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Infrared Atmospheric Pressure MALDI Ion Trap Mass Spectrometry of Frozen Samples Using a Peltier-Cooled Sample Stage

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Infrared atmospheric pressure matrix-assisted laser desorption/ionization on an ion trap mass spectrometer is used to analyze frozen samples generated using a Peltier-cooled sample stage. This allows for the analysis of samples in water without the addition of matrix, in near-native conditions, and with minimal loss of water due to evaporation. Analysis of frozen samples is extended to study peptides, carbohydrates, and glycolipids.

The matrix in matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) serves to absorb energy from the laser to promote ionization/desorption. Addition of the matrix, though, may alter the native environment of the analyte. Utilizing the energy-absorbing properties of water, a solvent inherent in most biologically relevant samples, would eliminate the need to add matrix, and it would increase the automation of atmospheric pressure (AP)-MALDI, making the technique more rapid and versatile.[1]

AP-MALDI is emerging as a preferred alternative to vacuum MALDI for the mass spectrometric analysis of compounds containing labile bonds. AP-MALDI has recently been coupled to ion trap mass spectrometers (ITMS) resulting in a combination of soft ionization, resulting from collisions with ambient gases, with the MS/MS capability of the ITMS.[2−8] Infrared (IR) AP-MALDI is an extension of this technique that capitalizes on water and glycerol as a matrix. The IR AP-MALDI process generates minimal fragmentation, as demonstrated in the analysis of sialylated carbohydrates, and is thus one of the softest ionization methods available.[9,10] The soft nature of IR AP-MALDI further affords the ability to study weak noncovalent complexes.[11,12] A practical advantage of the technique is the relative simplicity of sample preparation, as aqueous samples can be analyzed directly using infrared laser irradiation.[9−13] Since the H_2O solvent readily absorbs IR energy by excitation of the O−H bond, no separate energy-absorbing matrix is required for analyte ionization, and it is not necessary to dry the sample for co-crystallization or to ensure compatibility with a vacuum environment. This is particularly advantageous in the case of biological samples, for which analytes can be studied quickly under nearly physiological conditions.

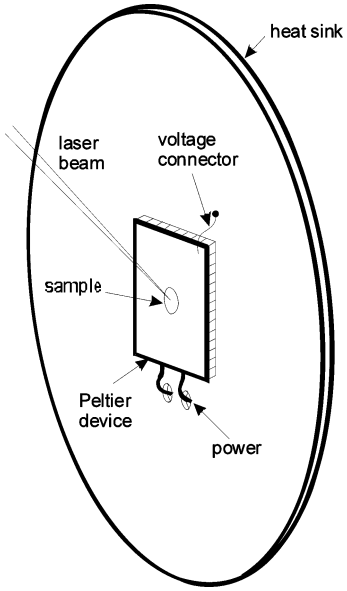

Figure 1. Schematic of Peltier-cooled sample stage.

A drawback in this type of analysis is the fact that water as a solvent is rapidly depleted due to evaporation as a result of IR irradiation, limiting the time over which the sample can be analyzed. Freezing can extend the sample lifetime, and the mass spectrometric analysis of frozen samples has been previously

