Research Article

Rapid Communications in Mass Spectrometry

Received: 18 December 2010 Revised: 4 February 2011 Accepted: 6 February 2011 Published online in Wiley Online Library

Rapid Commun. Mass Spectrom. **2011**, *25*, 1152–1158
(wileyonlinelibrary.com) DOI: 10.1002/rcm.4974

Matrix-assisted laser desorption/ionization mass spectrometric analysis of aliphatic biodegradable photoluminescent polymers using new ionic liquid matrices

Carlos A. Serrano[1], Yi Zhang[2], Jian Yang[2] and Kevin A. Schug[1]*

[1]Department of Chemistry & Biochemistry, The University of Texas at Arlington, Arlington, TX 76019, USA
[2]Department of Bioengineering, The University of Texas at Arlington, Arlington, TX 76019, USA

In this study, two novel ionic liquid matrices (ILMs), *N,N*-diisopropylethylammonium 3-oxocoumarate and *N,N*-diisopropylethylammonium dihydroxymonooxoacetophenoate, were tested for the structural elucidation of recently developed aliphatic biodegradable polymers by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). The polymers, formed by a condensation reaction of three components, citric acid, octane diol, and an amino acid, are fluorescent, but the exact mechanism behind their luminescent properties has not been fully elucidated. In the original studies, which introduced the polymer class (J. Yang *et al.*, *Proc. Natl. Acad. Sci. USA* 2009, *106*, 10086–10091), a hyper-conjugated cyclic structure was proposed as the source for the photoluminescent behavior. With the use of the two new ILMs, we present evidence that supports the presence of the proposed cyclization product. In addition, the new ILMs, when compared with a previously established ILM, *N,N*-diisopropylethylammonium α-cyano-3-hydroxycinnimate, provided similar signal intensities and maintained similar spectral profiles. This research also established that the new ILMs provided good spot-to-spot reproducibility and high ionization efficiency compared with corresponding crystalline matrix preparations. Many polymer features revealed through the use of the ILMs could not be observed with crystalline matrices. Ultimately, the new ILMs highlighted the composition of the synthetic polymers, as well as the loss of water that was expected for the formation of the proposed cyclic structure on the polymer backbone. Copyright © 2011 John Wiley & Sons, Ltd.

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), in its application for characterizing organic polymers, offers several benefits, including a large spectral window, low incidence of multiply charged states, choice of a wide range of viable matrices, and compatibility with a wide selection of solvents.[1–5] However, a successful analysis relies on the ability of the matrix to mix and co-crystallize with the polymer, as well as facilitate the desorption/ionization process.

Several theories exist on the mechanism of ion generation by MALDI.[6–15] The right choice in matrix is a critical step in garnering a successful analysis. Typically, solutions of the matrices are mixed with solutions of analytes, and the mixture is allowed to crystallize on a MALDI target plate.[16,17] Such a preparation has been shown to be widely amenable to the MS analysis of a variety of small and large, synthetically and biologically derived, molecules.[18–20]

An alternative to traditional crystalline matrices was presented by Armstrong *et al.* in 2001.[21] By coupling α-cyano-4-hydroxycinnamic acid (CHCA) with a variety of nitrogenous bases, a series of room temperature ionic liquids (RTILs), amenable for use as ionic liquid matrices (ILMs), was produced. RTILs are molten salts at room temperature and possess various interesting properties including negligible vapor pressure and versatile solubility.[22,23] ILMs for MALDI-MS can be created by combining the anionic forms of common crystalline matrices with a cation that inhibits crystal formation. ILMs have been noted for diminishing fragmentation of ionized analytes (softer ionization) and imbuing good shot-to-shot and spot-to-spot reproducibility due to their self-healing and solubilizing properties. The sensitivity provided by ILMs has been shown to be equally as good as that observed for traditional crystalline matrices.[23] In general, ILMs have been demonstrated for use in analyzing a wide variety of compounds including lipids, proteins, polymers, and carbohydrates.[24–29]

Biodegradable polymers are an emerging class of materials in the medical and clinical fields.[30–32] For example, innovations based on biodegradable polymers are used as replacement ligaments.[33] These types of polymers also have vast potential for bioimaging, drug delivery, and recreating biological niches, including controlled cellular differentiation, an idea that is becoming increasingly paramount to tissue engineering and regenerative medicine.[34–36]

Developing biodegradable polymers that intrinsically emit *in vivo* detectable fluorescence without incorporating additional (and often, toxic) organic dyestuffs or quantum dots has been a challenge. Recently, a breakthrough was

