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Differentiation of glucose-containing disaccharides by infrared multiple photon dissociation with a tunable CO_2 laser and Fourier transform ion cyclotron resonance mass spectrometry

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ABSTRACT

Lithiated glucose-containing disaccharides with various linkages and anomeric configurations, formed by electrospray ionization (ESI) and trapped in a Fourier transform ion cyclotron resonance (FTICR) mass spectrometer, were fragmented by infrared radiation from a tunable CO_2 laser. Irradiation over the wavelength range from 9.2 to 9.7 μm gave unique fragmentation patterns for each of the disaccharides. These fragmentation patterns can be used to easily determine the monosaccharide linkage position for each disaccharide. Once the linkage is determined, irradiation of the precursor ion (m/z 349) to produce a specific ratio of peak height for a particular fragment ion to that of the precursor ion is shown to yield unique ratio values that can be used to identify the different anomers of the same linkage at the 95% confidence level.

1. Introduction

Carbohydrates and their derivatives are biologically important. Some of their functions include mediation of cell–cell interactions, cell growth, fertilization, and inflammation [1–4]. The biological function of oligosaccharides is dictated by their structures, which include the stereochemistries, anomeric configurations, and linkages between monosaccharides that comprise them. Since numerous linkages and anomeric configurations are possible, even determining the exact structures of subcomponents of larger carbohydrates is a complicated task.

Nuclear magnetic resonance [5] (NMR) and mass spectrometry [6–10] have been used in the past to identify carbohydrates with different linkages and monosaccharide building blocks. Although NMR analysis allows stereochemistries and anomeric configuration of carbohydrates to be determined, it requires fairly large sample quantities and interpretation can be complex [11]. Mass spectrometry, on the other hand, requires less sample and provides spectral information with 3–4 orders of magnitude higher sensitivity than NMR [12]. One mass spectrometric method in particular is Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS). FTICR-MS provides unparalleled mass resolving power and mass accuracy, thus allowing the smallest of mass differences to be detected [13–16].

Since isomers and anomers have the same exact mass, even the high resolution of FTICR-MS cannot distinguish between them. Therefore, other information must be obtained to permit the differentiation of isomers. Recently, methods such as ion mobility [4,17] energy-resolved mass spectrometry [18], and fragmentation of saccharides have been coupled to mass spectrometry to differentiate carbohydrates [9,19–23].

Past research has used both collision-induced dissociation (CID) [19,20] and infrared multiple photon dissociation (IRMPD) [9,22,24] to promote fragmentation of saccharides. In the work reported here, fragmentation patterns of disaccharides have been examined since these oligosaccharides are the smallest unit still containing the glycosidic bond. Past experiments by Polfer et al. examined the fragmentation patterns of lithiated glucose-containing disaccharides using the *F*ree *E*lectron *L*aser for *I*nfrared e*X*periments (FELIX) at the FOM-Institute for Plasma Physics Rijnhuizen in The Netherlands [9]. They found different fragmentation patterns for the various linkages of glucose-containing disaccharides as a function of the (infrared) wavelength of irradiation and that the ratio of specific fragments (m/z 169/187) is higher for β-anomers than for α-anomers and thus may be used to differentiate the disaccharides. Although fragmentation patterns and some fragment peak height ratios were explored, no quantitative approach was developed to determine anomeric configurations. Lithium cation attachment has been found [9,19,20] to promote