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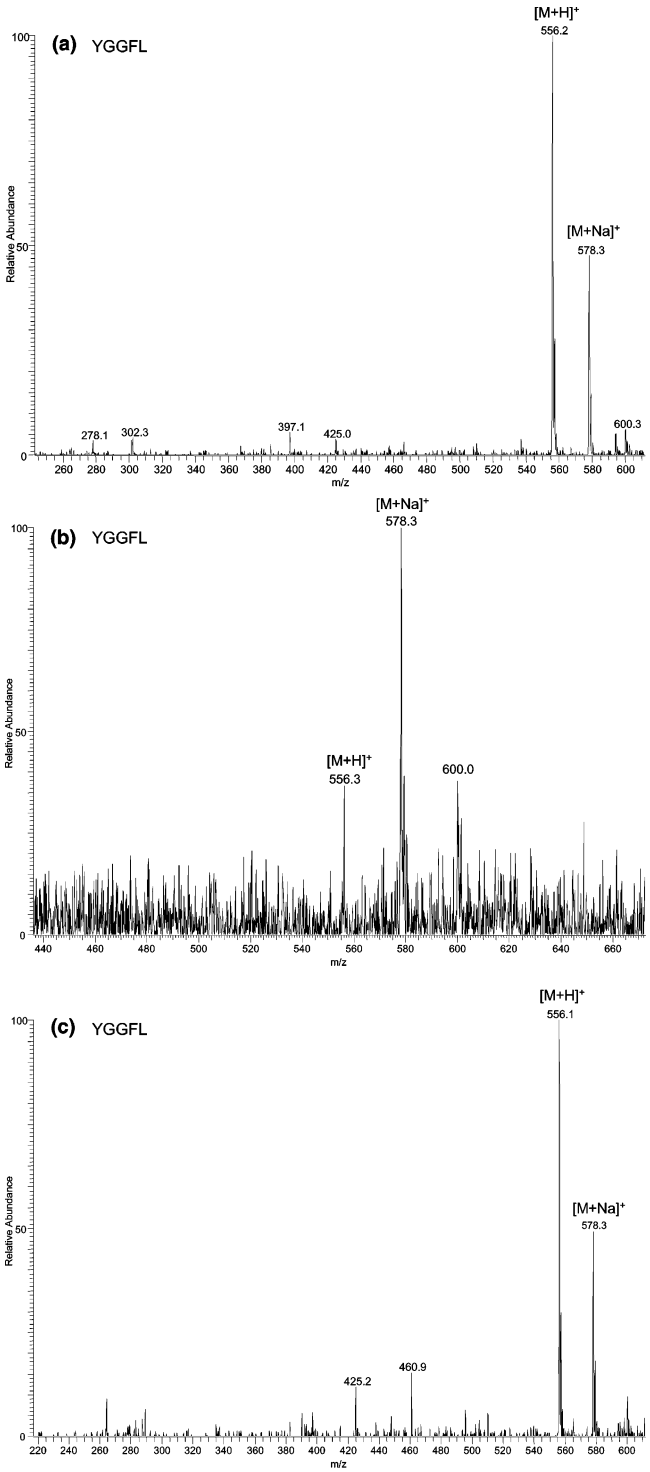

Figure 2. Positive ion mode analysis of YGGFL (m/w 555): (a) 1 nmol, (b) 1 pmol, and (c) 1 nmol with a 0-kV potential applied between the sample stage and the inlet capillary.

demonstrated.[14−22] Initial work utilized lasers with wavelengths in the visible region for the analysis of frozen thin-film DNA solutions.[14,15] This work was subsequently extended to peptide and protein analyses utilizing infrared lasers.[16,17] Ultraviolet irradiation has also been shown capable of generating ions from frozen samples, but this requires the addition of an organic solvent or other photoabsorbing compound prior to freezing.[18,19]

The processes used thus far to prepare frozen samples and to maintain their frozen state in the mass spectrometer have limited the applicability of this approach. Frozen samples can be produced