* *Correspondence to:* K. A. Schug, 700 Planetarium Pl., Box 19065, Arlington, TX 76019-0065, USA.
E-mail: kschug@uta.edu

made in the synthesis of the first aliphatic biodegradable photoluminescent polymers (BPLPs).[37] A series of polymers was created through the condensation of citric acid with 1,8-octanediol, serving as the initial pre-polymer (pre-POC), followed by condensation of the chosen amino acids to make the final product. The molecular weights of the polymers were characterized by MALDI-MS using an ILM, *N,N*-diisopropylethylammonium α-cyanocinnamate. A structural explanation for the luminescent properties was also proposed. The rationale includes a tertiary condensation reaction following addition of the amino acid. The carboxyl group of the amino acid and the hydroxyl moiety of citric acid join to cyclize the amino and carboxy groups of the amino acid with citric acid, as shown in Fig. 1. The result is speculated to be a hyperconjugated six-membered ring, which could support the observed fluorescence. Nuclear magnetic resonance (NMR) data supports this assignment.[37] Based on the choice of the amino acid, and presumably differences in side chain, the excitation and emission wavelengths can be tuned across a wide spectral range, giving this class of BPLPs enormous potential for supporting clinical and medical research. However, further structural analyses on BPLPs are needed to support this proposed fluorescence mechanism.

The aim of this study was to advance the MALDI-MS analysis of a series of BPLPs composed from different amino acids. Formation of the cyclic structure shown in Fig. 1, following addition of the amino acid, would result in the loss of water. In the search for an appropriate matrix which could differentiate the features of the polymers arising from the addition of different amino acids, and their subsequent cyclization, by MALDI-MS, we developed two new ionic liquid matrices, *N,N*-diisopropylethylammonium 3-oxocoumarate and *N,N*-diisopropylethylammonium dihydroxymonooxoacetophenoate. With these matrices, we were able to distinguish the different features of the BPLPs, including evidence for the loss of water. Such a finding provides support for the previously hypothesized fluorescence mechanism in BPLPs. In addition, the new ILMs were integral to revealing these features, providing more detailed spectra than those taken with other crystalline and ILMs.

EXPERIMENTAL

3-Hydroxycoumarin (3-HC) (98%), 2,4,6-trihydroxyacetophenone (THAP) (97%), α-cyano-4-hydroxycinnamic acid (CHCA) (99%), *N,N*-diisopropylethylamine(DIPEA) (>99.5%), and bradykinin acetate (BK) were purchased from Sigma Aldrich (St. Louis, MO, USA) and used without further purification. LC-MS grade methanol and HPLC grade tetrahydrofuran(THF) were from Burdick & Jackson (Muskegon, MI, USA) and Alfa Aesar (Ward Hill, MA, USA), respectively. The synthetic polymers were synthesized as previously reported from starting materials obtained commercially.[38] In this study, pre-POC and BPLP variants incorporating cysteine (Cys), serine (Ser), tyrosine (Tyr), methionine (Met), glycine (Gly), alanine (Ala), leucine (Leu), phenylalanine (Phe), valine (Val), glutamic acid (Glu), aspartic acid (Asp), glutamine (Gln), asparagine (Asn), and tryptophan (Trp) were studied by MALDI-MS. The amino acid content for the BPLP variants was 9.1% relative to pre-POC.

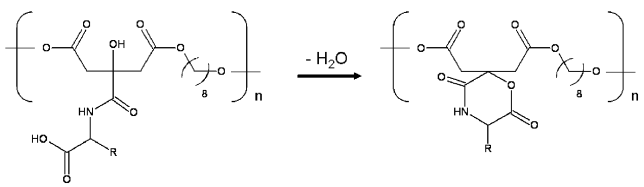

Figure 1. Proposed reaction scheme for formation of A hyperconjugated fluorescent cyclic moiety on the backbone of BPLPs.

Ionic liquids were prepared by combining methanolic solutions of 3-HC (2 g), THAP (2 g) or CHCA (2 g) with DIPEA in methanol (~12 mL) at 1:1 molar ratios. The paired solutions were allowed to mix on a Yamato RE200 rotary evaporator (Santa Clara, CA, USA) for 30 min and subsequently evaporated under reduced pressure at 50°C for 1 h. The dry ILMs were later suspended in methanol (1:2 m/v) and used as working solutions. From this preparation, three ILMs were created: *N,N*-diisopropylethylammonium 3-oxocoumarate (3-HC-DIPEA); *N,N*-diisopropylethylammonium dihydroxymonooxoacetophenoate (THAP-DIPEA); and *N, N*-diisopropylethylammonium α-cyano-3-hydroxycinnamate (CHCA-DIPEA).

Polymer samples were received in chemical grade THF at unknown concentrations and diluted systematically with THF to optimize the MS signal using the CHCA-DIPEA ILM. The same dilutions were used for analysis with the other ILMs. Preparations of BPLPs and ILMs for MALDI-MS analysis were made by mixing 10 μL of the diluted polymer solutions with 60 μL of ILM. Three 1 μL aliquots of each preparation were spotted directly onto an unmasked stainless steel plate and allowed to dry (evaporation of methanol) for approximately 10 min prior to analysis. BK was used as an internal standard for calibration of pre-POC spectra. The pre-POC spectra were later used to calibrate all other spectra by confirming coinciding peaks.

