,4'-MDA	2.5 ± 0.8%	70.6 ± 2.1%	11.5 ± 0.6%	6.8 ± 0.5%	1.7 ± 0.4%	6.8 ± 0.6%		
4,4'-MDA	3.1 ± 1.7%	51.4 ± 4.5%	21.5 ± 5.8%	10.1 ± 1.8%	1.3 ± 0.8%	12.6 ± 1.3%		
Isomer	106	180	Fragment Ions for $[M]^{+}$			196	197	198
			181 M-17	182 M-16	183 M-15			
2,2'-MDA	9.4 ± 1.6%	17.5 ± 1.0%	20.5 ± 2.3%	9.5 ± 0.4%	14.0 ± 1.7%	0.2 ± 0.1%	28.9 ± 2.1%	
2,4'-MDA	8.1 ± 1.3%	6.6 ± 0.3%	11.9 ± 1.3%	15.1 ± 0.9%	6.4 ± 0.7%	0.8 ± 0.2%	51.2 ± 2.0%	
4,4'-MDA	4.8 ± 0.7%	3.3 ± 0.9%	4.8 ± 1.1%	19.5 ± 2.1%	3.8 ± 2.7%	0.8 ± 0.8%	63.0 ± 3.1%	
Standard	106	180	Fragment Ions for 2,7-DAF			196	197	198
			181 M-16	182	183			
$[M+H]^+$	0.0 ± 0.0%	17.6 ± 7.0%	18.4 ± 6.1%	1.3 ± 1.5%	0.1 ± 0.2%	62.6 ± 8.7%		
$[M]^{+}$	0.0 ± 0.1%	95.1 ± 3.5%	3.5 ± 3.3%	1.0 ± 1.6%	0.3 ± 0.5%			