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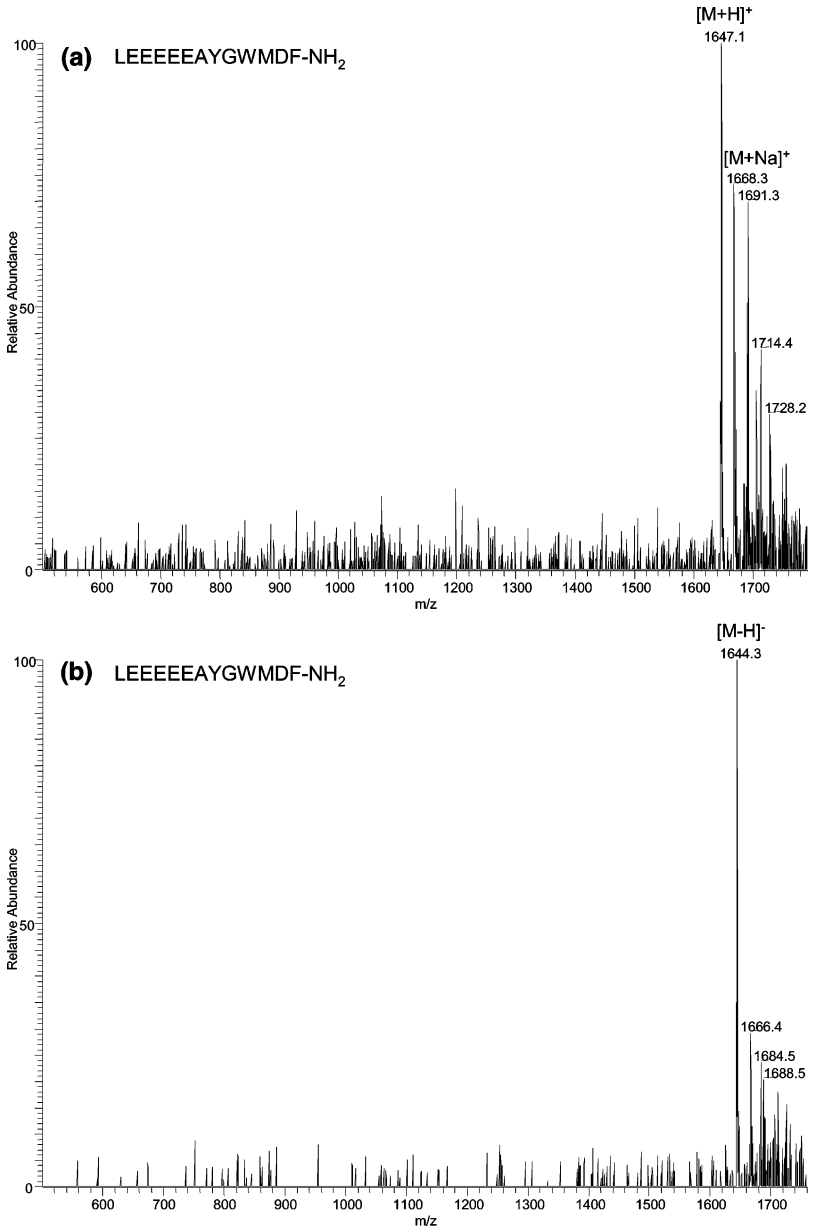

Figure 3. Mass spectrometric analysis of 1 nmol mini gastrin (m/w 1645) (a) in positive ion mode and (b) in negative ion mode.

by dipping samples in liquid nitrogen until frozen and subsequently placed in the mass spectrometer. This can result in sample loss or contamination, and the time over which the sample remains frozen after it is placed in the mass spectrometer may not be long enough to carry out the analysis. Frozen sample stages have been developed using circulating liquid nitrogen coolant, which maintains the frozen sample state in the instrument.[19−21] More recently, Naito and co-workers have incorporated a thermoelectric cooling stage for use with an FT-ICR instrument, providing a simple apparatus with controllable cooling capacity.[22]

Here, IR AP-MALDI is performed using a small Peltier-cooled stage to achieve the analysis of frozen samples in a quadrupole ion trap mass spectrometer. The IR laser desorbs the frozen sample in a time-controlled manner, resulting in an increase in sample lifetime. The effectiveness of the method is demonstrated in the analysis of peptides, carbohydrates, and glycolipids.

EXPERIMENTAL SECTION

Materials. Leucine enkephalin and mini gastrin I were purchased from Sigma (St. Louis, MO). 2′-Fucosyllactose was obtained from Glycotech Corp. (Rockville, MD). 3′-Sialyllactose and 6′-sialyllactose were acquired from V-Labs Inc. (Covington, LA) LS-tetrasaccharide b was purchased from Dextra Laboratories (Reading, U.K.). Ganglioside GM_1 was acquired from Calbiochem (San Diego, CA). All samples were dissolved to 1 mM concentration in 18 MΩ MilliPure water, unless otherwise stated.