For comparison, analyses of the polymers were also performed with traditional crystalline matrices, THAP, 3-HC, and CHCA. A series of volumetric mixtures was investigated. Sample solutions of 100 μL (10 mg/mL) of each crystalline matrix component in THF were mixed with 1, 5, 10, 15, 20, 30, 40, and 50 μL of each polymer solution to determine conditions for optimal ion response. In all cases, the mixture with 10 μL of polymer solution provided the best signal quality. The data presented were taken from 100 μL of matrix solution mixed with 10 μL of polymer in a microcentrifuge tube, which had been vortex mixed for 15 s. From this solution 2 μL was placed on a MALDI plate and allowed to dry for 3 min prior to analysis.

A Bruker Autoflex I MALDI time-of-flight mass spectrometer (Bruker-Daltonics, Billerica, MA, USA) housing a nitrogen laser (337 nm) was used in the positive ionization reflectron mode. The delayed extraction time was set at 20 ns. Acceleration, lens, and reflector voltages of 19 kV, 8.3 kV and 20 kV were implemented, respectively. The low mass cut-off was placed at *m/z* 400. The detector voltage was set at 1.808 kV. Each spectrum was collected as an average from 200 laser shots.

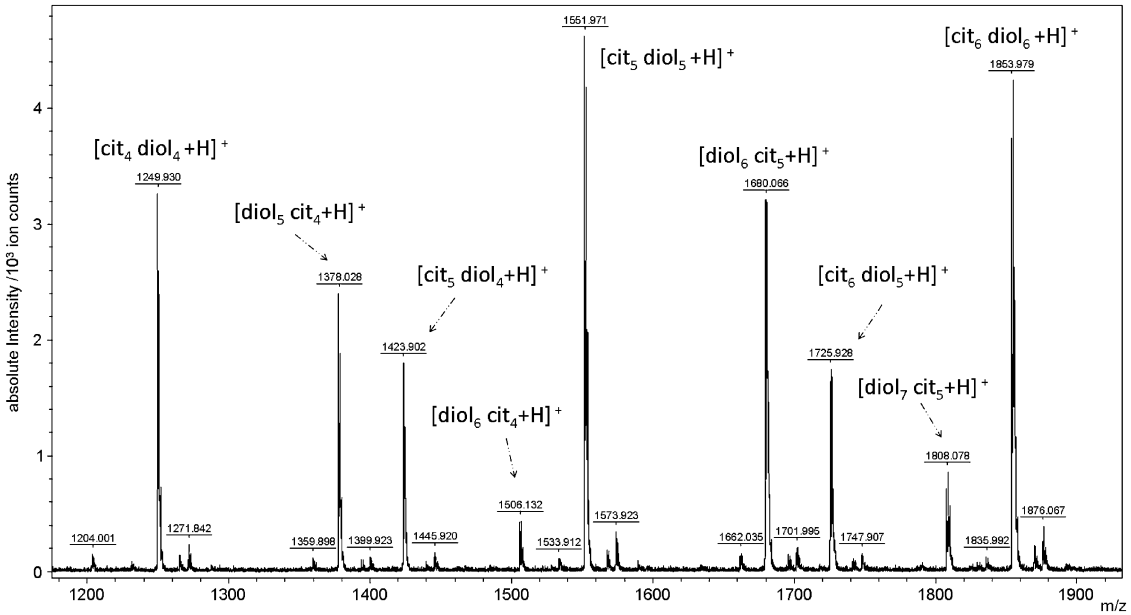

Figure 2. MALDI-MS spectra of pre-POC in 3-HC expanded over the region from *m/z* 1200 to 1900. Major peaks are assigned as units containing varying amounts of citrate and diol.

RESULTS AND DISCUSSION

A portion of a spectrum of pre-POC analyzed with the 3-HC-DIPEA ILM is shown in Fig. 2. This spectrum affords evidence for a citrate and diol poly-condensation reaction. This pattern was seen in the pre-POC samples analyzed in all three ILMs, as well in all the amino acid versions of BPLPs, excluding Lys-BPLP, as discussed later.

BPLP samples in CHCA-DIPEA optimally respond to laser fluencies equal to 30% of the maximum laser power (rating set at 129 μJ). This power setting is insufficient to elicit an appreciable signal for 3-HC-DIPEA and THAP-DIPEA. Therefore, each ILM was evaluated for an optimal setting. For 3-HC-DIPEA and THAP-DIPEA these settings were 50% and 60% attenuation, respectively, based on the resolution of isotopic peaks and signal intensity. Each ILM gradually produced an increasing ion current with an increase in percent attenuation of the laser power, and then declined after reaching a maximum. A maximum was reached at approximately 62% maximum laser power for 3-HC-DIPEA, 74% for THAP-DIPEA, and 47% for CHCA-DIPEA.