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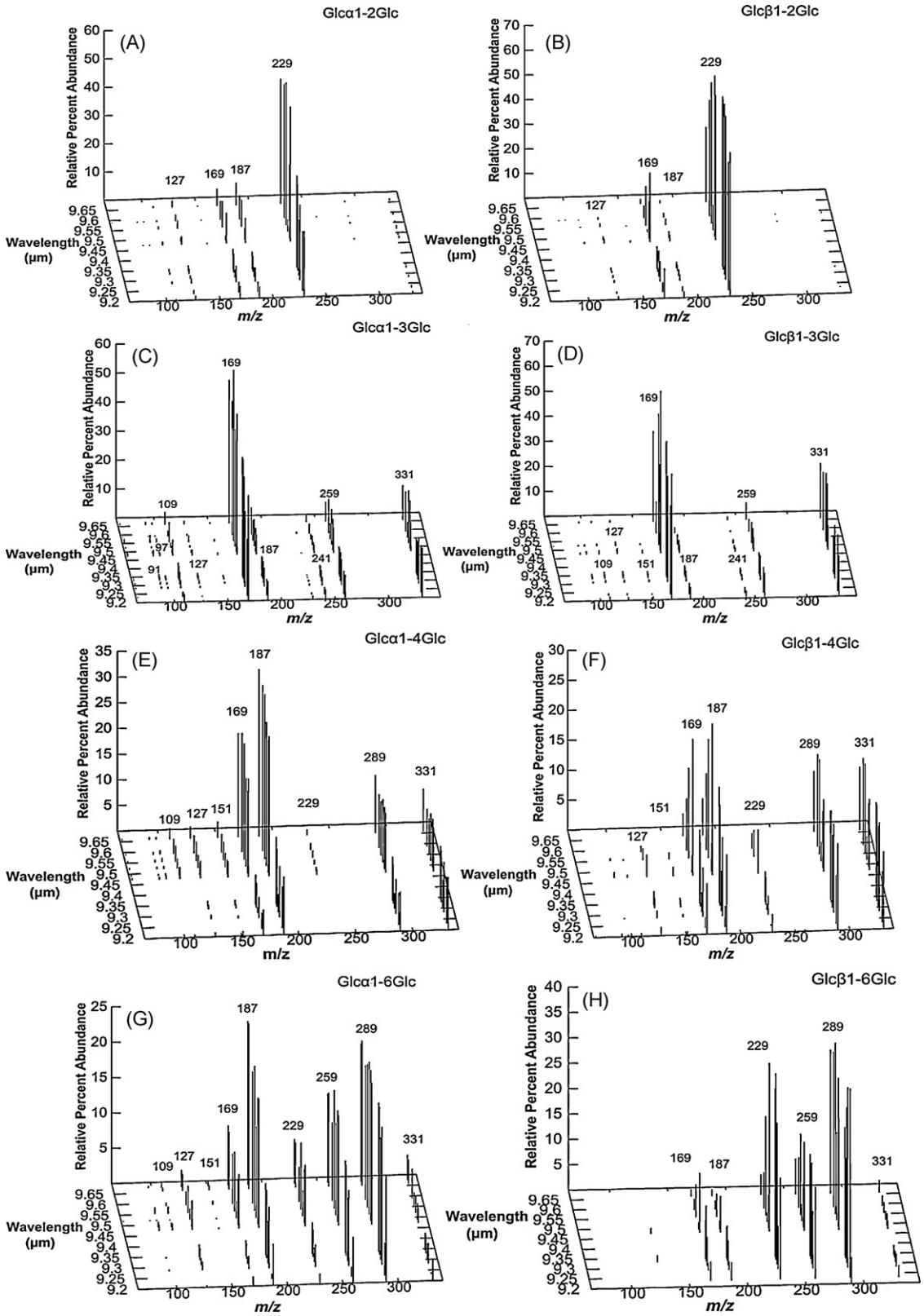


Fig. 1. Wavelength-dependent fragmentation for the various lithiated, glucose-containing disaccharides. (A) Kojibiose (α1–2), (B) sophorose (β1–2), (C) nigerose (α1–3), (D) laminaribiose (β1–3), (E) maltose (α1–4), (F) cellobiose (β1–4), (G) isomaltose (α1–6) and (H) gentiobiose (β1–6).

diagnostic fragmentation patterns for oligosaccharides following collisional or infrared multiple photon activation, so was chosen for the work reported here. Attachment of larger alkali cations most often results in loss of the cation as the only dissociation path upon ion activation, although such cation loss can be used to obtain diagnostic IRMPD spectra under favorable conditions [25].

The present research was directed toward developing a method that permits differentiation of lithiated disaccharides using a line-tunable CO_2 laser. In a previous paper we showed that dif-

ferentiation of lithiated monosaccharides is possible using ratios of peak heights of selected fragment ions produced by tunable CO_2 laser-IRMPD in the 9.2–9.7 μm wavelength range [26]. We report here wavelength-selective fragmentation of lithiated glucose-containing *disaccharide* anomers by IRMPD with a tunable CO_2 laser. A method to differentiate the disaccharides based on their fragmentation patterns and ratios of the peak heights of specific fragment ions is also presented.

