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Infrared Laser Isolation of Ions in Fourier Transform Mass Spectrometry

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A new method for isolating ions for tandem mass spectrometry analyses in Fourier transform mass spectrometry is illustrated. The method employs an infrared laser to dissociate completely the undesired ions. The selected ions are excited to an orbit away from the degradative portion of the laser beam. Ion isolation was accomplished and tandem mass spectrometry experiments were performed on model oligosaccharides and compounds from biological samples.

Ion isolation is the primary event in tandem mass spectrometry (MS). All current existing methods for ion isolation involve diverting the paths of undesired ions so that they do not proceed to the detector. In Fourier transform ion cyclotron resonance (FTICR) mass spectrometry, the ions are isolated by ejecting all undesired ions from the analyzer cell. This regimen is performed often with a combination of radiofrequency (rf) bursts and sweeps below and above the mass-to-charge (m/z) value of the desired ion. A more elegant method is the use of selected waveforms or arbitrary function generators that are programmed to isolate the desired ions.[1,2] The problem is that all these methods require considerable setup and manipulation that decrease the speed of the analysis. The isolation events are tailored for individual m/z and often require individually tuning for each m/z to obtain maximum efficiency. It complicates the coupling of separation methods such as liquid chromatography (LC) directly with tandem MS experiments.

There has been recent considerable interest in the use of infrared multiphoton dissociation (IRMPD) for producing fragment ions for analytical applications.[3−5] Although the introduction of IRMPD[6] predates collision-induced dissociation (CID),[7,8] its application has not been widely accepted. Nonetheless, IRMPD has several attractive features for FTICR. The absence of collision gas, which is necessary for collision-induced dissociation, decreases the acquisition time, allowing the accumulation of more scans. The energy transferred to the ions is also not limited by instrument parameters such as magnetic field strengths. Indeed, ions can be readily fragmented with sufficient laser power to eliminate all the ions from the observation window.

In this report, we employ the convenience of IRMPD to develop a new method for ion isolation employing infrared laser radiation. Infrared laser isolation (IRLI) is performed by exciting the desired ions to an orbit slightly larger than the dissociative region of the laser beam—out of the path of an intense infrared laser beam. Under the high-power conditions, the undesired ions are degraded to small ions that are either readily lost from the analyzer cell or fall below the observation window.

EXPERIMENTAL SECTION

The oligosaccharides (maltotetraose, maltohexaose, γ-cyclodextrin) were obtained from Sigma Chemical Co. (St. Louis, MO). The matrix, 2,5-dihydroxybenzoic acid (DHB), was obtained from Aldrich Chemical Co. (Milwaukee, WI). All reagents were purchased in the highest purity and used without further purification. O-Linked oligosaccharide alditols were isolated from egg jelly glycoproteins of the South African toad, *Xenopus laevis*, using a procedure developed in this laboratory and described in previous publications.[9,10]

For the IRLI experiments, two mixtures of oligosaccharides were prepared. One was an aqueous solution containing maltotetraose, maltohexaose, and γ-cyclodextrin (0.1 mg/mL each). The other was obtained from a HPLC effluent fraction containing a mixture of several O-linked oligosaccharide alditols released from egg jelly in acetonitrile/water. One microliter of the sample solution was placed on the MALDI probe followed by 1 μL of 0.01 M NaCl to enrich the Na^+ concentration and produce primarily sodiated species. One microliter of 0.4 M DHB in ethanol was added as matrix before the analysis. Warm forced air was used to quickly evaporate the mixture on the probe.