A comparison of the three ILMs was performed with the biopolymer Cys-BPLP as a representative analyte (Fig. 3). The performance of 3-HC-DIPEA and THAP-DIPEA was comparable with that of CHCA-DIPEA. The polymer characterization of Cys-BPLP yielded similar results for all three ILMs (Table 1). The extent to which high mass values appeared in each spectrum was also similar although, in this regard, 3-HC-DIPEA consistently revealed more lower-intensity high-mass signals than THAP-DIPEA throughout this study.

When the 3-HC-DIPEA and THAP-DIPEA were compared with the corresponding crystalline matrix preparations (HC and THAP, respectively) the signals obtained with the ILMs were more intense and a more significant distribution of masses, especially in the higher mass range, was visible (Fig. 4). While this difference may be attributable to different optimal matrix-to-sample ratios used for the crystalline matrix versus the ILMs, a range of matrix-to-sample ratios for the crystalline matrix preparation was surveyed, and the best results obtained are presented. In addition, in order to increase the data quality for the spectra captured following crystalline matrix preparation, the laser was steadily rastered across the spot, to minimize the exhaustion of the matrix at one spot. Rastering was not necessary to obtain high-quality data for the ILM preparations. In some cases, spectral features attributed to the incorporation of amino acids in the polymers could be seen with the use of crystalline matrices (data not shown). However, these signals had a very low signal-to-noise ratio. This was not the case for samples analyzed in conjunction with the ILMs, where clear evidence for the incorporation of amino acids could be seen in 15 of the 16 BPLP sample analyzed. The exception was Lys-BPLP, which did produce a robust spectral profile and distribution of peaks, but signals for pre-POC did not align with the previous standardized spectra. This is probably attributable to the contribution of the side-chain amine in Lys, which could extend and complicate the variation of chain structures accessible during the polymerization process.

The different varieties of BPLP could be distinguished by the amino acid incorporated into each synthesis with the use of both 3-HC-DIPEA (Fig. 5) and THAP-DIPEA (Fig. 6). Figure 5 shows that an amino acid is probably present in the BPLP and, secondly, that an additional loss of water occurs concurrently with the addition of that amino acid. For several of the BPLPs, the signal associated with loss of water is observed at higher intensity than that for the hydrated amino acid residue. This is most noticeable in the spectra of Trp-BPLP (Fig. 5(D)) and Tyr-BPLP (Fig. 5(E)), taken in 3-HC-DIPEA. The application of THAP-DIPEA to the BPLP family demonstrated similar results making

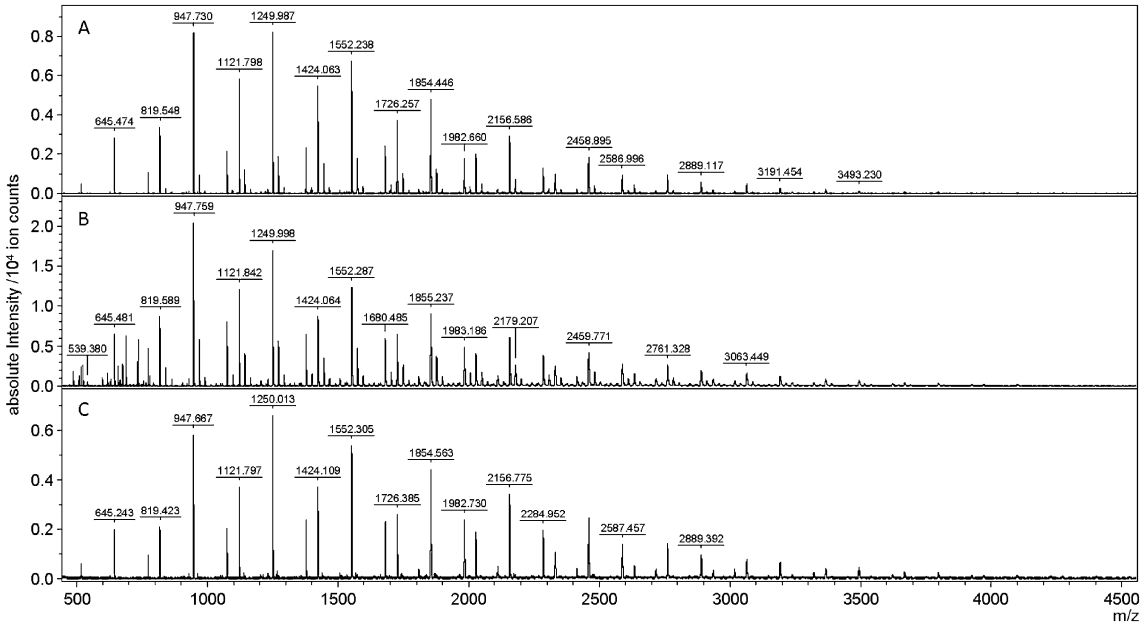

Figure 3. MALDI-MS spectra of Cys-BPLP taken in ILMs (A) CHCA-DIPEA, (B) 3-HC-DIPEA, and (C) THAP-DIPEA.