Infrared AP-MALDI Ion Trap Mass Spectrometry. Analyses were performed on a ThermoFinnigan (San Jose, CA) LCQ classic quadrupole ion trap mass spectrometer equipped with a modified Mass Technologies Inc. (Columbia, MD) atmospheric

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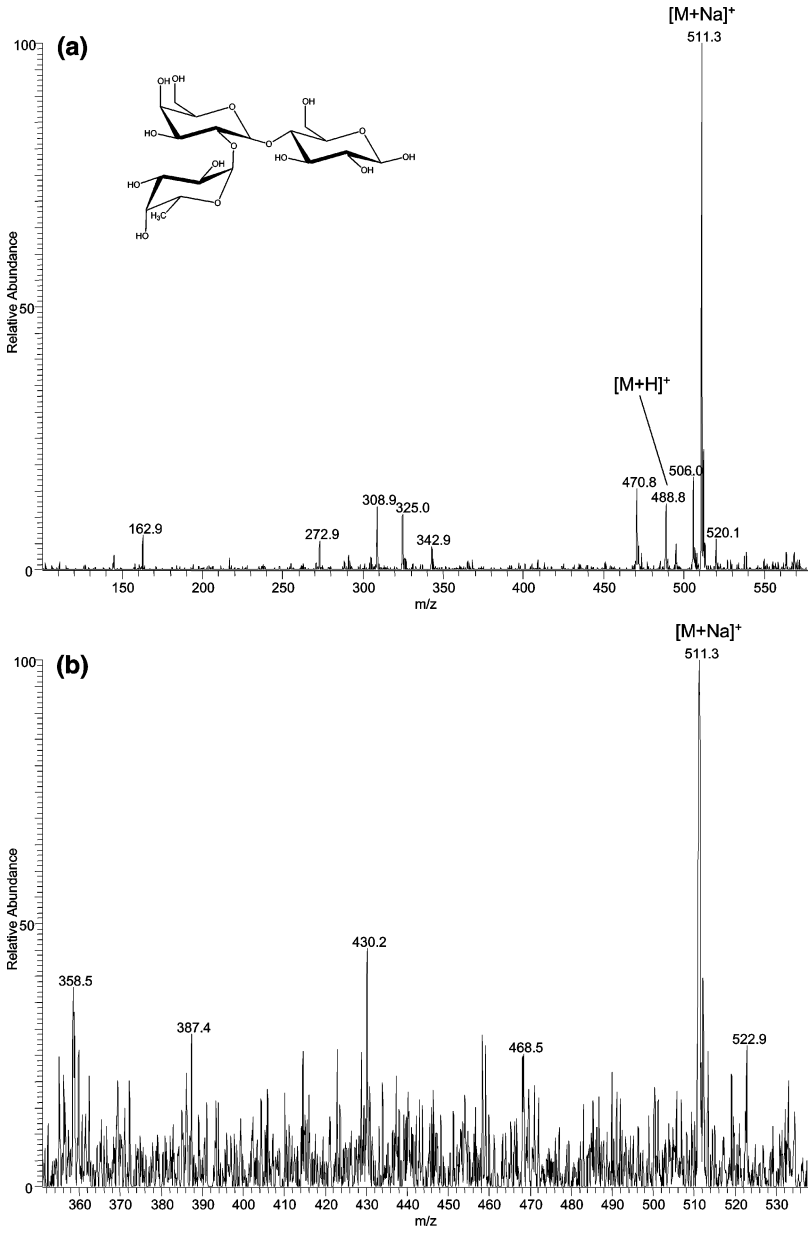

Figure 4. Positive ion mode analysis of 2′-fucosyllactose (m/w 489): (a) 1 nmol; (b) 10 pmol.