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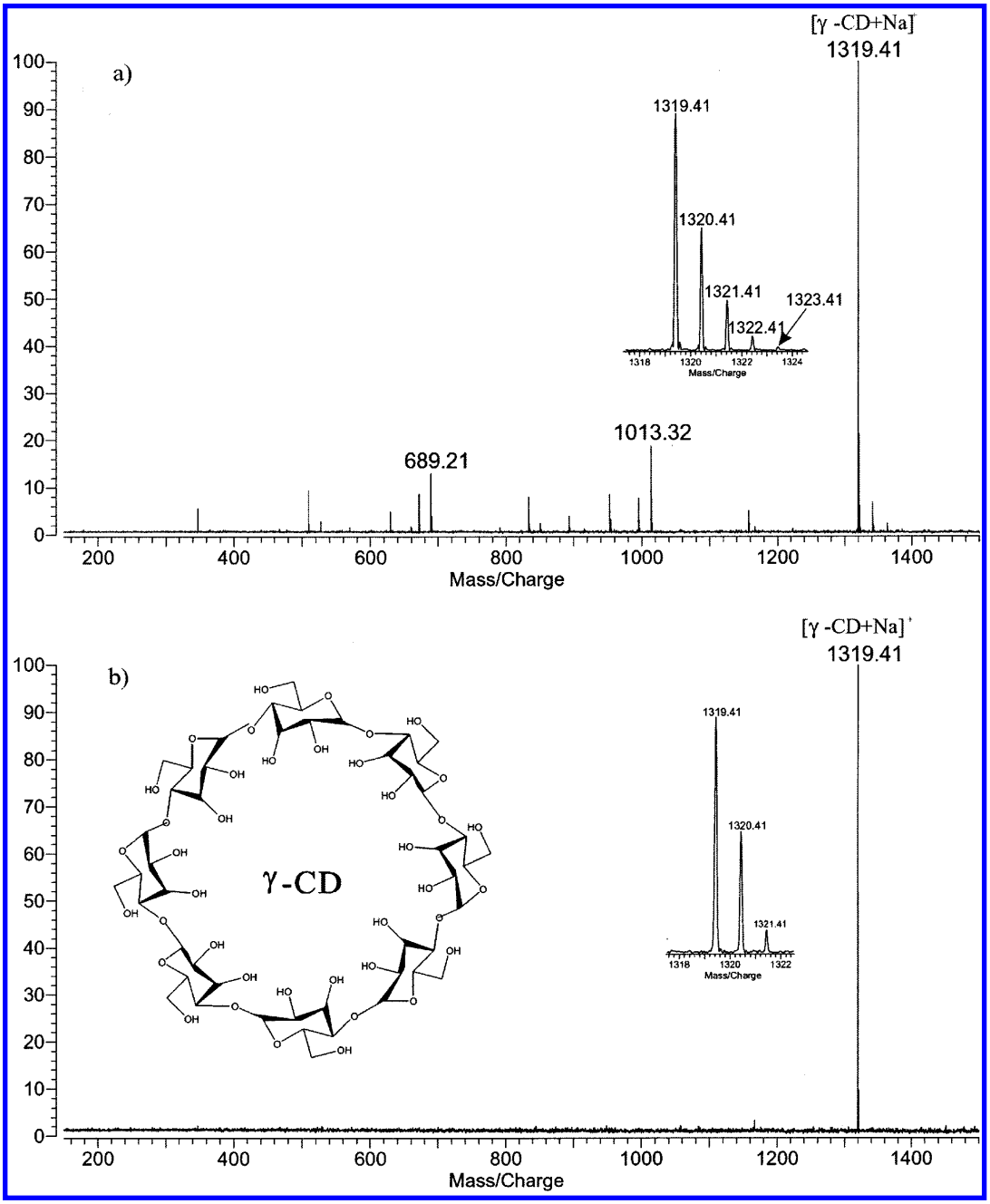

Figure 1. (a) MALDI-FTMS spectrum of a mixture containing three oligosaccharides, maltotetraose (*m*/*z* 689), maltohexaose (*m*/*z* 1013), and γ-cyclodextrin (*m*/*z* 1319). The quasimolecular ions correspond to the sodium-coordinated species. (b) Isolation spectrum with IRLI. The CO_2 laser was turned on for a period of 450 ms at 11 W.

MALDI-FTMS analyses were performed on an IonSpec (Irvine, CA) instrument equipped with a 4.7-T superconducting magnet. The details of the instrument are published elsewhere.[9−11] The standard nitrogen laser was replaced with a Nd:YAG (New Wave Research Inc., Fremont, CA) laser with a 355-nm output.

CID experiments were performed in the off-resonance mode. The desired ion was isolated in the ion cyclotron resonance (ICR) cell by the use of arbitrary waveform generation and synthesizer excitation. The ions were excited at a frequency 1000 Hz greater than the effective cyclotron frequency for 1000 ms at 2−7 V (base to peak) depending on the desired level of fragmentation and the size of oligosaccharides. Two argon pulses were used during the CID event to maintain a pressure of 10^{-5} Torr.