Table 1. Number average molecular weights (M_n), weight average molecular weights (M_w), and polydispersities (M_w/M_n) of pre-POC from analyses using three ILMs

	M_n	M_w	PD
3-HC-DIPEA	1510	1710	1.13
THAP-DIPEA	1710	2030	1.19
CHCA- DIPEA	1460	1670	1.14

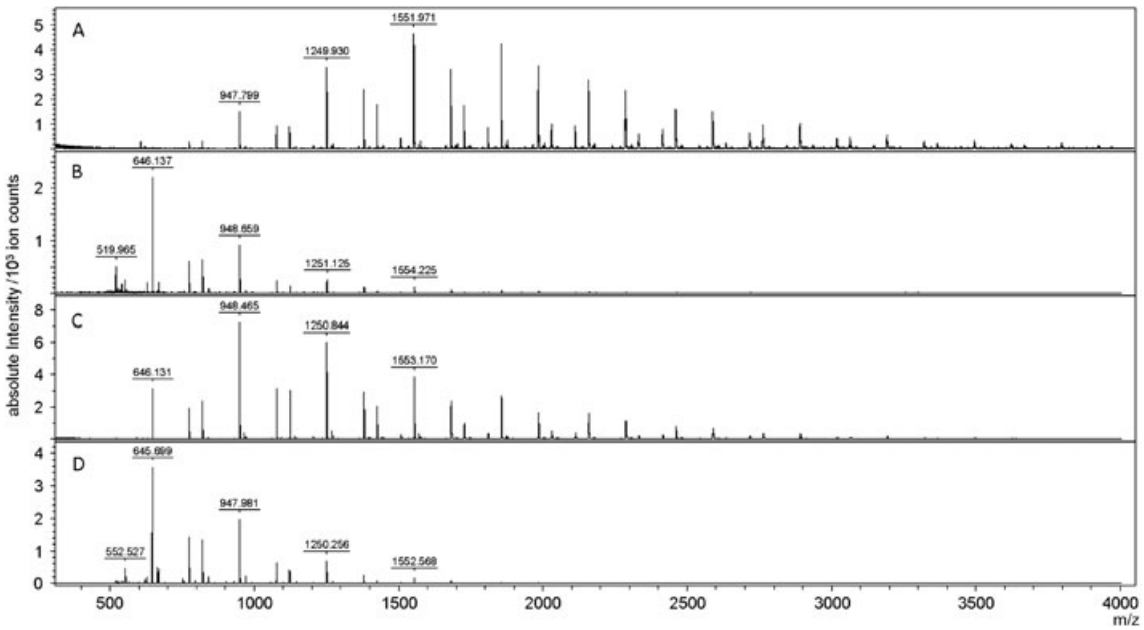

Figure 4. MALDI-MS spectra of pre-POC taken in (A) 3-HC-DIPEA, (B) a dry preparation of 3-HC, (C) THAP-DIPEA, and (D) a dry preparation of THAP.