pressure MALDI source. This system was equipped with a Bioptic Lasersysteme (Berlin, Germany) Bioscope UV+ laser system containing an Er:YAG laser (2940 nm). The laser beam was focused using a sapphire lens ($F = 100$ mm). The Er:YAG laser generated ~100-ns pulses at 5 Hz with ~400 μJ/pulse. The laser was run asynchronously with the mass spectrometer trapping cycle, and spectra were acquired at 300 ms/scan in positive mode and 400 ms/scan in negative mode. A potential of 2.5 kV was applied between the sample plate and the inlet capillary. The capillary temperature was set to 200 °C.

Peltier Device. The sample stage consisted of a 0.015-in.-thick stainless steel sheet adhered to a 14.6 × 14.6 mm TECA Corp. (Chicago, IL) ceramic thermoelectric (Peltier) cooling plate using a ITW Chemtronics (Kennesaw, GA) thermally conductive epoxy. The sample stage geometry was orthogonal to the inlet capillary and separated by a distance of ~3 mm. This geometry was maintained as compared to previous IR AP-MALDI experiments.[9−12] The heating side of the thermoelectric device was adhered in the same manner to a 4-in.-diameter, 0.2-in.-thick grooved aluminum disk that served as a heat sink (Figure 1). This heat sink assured proper dispersion of heat, allowing temperatures to be maintained below the freezing point of all analyte solutions tested. Without this heat sink, the frozen sample could not be maintained during laser excitation. The thermoelectric device was electrically connected to the heat sink and reference voltage using a small wire lead. The entire assembly could be electrically floated to 2.5 kV for optimum ion focusing into the mass spectrometer. The 1-μL samples were spotted directly on the stainless steel plate at room temperature. Once the thermoelectric device was turned on, the samples froze in ~20 s and remained frozen at temperatures of ~−10 °C over the duration of the analyses. A minor frost coating appeared following several minutes of Peltier use with no detrimental effect to the sample or ion signal intensity. While localized melting could be observed on the sample at the point of

