Characterization of Branching in Aramid Polymers Studied by MALDI−Ion Mobility/Mass Spectrometry

Anthony P. Gies,* Michal Kliman, John A. McLean, and David M. Hercules

Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37235

Received August 27, 2008
Revised Manuscript Received October 15, 2008

Introduction. Conventional methods of analysis (i.e., viscometry, titration, TGA, DSC, DMA, GPC, NMR, IR, Py-GC/MS)[1−3] have proven inadequate for providing desired information on the molecular mass distribution, chemical distribution, and structural heterogeneities present in aramid fibers.[4] In large part, this is due to weak signals generated from low concentrations of end groups and byproduct; background noise often masks these weak signals.[5] However, MALDI-TOF MS provides the signal level, mass accuracy and resolution, or direct structural determination of the species and end groups present in a complex polymeric sample.[4] The greatest obstacle in characterizing aramids with mass spectrometry has been their poor solubility in common solvents.[1,6] Even when a suitable solvent is found for the aramids (e.g., concentrated sulfuric acid), it is not compatible with mass spectrometry. The evaporation−grinding method[7−9] (E-G method) has been developed as a practical technique for MALDI-TOF MS analysis of aramids and other insoluble polymers.

A general problem for polymer analysis by MALDI is that peaks from branched and linear species occur in the mass spectra, which can potentially be isobaric. Separation of these species would be valuable to improve peak identification and to study branching mechanisms. Gel permeation chromatography (GPC) is ineffective because of low resolution and solvent incompatibility. To overcome these challenges, we have investigated the utility of MALDI−ion mobility (IM)/MS and MALDI-TOF/TOF CID fragmentation to structurally distinguish peaks from branched and linear species. The combination of IM/MS is analogous to performing postionization gas-phase electrophoresis, i.e., separations on the basis of apparent surface area, followed by separations by TOF MS on the basis of mass-to-charge (*m*/*z*).

Briefly, in IM/MS separations, ions are generated by conventional means such as MALDI at reduced pressure (e.g., 3−5 Torr of He). The ions are then directed into the IM drift tube and migrate under the influence of weak electrostatic fields (e.g., 10−30 V cm^{-1} Torr^{-1}). The velocity of the ions across the drift tube is impeded by nearly thermal collisions with the background drift gas. Separation is then achieved by the number of collisions a particular analyte species experiences, typically >10^4−10^6 collisions.[10−12] Thus, separation selectivity in IM, as in GPC, is determined by ion volume rather than by mass. Therefore, for two ions of identical mass but of different gas-phase volumes, the smaller of the two will move through the ion mobility chamber more rapidly. The resolving power of IM (ca. 10−100, $t_d/\Delta t_d$ where t_d is ion drift time) lies between that of gas chromatography and GPC.

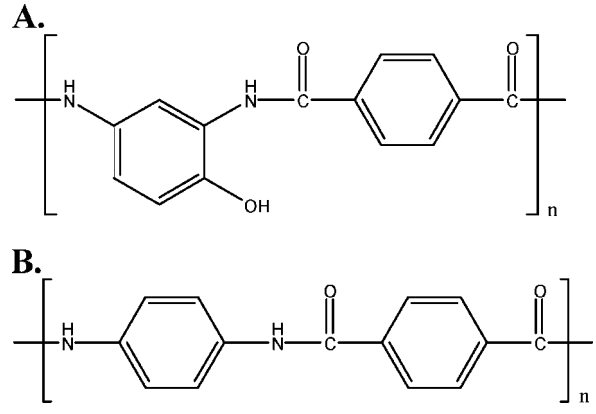
The time scale for separations in the IM dimension are 100 μs to 10 ms, which is ideally suited for interfacing with an orthogonal-TOF MS, which provides separations on the order of 10 μs. Thus, many TOF MS spectra are acquired across the IM elution profile and are subsequently stitched together to generate a 2-D IM/MS plot of conformation space, whereby the *y*-axis corresponds to ion drift time and the *x*-axis corresponds to *m*/*z*. Also, for different biopolymer species, distinct correlations of IM versus MS are observed, which establishes a so-called conformation space trendline.[13] In these studies, we investigate the utility of IM/MS separations to distinguish branching versus linear species in the analysis of aramids.