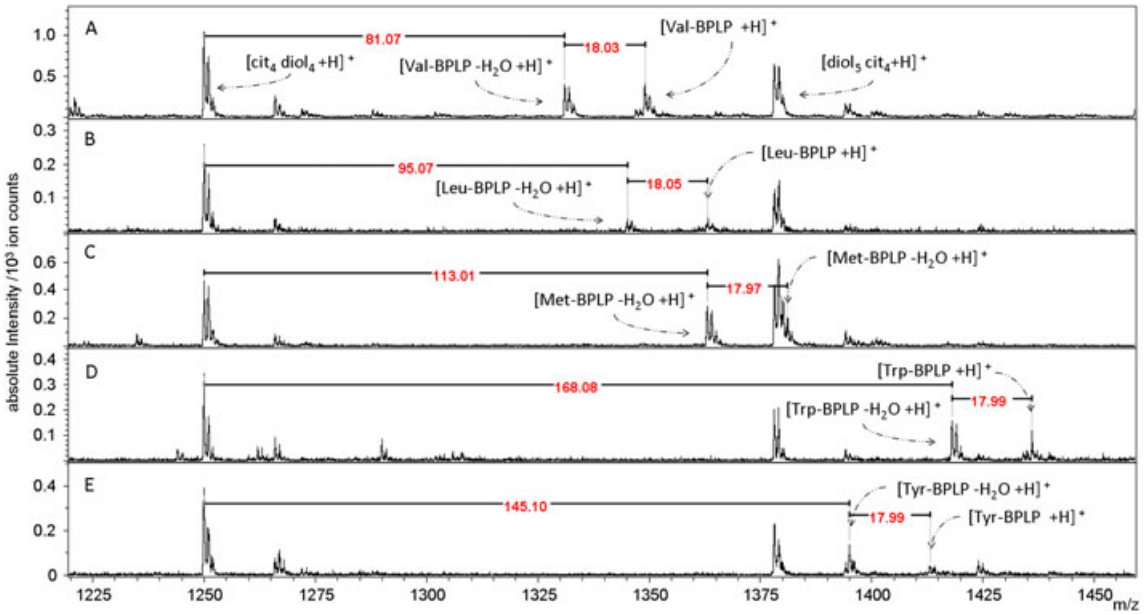

Figure 5. MALDI-MS spectra, expanded over the region from *m/z* 1225 to 1450, of BPLPs incorporating (A) valine, (B) leucine, (C) methionine, (D) tryptophan, and (E) tyrosine, collected using the ILM 3-HC-DIPEA.

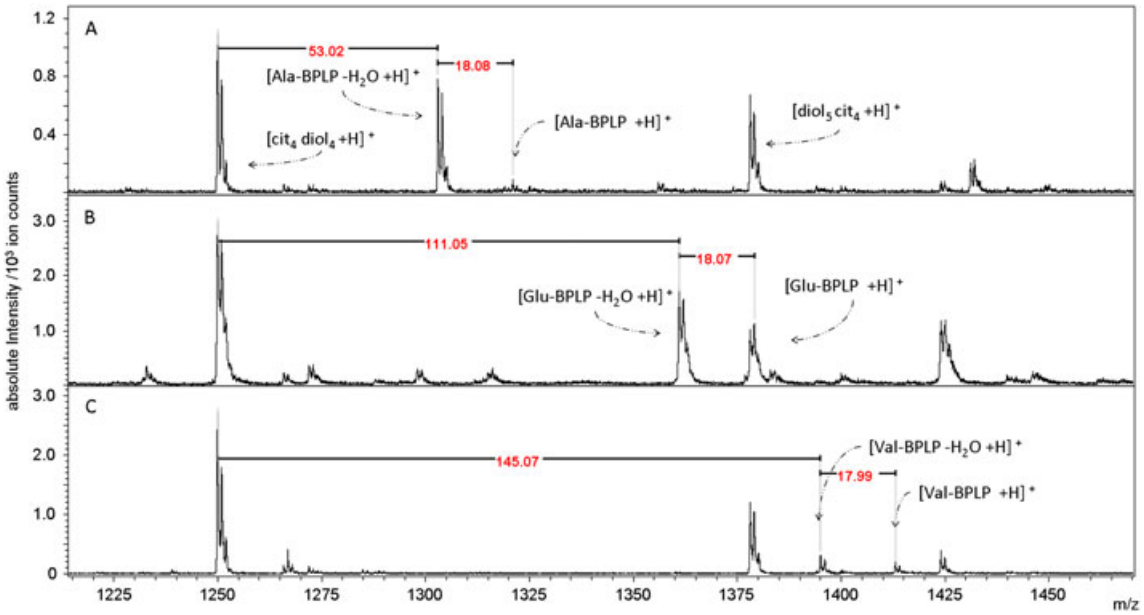

Figure 6. MALDI-MS spectra, expanded over the region from *m/z* 1225 to 1450, of BPLPs incorporating (A) alanine, (B) glutamic acid, and (C) tyrosine. collected using the ILM THAP-DIPEA.

it possible to distinguish the members of the BPLPs by the amino acid incorporated during synthesis (Fig. 6). A similar incorporation of amino acid is observed, along with a loss of water; however, some signal intensities were diminished relative to that obtained for 3-HC-DIPEA. An exception is seen for Glu-BPLP (Fig. 6(B)), where an overlap of Glu-BPLP signals was observed with pre-POC signals (signal envelope at *m/z* 1378–1380). Other BPLP variants, not shown, also demonstrated the presence of an amino acid as a part of the polymer molecule when both 3-HC-DIPEA and THAP-DIPEA ILMs were used.

While a loss in water, as shown in these spectra, is not definitive evidence, these findings do not contradict the proposal for a condensed six-membered ring formed between the residual hydroxyl group on citrate and the free carboxylic acid group on the amino acid during synthesis, as shown in Fig. 1.[37] Addition of the amino acid to terminal groups on the citrate during synthesis may provide some ambiguities, and maybe the reason why only some of the amino acids cyclize. This evidence is one more step in understanding the interesting chemistry which produces the extraordinary fluorescent properties of the aliphatic BPLP polymer class.