2. Experimental

2.1. Reagents and materials

Samples of glucose-containing disaccharides including kojibiose (α1–2), sophorose (β1–2), nigerose (α1–3), laminaribiose (β1–3), maltose (α1–4), cellobiose (β1–4), isomaltose (α1–6) and gentiobiose (β1–6) disaccharides were obtained from Dr. Brad Bendiak of the Department of Cellular and Structural Biology, University of Colorado Health Sciences Center. Solutions of the disaccharides were prepared at a concentration of 0.1 mM with 0.1 mM LiCl in a solution of general-use grade methanol and MilliQ ultra-pure water (80:20%).

2.2. Experimental set-up

The lithiated disaccharides were ionized by a commercial electrospray ionization (ESI) source (Analytica of Branford, Branford, CT, USA) with a user-modified heated metal capillary [27–29] with a conical capillary inlet [30] set at a temperature of 125 °C. Experiments were carried out on a Bruker 47e FTICR mass spectrometer (Bruker Daltonics; Billerica, MA, USA) with a 4.7 T superconducting magnet (Magnex Scientific Ltd.; Abington, UK) and Infinity™ cell [31]. Lithiated disaccharides (*m*/*z* 349) were mass isolated and irradiated with a Lasy-20G tunable continuous wave CO_2 laser (Access Laser Co., Everett, WA, USA) with power range of 0–20 W and wavelength range of 9.20–10.80 μm.

2.3. Procedure

To obtain IRMPD fragmentation patterns (Fig. 1) as a function of CO_2 laser wavelength, solutions of each of the various lithiated disaccharides were electrosprayed and the precursor ions (*m*/*z* 349) were mass isolated. Once isolated, the precursor ions were irradiated with a specific wavelength output of the CO_2 laser for 1 s. A laboratory-constructed mechanical mirror was used to allow light to pass through an anti-reflectivity coated ZnSe window on the FTICR vacuum chamber and into the Infinity™ cell. For each experiment, the laser wavelength was varied over the range of 9.2–9.7 μm. To ensure reproducibility, an internal calibration for fluctuating laser power was carried out by monitoring the appearance of the *m*/*z* 229 fragment ion and disappearance of *m*/*z* 349 precursor ion for sophorose (β1–2) at 9.588 μm. The power required for a ratio of *m*/*z* 229 peak height to *m*/*z* 349 peak height of 1.04 ± 0.16 to 1 was determined daily. This standard laser power was used and kept constant for all experiments on that day. Three sets of fifteen scans of 512 K datasets were collected and averaged at each wavelength. To determine the reproducibility, IRMPD spectra of sophorose at several wavelengths were taken 2 months apart and compared, Fig. 2. As seen in Fig. 2, although the relative percent abundance of each fragment for lithiated sophorose varied somewhat day-to-day, the identity of the fragments produced when the precursor ion was irradiated remained the same. Significance of results was based on the 95% confidence interval of the mean [32].

For differentiation of a set of unknown disaccharides, the method used to identify their linkage positions is given in Scheme 1. Once the linkage was determined, the precursor ion was irradiated to obtain a 1:2 ratio of its peak height to that of a particular fragment ion (*m*/*z* 229 for 1–2 linked, *m*/*z* 187 for 1–4 linked and *m*/*z* 169 for both 1–3 and 1–6 linked disaccharides). The resulting fragment ions were compared and the anomericity of the disaccharides was determined according to the ratios of fragment ions as shown in Scheme 2.

2.4. Reproducibility

For each wavelength, the average precursor peak height and the measured laser power were kept constant daily for each experimental run. For the IRMPD fragmentation patterns, a daily calibration was performed at 9.588 μm for sophorose at the start of the day and was re-checked periodically throughout the day. For the study involving unknown disaccharides the ratios of the peak height for the precursor ion to those of specific fragment ions were used to keep the extent of fragmentation constant. Fluctuations in laser power and in ionization by the ESI source are primary causes of the variations in day-to-day percent abundances seen in Fig. 2.