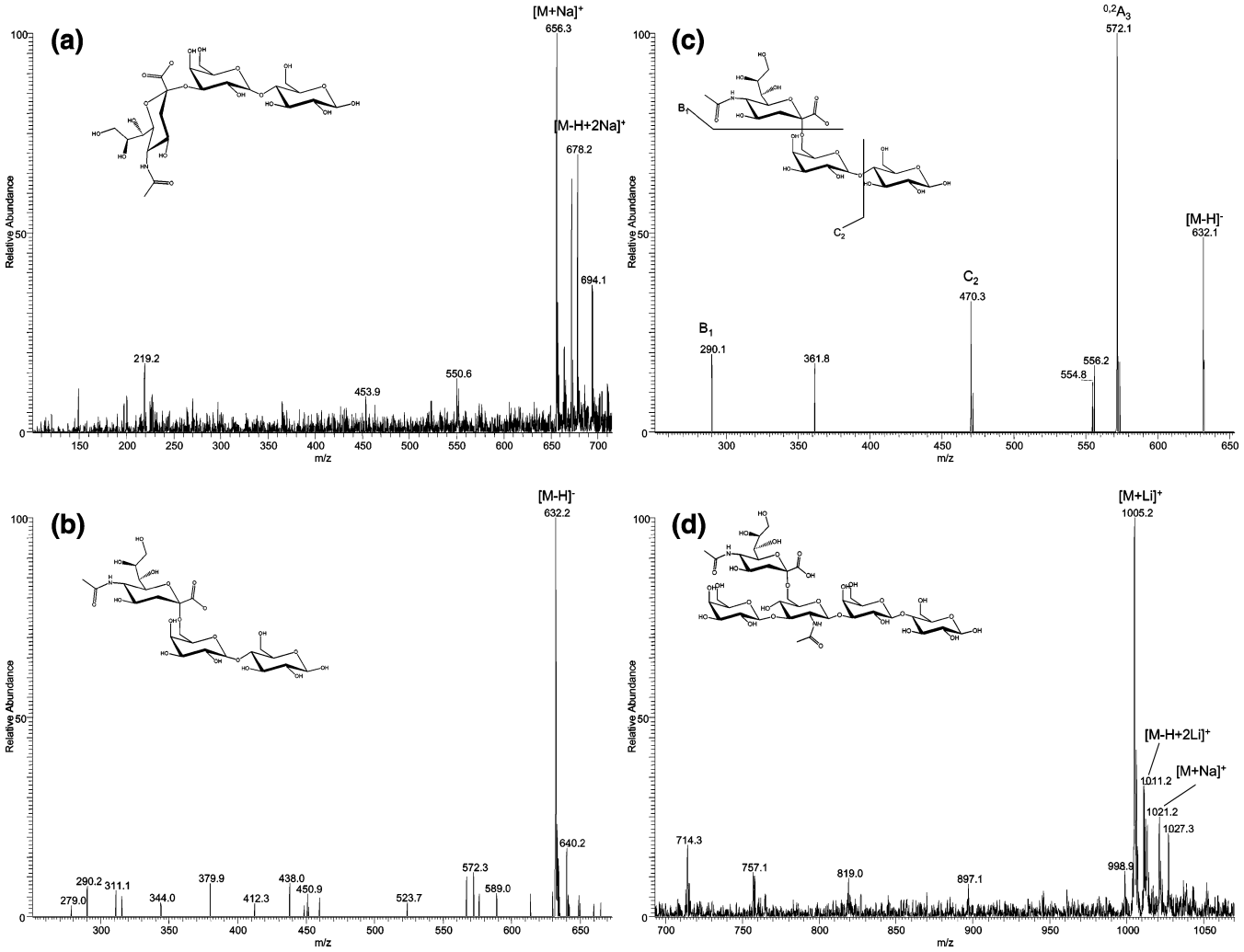

Figure 5. IR AP-MALDI of (a) 3′-sialyllactose in positive ion mode, (b) 6′-sialyllactose in negative ion mode, (c) MS/MS of 6′-sialyllactose (*m*/*z* 632), and (d) lithium-doped LS-tetrasaccharide b in positive ion mode.

laser interaction, no sample-wide melting was observed, and all local melted spots quickly refroze once the laser beam was moved to another part of the sample.

RESULTS AND DISCUSSION

Analysis of biological samples began with the study of small peptides. Figure 2 is a mass spectrum of leucine enkephalin ionized from ice with no additional matrix added. Strong signal was observed for 1 nmol of sample (Figure 2a). Signal could be maintained for as little as 1 pmol of total sample (Figure 2b). Both spectra show a strong sodiated peak at m/z 578, as well as the predominant $[M + H]^+$ peak at m/z 556. Figure 2c shows the mass spectrum of leucine enkephalin with a 0-kV potential applied between the sample plate and inlet capillary. The rationale for this test is that we anticipate that the IR AP-MALDI technique might be applied to the analysis "in the field" of samples in ice and in situ. In such an environment, there would not be an electrically floatable sample stage. The ions rely on air flow to be guided into the capillary. Little decrease in signal results from removal of the electric potential. Further, analysis from ice produced spectra with moderate shot-to-shot variation. Although ion signal intensities were not as repeatable as with liquid IR AP-MALDI, the averaging of spectra resulted in sample-to-sample repeatability.

While one potential advantage of utilizing ice as a matrix is to eliminate matrix ion interference for small analytes, larger peptides were also tested to determine the m/z range of the analytical technique. Figure 3 shows the results for mini gastrin I in both positive and negative ion modes. Major peaks result from protonation and single and multiple sodium adductions in positive ion mode (Figure 3a). The negative ion mode spectrum shows a strong $[M - H]^-$ peak, as well as sodiated adducts, which are common in IR AP-MALDI (Figure 3b). This example demonstrates that peptide analysis directly from ice is not limited to positive ion mode spectra, as strong signal is seen in both positive and negative ion modes.