Experimental Section. *Materials.* Poly(*o*-hydroxyamide) (MP-amide) fibers were synthesized as described earlier.[14] The oligomer structure is shown in Chart 1A. The commercial aromatic polyamide used in this study was Kevlar aramid fiber. The structure of the polymer is shown in Chart 1B.

Instrument Measurements. Aramid samples were analyzed using a MALDI-IM/TOF MS instrument (constructed in collaboration with Ionwerks, Inc., Houston, TX) which was described elsewhere.[11] The instrument is equipped with a Nd:YLF laser (349 nm). Spectra were obtained in the positive ion mode using a laser intensity of ∼10% greater than threshold and performing MALDI at 300 Hz repetition rate. IM measurements used a 13.9 cm long helium-filled ion mobility drift cell, maintained at 3.8 Torr, with separation field strengths of 23 V/(cm Torr). Mass spectra were acquired in the reflectron mode with a mass resolution greater than 3000 fwhm; isotopic resolution was observed throughout the entire mass range. MALDI-TOF/TOF CID used an Applied Biosystems 4700 Proteomics Analyzer (Applied Biosystems, Framingham, MA) equipped with a Nd:YAG (355 nm) laser. Spectra were obtained in the positive ion mode using an accelerating voltage of 8 kV for the first source and 15 kV for the second source and a laser intensity of ∼10% greater than threshold. External mass calibration was performed using protein standards from a Sequazyme Peptide Mass Standard Kit (Applied Biosystems). The instrument was calibrated before every measurement. All polyamide samples were prepared by the E-G method using a 3-aminoquinoline (3AQ, Aldrich) matrix doped with sodium trifluoroacetate (NaTFA, Aldrich).

Results and Discussion. Our earlier MALDI-TOF MS studies of poly(*p*-phenylene terephthalamide) (PPD-T)[15] and polybenzoxazole (PBO)[14] fibers indicated that branching was occurring in these materials, but definitive information was lacking.

Chart 1. Chemical Structures for (A) Poly(*o*-hydroxyamide) (MP-amide) and (B) Kevlar Aramid Fibers



* Corresponding author: Tel (615) 343-5980; e-mail a.gies@vanderbilt.edu.

10.1021/ma8019464 CCC: $40.75
Published on Web 11/01/2008

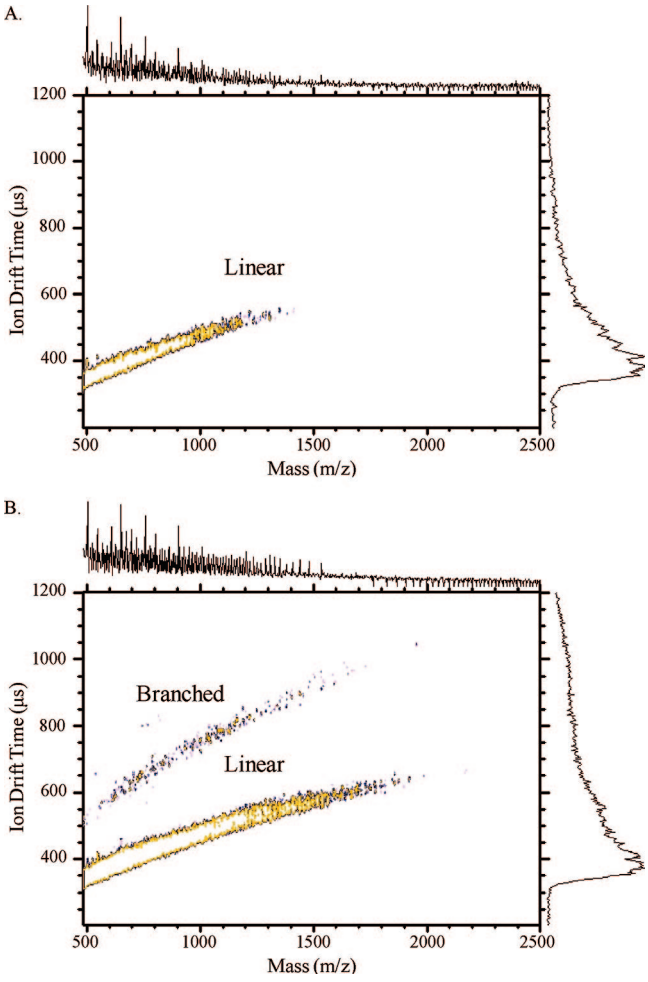


Figure 1. MALDI-IM/MS two-dimensional plot of (A) linear and (B) branched MP-amide fibers prepared by the E-G method. Mass range 460−2500 Da, 3AQ matrix, and cationized with NaTFA.