To perform IRMPD experiments, modifications were performed on the ICR cell and the vacuum chamber. The trapping plate with the electron filament was removed and replaced with a copper plate containing a 13-mm hole in the center. Four copper wires (0.24 gauge) were fixed by screws on the plate, two horizontally and two vertically, over the hole and set 3.6 mm apart. The existing aluminum vacuum chamber was replaced with a smaller diameter (100-mm o.d.) tube containing a 70-mm BaF_2 window (Bicron Corp., Newbury, OH). A Parallax continuous-wave

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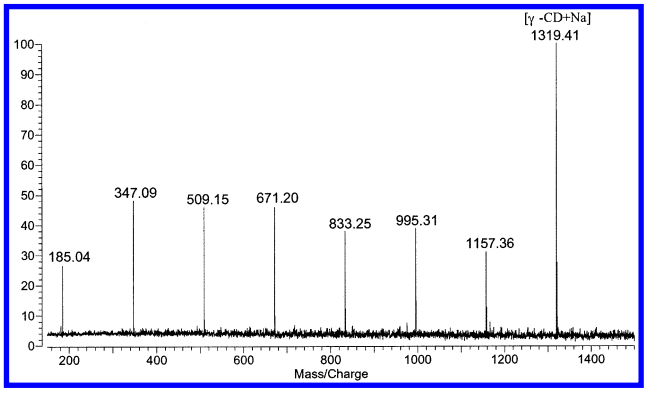

Figure 2. Collision-induced dissociation spectrum of γ-cyclodextrin [γ-CD + Na]$^+$ isolated by IRLI. Fragments are representative of all glycosidic bond cleavages.

CO_2 laser (Waltham, MA) with 20-W maximum power was mounted onto the magnet and aimed directly to the center of the analyzer cell.

RESULTS

IRLI is illustrated for samples containing mixtures of oligosaccharides. A mixture of three oligosaccharides (maltotetraose, maltohexaose, γ-cyclodextrin) was prepared and analyzed by MALDI. The resulting spectrum (Figure 1a) shows the quasi-molecular ion for each component ([M + Na]$^+$) as well as a number of fragment ions. The sodiated parent of the largest component, γ-cyclodextrin (m/z 1319.41), was selected by exciting the ion on-resonance for a period of 3 ms with an rf (1.5 V_{b-p} amplitude) corresponding to that of the ion. The CO_2 laser was turned on for a period of 450 ms at 11 W. The isolation spectrum (Figure 1b) employing the IRLI event shows essentially no ions either below or above the desired mass range. An interesting feature of the spectrum is the suppression of some isotopic peaks in the mass spectrum (insert). The rf event was selected to excite specifically the monoisotopic ion peak (m/z 1319.41). The inset in Figure 1a shows the natural isotopic distribution. Signals are observed up to m/z 1323.41 corresponding to four ^{13}C's. The isotopic distribution of the species isolated by IRLI shows the monoisotopic peak along with up to two ^{13}C's. The m/z 1320.41 peak was not affected, but the m/z 1321.41 peak and larger isotopmers were decreased. These isotopmers were not sufficiently excited translationally and were dissociated by the laser beam.

Excitation of the ion to a diameter approximately equal to that of the beam resulted in optimal signal intensities. For the experiments of Figure 1, the ions were excited to a diameter of ~5.7 mm as determined by the equation

$$r = V_{p-p}T_{excite}/2dB_o$$

where V_{p-p} is the amplitude of the rf pulse, T_{excite} is the excitation time, d is the diameter of the analyzer cell, and B_o is the magnetic field.[12,13] This value matches well with the $1/e^2$ diameter of the beam, which is 6 mm as specified by the manufacturer. The absence of mass in the equation means all the ions, regardless of m/z, are excited to the same orbit thereby eliminating the need for considerable tuning.