3. Results and discussion

For each of the eight lithiated disaccharides, the precursor ion (*m*/*z* 349) was irradiated and the relative percent abundances of the product ions were found and plotted for wavelengths between 9.2 and 9.7 μm. As seen in Fig. 1, IRMPD of the precursor ions over a range of wavelengths from 9.2 to 9.7 μm produced a unique fragmentation pattern for each disaccharide. Over the limited wavelength range of the CO_2 laser used, these fragmentation patterns were quite similar to those obtained by Polfer et al. using a free electron laser (FEL) [9]. While the IRMPD fragments seen for the different disaccharides are the same in our work and the earlier FEL study, the relative percent abundances are not. This is most likely due to differences in laser power and nature of laser irradiation (continuous wave CO_2 laser *vs*. several macropulses composed of high peak power micropulses for the FEL) [33]. In addition to the wavelength range from 9.2 to 9.7 μm, for which results are shown in Fig. 1, the wavelength of fixed frequency CO_2 lasers (10.6 μm) was also used for IRMPD and very little (if any) fragmentation was seen with the laser power used to fragment the disaccharides from 9.2 to 9.7 μm. Longer irradiation times and/or very high laser powers are needed to observe the fragmentation that occurs at this wavelength. Thus, in terms of fragmentation efficiency, the output of fixed frequency CO_2 lasers (10.6 μm) is not optimal for IRMPD differentiation of various disaccharide isomers.

While the fragmentation patterns produced by irradiation of the precursor ions at the specified CO_2 laser wavelengths shown in Fig. 1 are unique for the various linkages of the disaccharides, they are in general quite complicated to inspect visually and also not unique for anomers with the same linkage. Therefore, additional information is needed to differentiate the various anomers. A procedure for anomeric differentiation of the disaccharides was developed in which the laser power was adjusted to produce 1:2 ratios of the peak height of the precursor ion (*m*/*z* 349) to that of a specific fragment ion (*m*/*z* 229 for 1–2 linked, *m*/*z* 187 for 1–4 linked and *m*/*z* 169 for both 1–3 and 1–6 linked disaccharides). The abundance ratio of these fragment ions to the precursor ion was used for differentiation since the abundances of these fragments were particularly sensitive to the depletion of the precursor ion. Each disaccharide was individually irradiated at wavelengths of 9.342, 9.473, 9.588 and 10.611 μm. Once the precursor ion was fragmented, the relative percent abundances of all the fragments were found at the four wavelengths. Examining the ratio of several fragment ions at wavelengths 9.342,