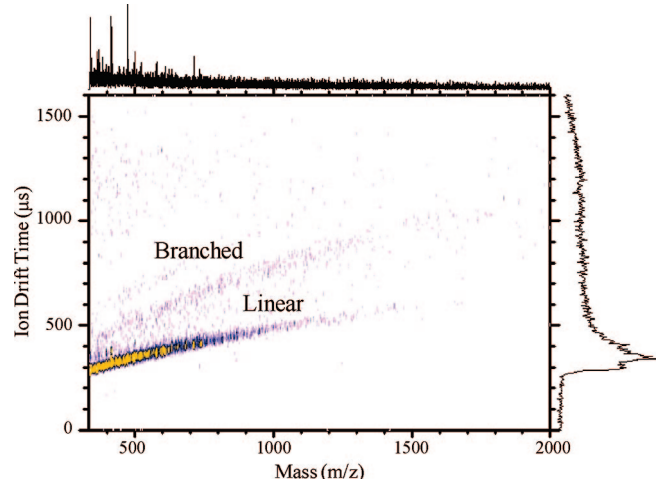


Figure 2. MALDI-IM/MS two-dimensional plot of Kevlar fibers prepared by the E-G method. Mass range 300−2000 Da, 3AQ matrix, and cationized with NaTFA.

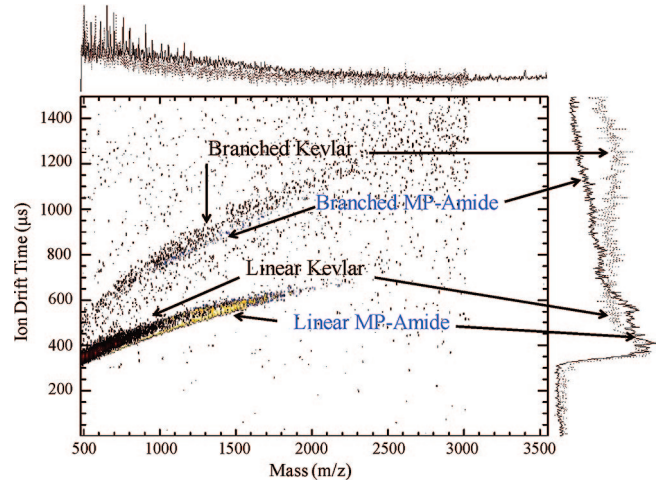


Figure 3. MALDI-IM/MS two-dimensional plot of MP-amide vs Kevlar fibers prepared by the E-G method. Mass range 460−3500 Da, 3AQ matrix, and cationized with NaTFA.

Difficulties in mass spectral identification of branched species include (1) low ion intensities for many branched species, (2) peaks from branched species can be isobaric with linear peaks, and (3) the inability of conventional MALDI-TOF MS to selectively identify the branch points in the polymers. The combination of MALDI-IM/TOF MS has the potential capability of addressing all of these problems. To show the potential usefulness of ion mobility separations for routine polymer analysis, we report here data on the MALDI-IM/TOF MS separation of linear and branched aramids and describe how it enhances the use of MS/MS fragmentation to learn about oligomer structure.

MP-amide. Figure 1 shows 2-D IM/MS plots obtained for MP-amide. The overall mass spectrum is shown at the top with the mass scale at the bottom. The right-hand *y*-axis is the overall IM spectrum with the IM drift time on the left *y*-axis. The diagonal correlated data are trendlines for MALDI-IM/MS of specific structural species. Figure 1A is a 2-D plot for an MP-amide polymer (Chart 1A) synthesized under conditions known to produce primarily linear species. Note that there is only a single trend line in this plot. Figure 1B shows a 2-D plot for an MP-amide polymer (Chart 1A) synthesized using conditions known to induce branching. Note that there are two trendlines in Figure 1B: the lower one coincident with the trendline in Figure 1A, for linear species, and the upper one which is due to branched species. When the mass spectra of the branched species are examined, their peaks are readily identified in the combined mass spectrum.