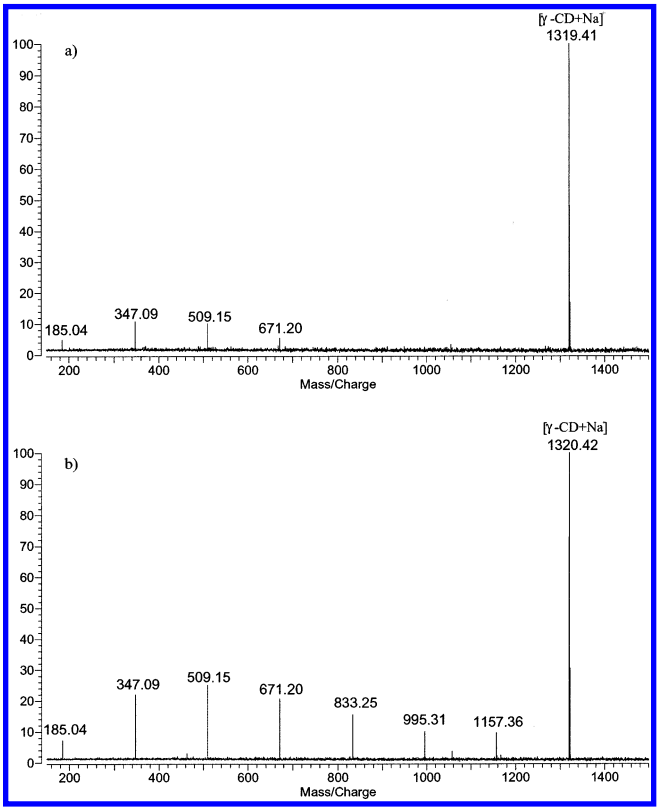

Figure 3. (a) Infrared laser isolation of γ-cyclodextrin with off-resonance excitation to move the ion in and out of the laser beam. The ion is simultaneously isolated and dissociated by IRMPD. Small fragments due to glycosidic bond cleavages are the major products. (b) The same experiment in (a) with the addition of an on-resonance excitation followed by off-resonance excitation. Better control of the dissociation is obtained with every glycosidic bond cleavage represented.

Examination of several spectra with varying laser power showed that the product ion distribution shifted continually to smaller fragments with higher power (not shown). When Rb^+ and Cs^+ ions were used to coordinate to the oligosaccharides, we found that the metal ions were the predominant products. With the Na^+ coordinated species, we presume that Na^+ ions are the ultimate products during the irradiation event; however, these ions could not be detected due to limitations in data system. The small ions do not interfere further with the analysis and are more easily lost from the ICR cell.

After the isolation event, standard tandem MS experiments could be applied to the isolated ion. CID was performed on the ion in Figure 1b with the resulting spectrum shown in Figure 2. The CID of the isolated ions produced fragments that are consistent with the known CID products of this molecule. All peaks assignable to known fragments were labeled.

We explored the use of IRMPD to produce fragments in conjunction with IRLI. A tandem MS event that would employ IRMPD rather than CID would also be highly beneficial. It would decrease the accumulation time significantly by eliminating a second pulsed gas event and the subsequent pumpdown period.

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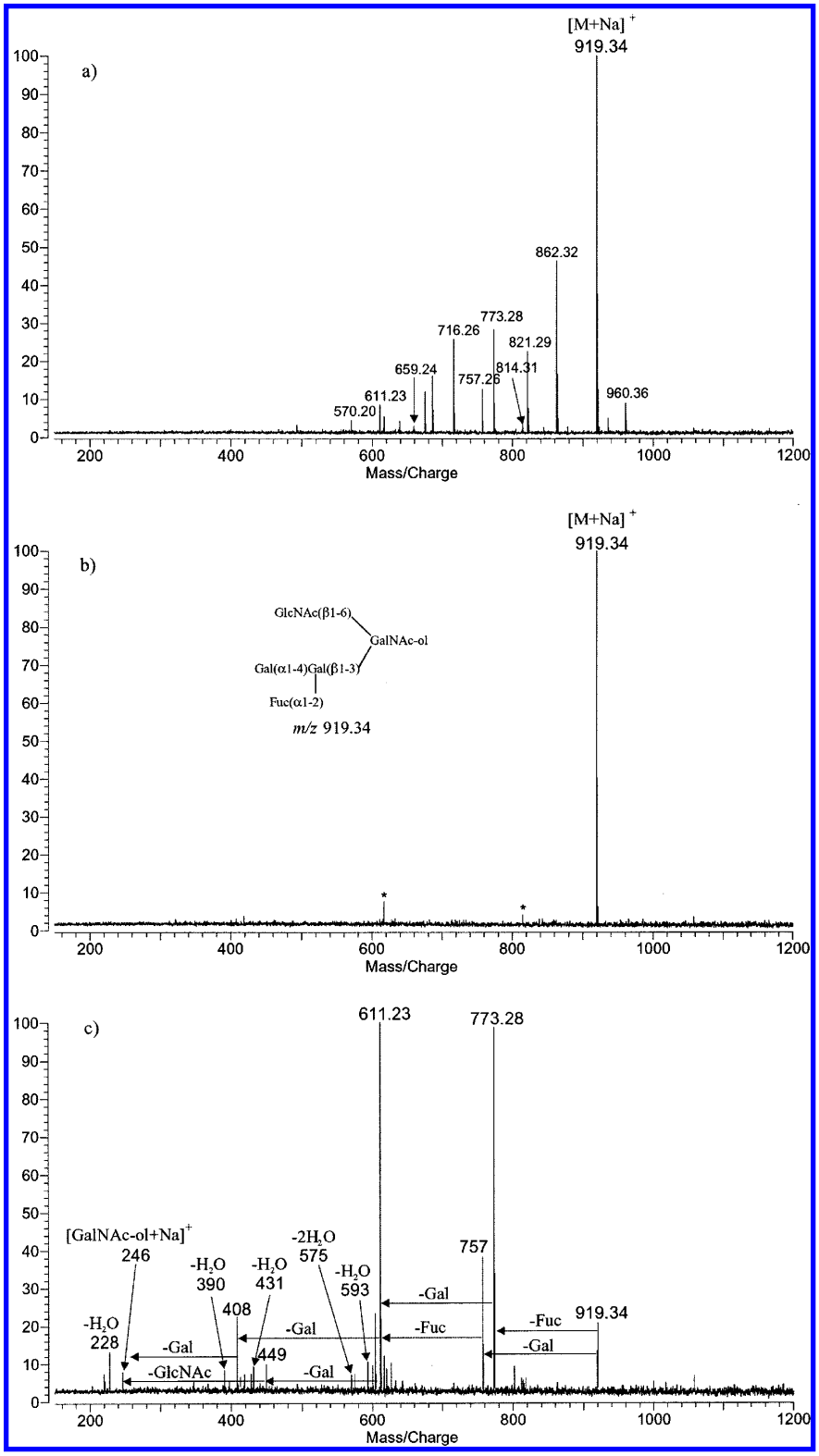