IR AP-MALDI is also becoming increasingly common in the study of oligosaccharides.[10−11] Figure 4 shows the positive mode ionization of 2′-fucosyllactose (2′FL) frozen in ice. Strong signal is observed for 1 nmol of sample applied to the sample plate (Figure 4a). Significant signal strength remains for as little as 10 pmol of sample spotted (Figure 4b). Both spectra show a strong signal for the $[M + Na]^+$ peak, which is often the strongest signal for neutral oligosaccharides. 2′FL was also analyzed with a 0-kV potential across the sample plate, resulting in minimal loss of signal intensity (data not shown).

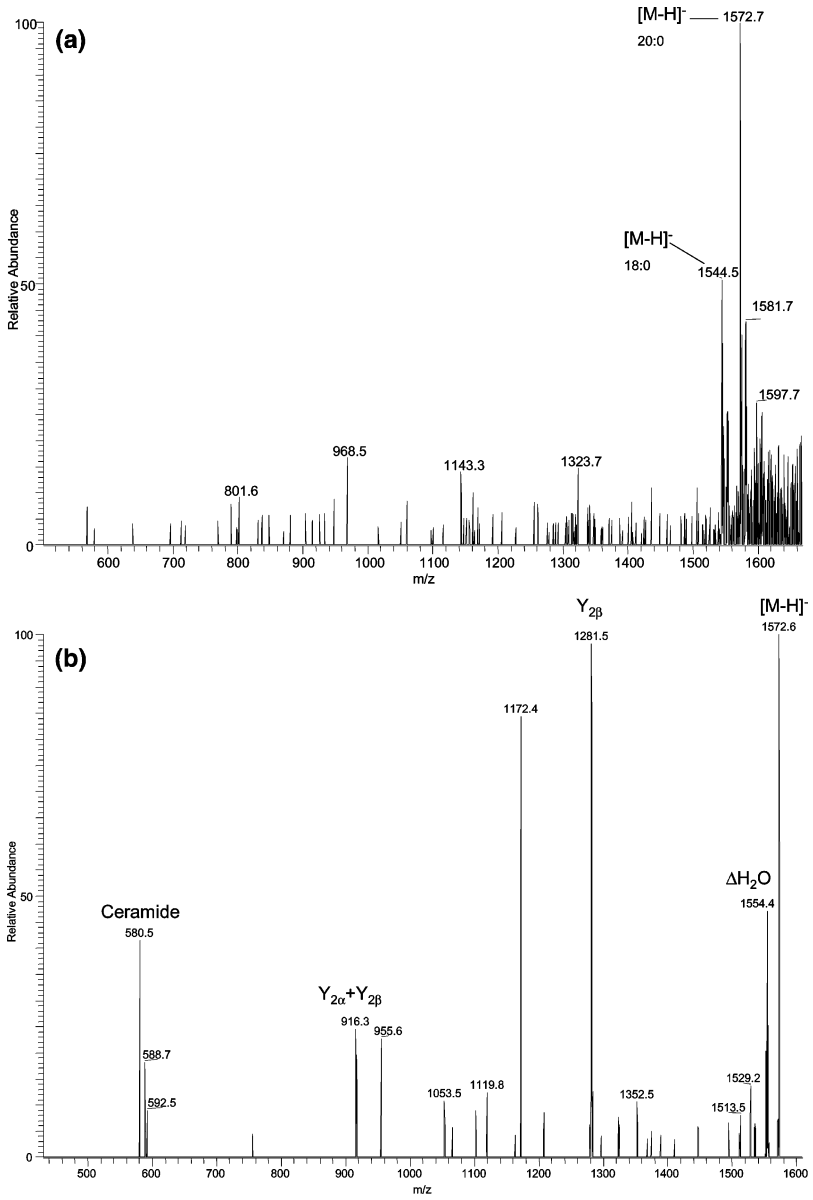

Figure 6. Negative ion mode analysis of (a) 300 pmol GM_1 and (b) MS/MS of 300 pmol of GM_1.