Kevlar. Figure 2 shows a 2-D MALDI-IM/MS plot obtained from Kevlar fibers (Chart 1B) by combining the E-G MALDI sample preparation technique with MALDI-IM/MS. Figure 2 clearly shows the separation of linear (lower trendline) and branched (upper trendline) species. Although the trendline for the branched species shows considerable scatter (noise), branched species are definitely confirmed. Comparison of the upper trendlines in Figures 1B and 2 shows that they have nearly identical ion drift times, providing further evidence for the presence of branched species in Kevlar fibers.

An overlay of Figures 1B and 2 reveals further distinction, as shown in Figure 3. The MP-amide trendline for linear species (yellow) is slightly lower than the same trendline for Kevlar (black). Similarly, the upper trendlines for the branched species show that the MP-amide trendline (blue) is slightly lower than that for Kevlar. In summary, MALDI-IM/MS provides clear separation of linear and branched species and distinction between the branched meta−para-aramid line and the branched Kevlar line. Of practical importance, this analysis was performed in less than 1 h including sample preparation and cleanup.

Figure 4 shows MALDI-TOF/TOF CID spectra for linear (A), carboxyl terminated (697.2 Da) and branched (B) carboxyl

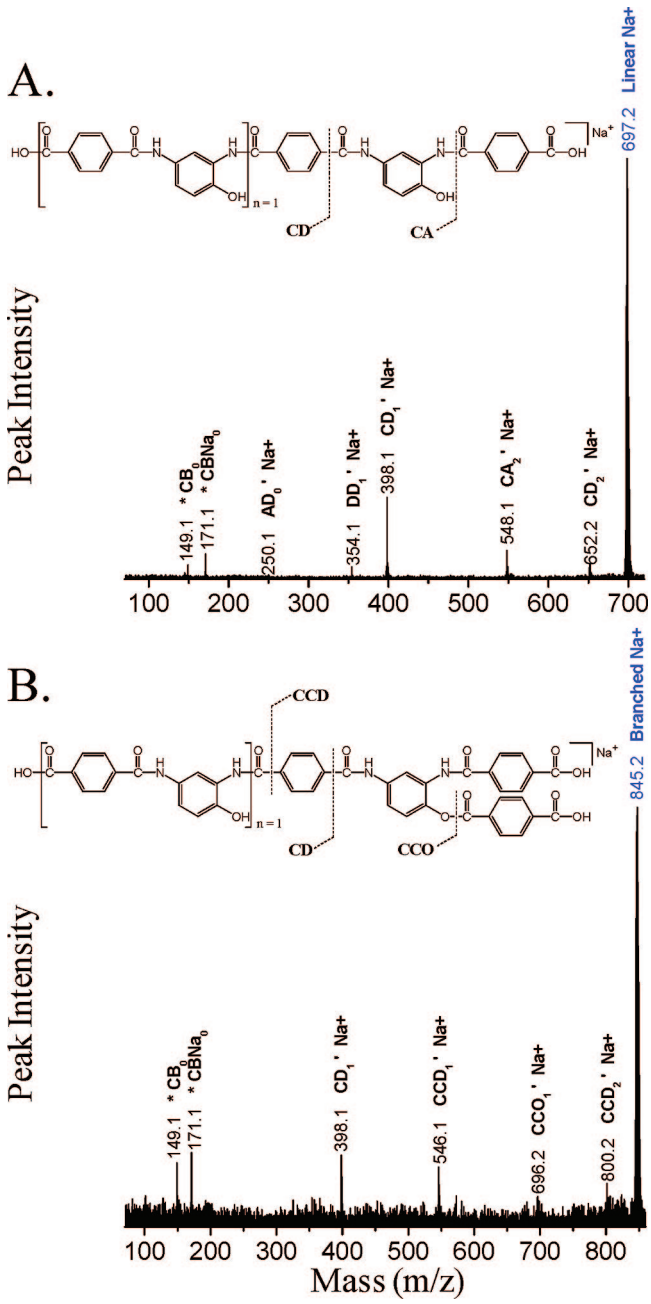


Figure 4. MALDI-TOF/TOF CID mass spectrum of (A) linear and (B) branched MP-amide fibers prepared by the E-G method.