Figure 4. (a) MALDI-FTMS spectrum of a mixture of oligosaccharides from an HPLC run. The samples were obtained from the egg jelly of *X. laevis*. (b) Spectrum of component *m*/*z* 919.34 (structure inset) isolated by IRLI. Complete isolation was obtained. The minor signals were due to electronic noise (asterisk). (c) CID spectrum of isolated species. For clarity, only nominal masses were provided.

Such a method would find immediate use in coupling liquid chromatography to tandem MS to produce LC/MS/MS methods. IRMPD after the IRLI event required the ability to change the laser power remotely and automatically. In theory, it should be possible to use the same power for IRLI and IRMPD, with a significantly shorter irradiation period for IRMPD. In our experience, conventional IRMPD is performed best at lower power than IRLI. The lower power (typically 4−6 W) provides better control of the dissociation. In addition, a pulsed gas event was necessary to cool the ions toward the center of the cell. However, the introduction of more collision gas would increase the analysis time, negating the advantage of IRMPD over CID.

The IRMPD event could be coupled with IRLI if the ions are "parked" in an orbit where the fluence is less, allowing fragmentation only of the selected species rather than complete dissociation. Because the beam profile is a Gaussian, it should be, in principle, possible to find the proper orbit. Instead, we explored the use of off-resonance excitation, which simultaneously increases and decreases the orbital radius of the ion. In this way, the ion moves in and out of the high-energy beam, limiting its exposure to the highly dissociative radiation. The spectrum in Figure 3a was obtained in this manner. Fragments similar to the CID were obtained; however, they were distributed toward the low masses. Larger fragments were not readily observed, and it was difficult to decrease the extent of fragmentation. An alternative method was to excite the ion to a larger radius by on-resonance excitation followed by off-resonance excitation at the excited radius. This moved the ion further away from the highly degradative beam and allowed it to intercept only the lower intensity regions intermittently. The resulting spectrum is shown in Figure 3b. Note the fragmentation is extensive and all glycosidic bond cleavages are represented providing the complete sequence of the compound.

Biological samples were also examined to illustrate the general utility of IRLI. A complex mixture of O-linked oligosaccharide alditols was released from mucin-type glycoproteins using alkaline borohydride reduction. A fraction of the HPLC run was analyzed by MALDI FTICR MS (Figure 4a). The MALDI spectrum contains a number of oligosaccharide signals including the desired ion, m/z 919.34, corresponding to a pentasaccharide (structure inset Figure 4b). The ion was readily isolated by IRLI. The CID spectrum of the isolated m/z 919.34 yielded a fragmentation pattern that corresponded well to that obtained earlier by employing traditional isolation and CID events (Figure 4c).[10,11]

CONCLUSION

IRLI provides a novel method for ion isolation in FTICR MS. A major advantage of this over existing ion isolation methods is that it is not specific to m/z. That is, the selected ion is excited to the same orbital radius regardless of its m/z. The tuning time therefore is considerably decreased. When used in conjunction with IRMPD, IRLI may potentially be useful for applications where short acquisition times are a premium such as the coupling chromatographic or electrophoretic separation with tandem FTICR MS.

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