IR AP-MALDI has shown promising results as a soft ionization tool for the study of oligosaccharides containing sialic acid. Figure 5 illustrates the effectiveness of freezing samples for the analysis of 3-sialic acid containing oligosaccharides 3′-sialyllactose and 6′-sialyllactose and LS-tetrasaccharide b (LST b). The positive ion mode analysis of 3′-sialyllactose shows both singly and doubly sodiated peaks at m/z 656.3 and 678.2 (Figure 5a). Negative ion mode analysis of sialylated oligosaccharides is also possible (Figure 5b). Negative ion mode analysis of 6′-sialyllactose generates enough signal to also perform MS/MS analysis (Figure 5c). This step is crucial for the structural determination of oligosaccharides, due to the mass redundancy of many common carbohydrate structures. Cross-ring fragments result from the MS/MS analysis ($^{0,2}A_3$ at m/z 572.1), as well as cleavages at both glycosidic linkages (B_1 at m/z 290.1 and C_2 at m/z 470.3).[23] This information is extremely important for proper sugar identification and is easily achieved from the frozen sample.

One additional method of analysis for oligosaccharides is the differential fragmentation that results from cation adduction. Carbohydrates can fragment in alternate manners based on the number and type of cations adducted to the sugar.[10,24] This becomes particularly important when isomeric sugars have identical molecular weights. Freezing samples directly from solution allows for the opportunity to dope aqueous samples with salts directly to promote alternative cationization. Figure 5d shows the spectrum of lithium-doped LST b. Lithium chloride was added directly to the sample spot, and the resulting major peaks represent singly and doubly lithiated species at m/z 1005.2 and 1011.2. These ions can be isolated in the ion trap for further analysis and different fragmentation results from singly and doubly lithiated sugars (data not shown). The facile means of cation adduction can be extended to other cations or buffers to promote the production of ions of interest.

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IR AP-MALDI can analyze glycolipids directly from ice. Figure 6 shows the negative mode analysis of 300 pmol of the ganglioside GM_1 frozen on the Peltier-cooled stage. No significant loss of sialic acid is seen, due to the soft nature of the ionization. The major $[M - H]^-$ peaks result from a 18:0 and a 20:0 sterol at m/z 1544.5 and 1572.7, and these ions can be further isolated and fragmented to provide additional structural information. Figure 6b is the MS/MS analysis of GM_1. This spectrum shows several of the major glycosidic fragments ($Y_{2\beta}$ at m/z 1281.5 and $Y_{2\alpha}/Y_{2\beta}$ at m/z 916.3), as well as sterol fragments, which can be used for further structural characterization of the glycolipid.

CONCLUSIONS

IR AP-MALDI is ideally suited for combination with a Peltier-cooled sample stage for the analysis of frozen aqueous samples. The IR laser is tuned for the excitation of the O−H stretching frequency. Analysis directly from water rapidly evaporates the solvent. Freezing the sample provides a means to maintain solvent, while at the same time providing a suitable matrix for ionization. Samples remain in a near-native state, without the addition of an external matrix. This is important not only to maintain "native" sample conditions but also to use for more rapid high-throughput analyses. Eliminating the matrix addition step can decrease the total time needed for individual sample testing.

The detection limit is currently not ideal for the frozen sample stage. IR AP-MALDI remains less sensitive than vacuum MALDI, and these experiments show a further decrease in limit of detection. Nonetheless, the detection limit, as little as 1 pmol, is approaching that of other IR AP-MALDI techniques. Further work remains to optimize analysis from ice, which might include incorporation of ion focusing optics or other ion guiding mechanisms. IR AP-MALDI from frozen samples also leads to the possibility to study frozen tissue samples or for studies of ice samples "in the field". As the detection limit of the technique improves, so will the range of possible applications.

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