terminated (845.2 Da) species of MP-amide (Chart 1A). The peaks for CID fragmentation were selected, based on their location in the 2-D plot in Figure 1B. Detailed analysis of the MS/MS spectra is beyond the scope of the present work. The TOF/TOF fragmentation studies have identified a weak link at the carbonyl−phenyl bond. Both the linear and branched spectra display a significant peak at 398.1 Da due to a carboxyl−decarboxyl fragment labeled CD. However, due to the introduction of an "extra" carboxyl group, the branched MP-amide spectrum displays additional "fingerprint" peaks at 546.1 and 800.2 Da that represent a carboxyl−decarboxyl terminated fragment with an additional carboxyl end, CCD. The branched MP-amide spectrum has an additional, less intense, "fingerprint" peak at 696.2 Da (species CCO) due to fragmentation at the ester branch point. This ester fragmentation point also explains why the branched spectrum shows a more intense carboxyl−carbonyl carbocation (CC) peak at 149.1 Da, relative to the linear MP-amide spectrum.

Conclusions. The combination of MALDI-IM/TOF MS shows great potential for separating and identifying polymer species which have identical mass but different molecular volumes. What polymer and instrumental parameters will be most important for IM separation needs to be defined, although IM shows greater discriminating power than GPC. Note that because IM/MS separations are performed postionization, it eliminates the need for fraction collection as in GPC. MALDI MS/MS can be performed either online or separately after peak identification from the 2-D plot. One thing is certainly clear: the combination of MALDI-TOF MS with ion mobility separation and CID fragmentation will have a significant impact on synthetic polymer characterization.

Acknowledgment. We thank Teijin Aramid for partial support of this work.

References and Notes

(1) Yang, H. H. *Aromatic High-Strength Fibers*; John Wiley & Sons: New York, 1989; pp 178−206.
(2) Evers, R. C.; Arnold, F. E.; Helminiak, T. E. *Macromolecules* **1981**, *14*, 925–930.
(3) Choe, E. W.; Kim, S. N. *Macromolecules* **1981**, *14*, 920–924.
(4) Montaudo, G.; Lattimer, R. P. *Mass Spectrometry of Polymers*; CRC Press: Boca Raton, FL, 2001; p 455.
(5) Ji, H.; Nonidez, W. K.; Mays, J. W.; Advincula, R. *Macromolecules* **2005**, *38*, 9950–9956.
(6) Takatsuka, R.; Uno, K.; Toda, F.; Iwakura, Y. *J. Polym. Sci., Polym. Chem. Ed.* **1977**, *15*, 1905–1915.
(7) Gies, A. P.; Nonidez, W. K.; Anthamatten, M.; Cook, R. C.; Mays, J. W. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 1903–1910.
(8) Gies, A. P.; Nonidez, W. K. *Anal. Chem.* **2004**, *76*, 1991–1997.
(9) Gies, A. P.; Nonidez, W. K.; Anthamatten, M.; Cook, R. C. *Macromolecules* **2004**, *37*, 5923–5929.
(10) Eiceman, G. A.; Karpas, Z. *Ion Mobility Spectrometry*, 2nd ed.; Taylor & Francis Group: Boca Raton, FL, 2005; pp 39−66.
(11) McLean, J. A.; Ruotolo, B. T.; Gillig, K. J.; Russell, D. H. *Int. J. Mass Spectrom.* **2005**, *240*, 301–315.
(12) McDaniel, E. W.; Mason, E. A. *The Mobility and Diffusion of Ions in Gases*; John Wiley & Sons: New York, 1973; pp 29−84.
(13) Fenn, L. S.; McLean, J. A. *Anal. Bioanal. Chem.* **2008**, *391*, 905–909.
(14) Gies, A. P.; Hercules, D. M. *Macromolecules* **2006**, *39*, 2488–2500.
(15) Gies, A. P.; Hercules, D. M.; Ellison, S. T.; Nonidez, W. K. *Macromolecules* **2006**, *39*, 941–947.

MA8019464