CONCLUSIONS

This study introduced two new ILMs, 3-HC DIPEA and THAP-DIPEA, as viable alternatives for characterizing fluorescent aliphatic BPLPs. Their performance was comparable with that of the CHCA-DIPEA ILM, reported previously, and they may find additional uses in future applications. In all cases, the ILMs showed enhanced spectral intensity and characterization data quality compared with crystalline matrix preparations. In addition, evidence was outlined to support the potential for a cyclization product involving the citrate backbone and amino acid terminus to yield the structure put forward by the original authors as the source of fluorescence in this polymer class.

Acknowledgements

CAS and KAS acknowledge support from Eli Lilly and Company and the University of Texas at Arlington. JY and YZ acknowledge support from the National Science Foundation (0954109) and the National Institutes of Biomedical Imaging and Engineering (EB009795).

REFERENCES

[1] G. Montaudo, F. Samperi, M. Montaudo. Characterization of synthetic polymers by MALDI-MS. *Prog. Polym. Sci.* **2006**, *31*, 277.
[2] S. Wetxel, C. Guttman, J. Girard. The influence of matrix and laser energy on the molecular mass distribution of synthetic polymers obtained by MALDI-TOF-MS. *Int. J. Mass Spectrom.* **2004**, *238*, 215.
[3] R. Murgasoca, D. M. Hercules. MALDI of synthetic polymers – an update. *Int. J. MassSpectrom.* **2003**, *226*, 151.
[4] K. Wu, R. Odom. Characterizing synthetic polymers by MALDI-MS. *Anal. Chem.* **1998**, *70*, 456.
[5] H. Pash, W. Schrepp. *MALDI-TOF Mass Spectrometry of Synthetic Polymers*, Springer-Verlag, Heidelberg, **2003**.
[6] M. Karas, U. Bahr, I. Fourrnier, M. Gluckmann, A. Pfenninger. The initial-ion velocity as a marker for different desorption-ionization mechanisms in MALDI. *Int. J. Mass Spectrom.* **2003**, *226*, 239.
[7] T. W. Jaskolla, M. Karas. Using fluorescence dyes as a tool for analyzing the MALDI process. *J. Am. Soc. Mass Spectrom.* **2005**, *109*, 4030.
[8] R. Knochenmuss, R. Zenobi. MALDI ionization: the role of in-plume processes. *Chem. Rev.* **2003**, *103*, 441.
[9] R. Knochenmuss, A. Vertes. Time-delayed 2-pulse studies of MALDI matrix ionization mechanisms. *J. Phys. Chem. B* **2000**, *104*, 5406.
[10] A. Hoteling, W. Nichols, D. Giesen, J. Lenhard, R. Knochenmuss. Electron transfer reaction in laser desorption/ionization and matrix-assisted laser desorption/ionization: factors influencing matrix and analyte ion intensities. *Eur. J. Mass Spectrom.* **2006**, *12*, 345.
[11] S. Bourcier, S. Bouchonnet, Y. Hoppiliard. Ionization of 2,5-dihydroxybenzoic acid (dhb) matrix-assisted laser desorption ionization experiments and theoretical study. *Int. J. Mass Spectrom.* **2001**, *210/211*, 59.
[12] A. A. Puretzky, D. B. Geohegan, G. B. Hurtst, M. V. Buchanan. Imaging of vapor plumes produced by matrix assisted laser desorption: a plume sharpening effect. *Phys. Rev. Lett.* **1999**, *83*, 444.
[13] I. Fournier, A. Brunot, J. C. Tabet, G. Bolbach. Delayed extraction experiments using a repulsive potential before ion extraction: evidence of clusters as ion precursors in UV-MALDI. Part I: Dynamical effects with the matrix 2,5-dihydroxybenzoic acid. *Int. J. Mass Spectrom.* **2002**, *213*, 203.
[14] J. H. Gross. Molecular ions of liquids in the gas phase. *J. Am. Soc. Mass Spectrom.* **2008**, *19*, 1347.
[15] K. Dreisewerd. The desorption process in MALDI. *Chem. Rev.* **2003**, *103*, 395.
[16] T. Jaskolla, M. Karas, U. Roth, S. Kerstin, C. Menzel, K. Reihs. Comparison between vacuum sublimed matrices and conventional dried droplet preparation in MALDI-TOF mass spectrometry. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 1104.
[17] S. Sauer. The essence of DNA sample preparation for MALDI mass spectrometry. *J. Biochem. Biophys. Methods* **2007**, *70*, 311.
[18] S. Trimpin, S. Keune, K. Mullen, H. J. Rader. Solvent-free MALDI-MS: development improvements in the reliability and the potential of MALDI in the analysis of synthetic polymer and giant organic molecules. *J. Am. Soc. Mass Spectrom.* **2006**, *17*, 661.
[19] Z. Zhang, L. Zhou, S. Zhao, H. Deng. 3-Hydroxycoumarin as a new matrix for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of DNA. *J. Am. Soc. Mass Spectrom.* **2006**, *17*, 1665.
[20] P. Sporns, J. Wang. Exploring new frontiers in food analysis using MALDI-MS. *Food Res. Int.* **1999**, *31*, 181.
[21] D. W. Armstrong, L. Zhang, L. He, M. L. Gross. Ionic liquids as matrixes for matrix-assisted laser desorption/ionization mass spectrometry. *Anal. Chem.* **2001**, *73*, 3679.
[22] D. W. Armstrong, P. Sun. Ionic liquids in analytical chemistry. *Anal. Chim. Acta* **2010**, *661*, 1.
[23] A. Tholey, E. Heinzle. Ionic (liquid) matrices for matrix-assisted laser desorption/ionization mass spectrometry–applications and perspectives. *Anal. Bioanal. Chem.* **2006**, *386*, 24.
[24] Y. Li, M. Gross. Ionic-liquid matrices for improved analysis of phospholipids by MALDI-TOF mass spectrometry. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 679.
[25] K. Chan, P. Lanthier, X. Liu, J. Sandhu, D. Stanimirovic, J. Li. MALDI mass spectrometry imaging of gangliosides in mouse brain using ionic liquid matrix. *Anal. Chim. Acta* **2009**, *639*, 57.
[26] R. Ullmer, A. M. Rizzi. Use of a novel ionic liquid matrix for MALDI-MS analysis of glycopeptides and glycans out of total tryptic digests. *J. Mass Spectrom.* **2009**, *44*, 1596.
[27] A. Berthod, J. A. Crank, K. L. Rundlett, D. W. Armstrong. A second-generation ionic liquid matrix-assisted desorption/ionization matrix for effective mass spectrometric analysis of biodegradable polymers. *Rapid Commun. Mass Spectrom.* **2009**, *23*, 3409.
[28] C. Przybylski, F. Gonnet, D. Bonnaffe, Y. Hersant, H. Lortat-Jacob, R. Danial. HABA-based ionic liquid matrices for UV-MALDI-MS analysis of heparin and heparin sulfate oligosaccharides. *Glycobiol.* **2010**, *20*, 224.
[29] T. N. Laremore, F. Zhang, R. J. Linhardt. Ionic liquid matrix for direct UV-MALDI-TOF-MS analysis of dermatan sulfate and chondroitin sulfate oligosaccharides. *Anal. Chem.* **2007**, *79*, 1604.
[30] L. Nair, C. Laurencin. Biodegradable polymers as biomaterials. *Prog. Polym. Sci.* **2007**, *32*, 762.
[31] J. Kim, K. Park, H. Nam, S. Lee, K. Kim, I. Kwon. Polymers for bioimaging. *Prog. Polym. Sci.* **2007**, *32*, 1031.
[32] G. Zhang, L. Suggs. Matrices and scaffolds for drug delivery in vascular tissue engineering. *Adv. Drug Deliv. Rev.* **2007**, *59*, 360.
[33] J. Cooper, J. Sahota, W. Gorum, J. Carter, S. Doty, C. Laurencin. Biomimetic tissue-engineered anterior cruciate ligament replacement. *Proc. Nat. Acad. Sci. USA* **2007**, *104*, 3049.

[34] E. Yim, J. Wen, K. Leong. Enhanced extracellular matrix production and differentiation of human embryonic germ cell derivatives in biodegradable poly-(caprolactone-co-ethyl phosphate) scaffold. *Acta Biomaterialia* **2006**, *2*, 365.
[35] R. Sands, D. Mooney. Polymer to direct cell fate by controlling the microenvironment. *Curr. Opin. Biotechnol.* **2007**, *18*, 448.
[36] C. Nuttelman, M. Rice, A. Rydholm, C. Salinas, D. Shah, K. Anseth. Macromolecular monomers for the synthesis of hydrogel niches and their application in cell encapsulation and tissue engineering. *Prog. Polym. Sci.* **2008**, *33*, 167.
[37] J. Yang, Y. Zhang, S. Gautam, L. Liu, J. Dey, W. Chen, R. P. Mason, C. A. Serrano, K. A. Schug, L. Tang. Development of aliphatic biodegradable photoluminescent polymers. *Proc. Nat. Acad. Sci. USA* **2009**, *106*, 10086.
[38] J. Yang, A. Webb, S. Pickerill, G. Hageman, G. Ameer. Synthesis and evaluation of poly(diol citrate) biodegradable elastomers. *Biomaterials* **2006**, *27*, 1889.