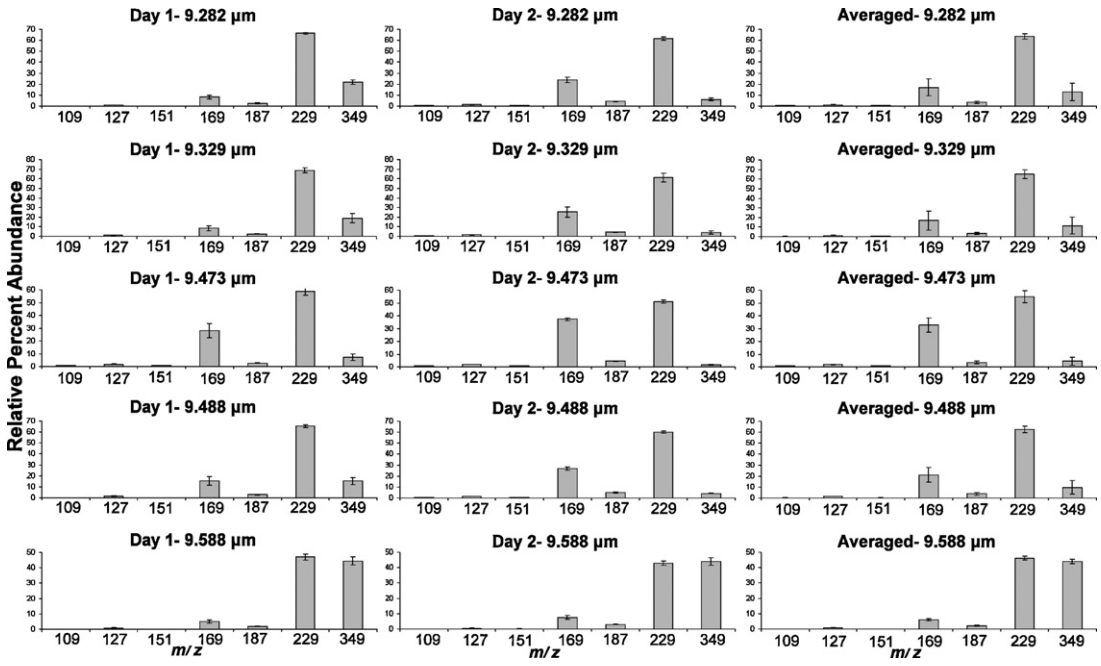
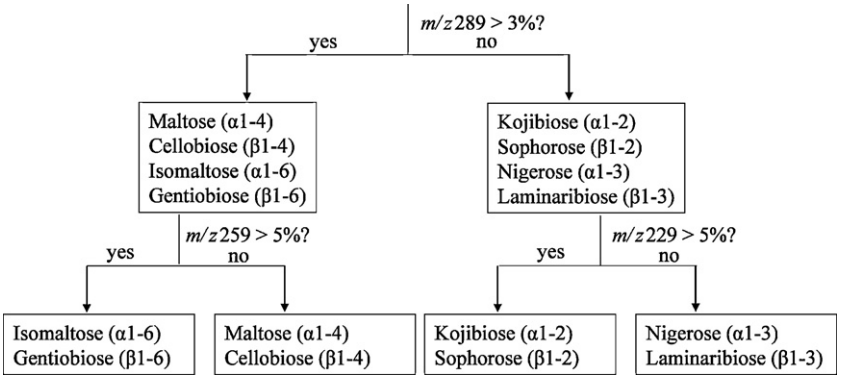


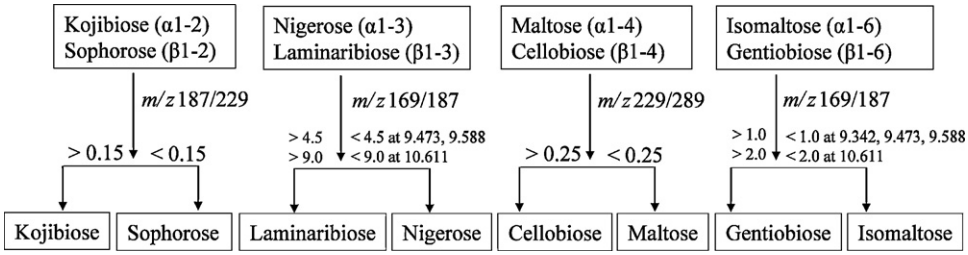
Fig. 2. Relative percent abundances for fragment ions produced from lithiated sophorose (β1–2) at several wavelengths obtained on two separate days and the average relative percent abundance from these 2 days. The error bars represent the 95% confidence limits of the mean.

9.473, 9.588 and 10.611 μm, Scheme 2, permitted the various anomers to be differentiated. For example, to differentiate maltose (α1–4 linkage) from cellobiose (β1–4 linkage) the ratio of the *m*/*z* 229 to *m*/*z* 289 IRMPD fragment ion peak heights is examined. If the ratio is <0.25, the anomer is maltose, while if it is >0.25 the anomer is cellobiose, no matter which of the four wavelengths is used. While wavelengths 9.342, 9.473 and 9.588 μm required less than a watt of power for fragmentation, approximately 4–6 W were required to fragment the disaccharides at 10.611 μm.

To test this approach of disaccharide identification, two blind studies were performed on separate days. For each study, the unknowns were irradiated individually at all the four wavelengths and their identity predicted by first determining the linkage and then determining the anomeric configuration based on the approach detailed above. Once the linkage was determined, the



Scheme 1. Decision flow chart used to determine the linkage of the disaccharides based on the identity of fragment ions produced by irradiating the precursor ion (*m*/*z* 349) so that it was nearly depleted (where approximately ≤5% of the precursor ion remained).



Scheme 2. Schematic showing the fragment ion peak height ratios used to determine the anomer identification of the disaccharides. The numbers next to the greater/less than symbols are the ratios of the peak height of fragment ions that are shown next to the arrows.

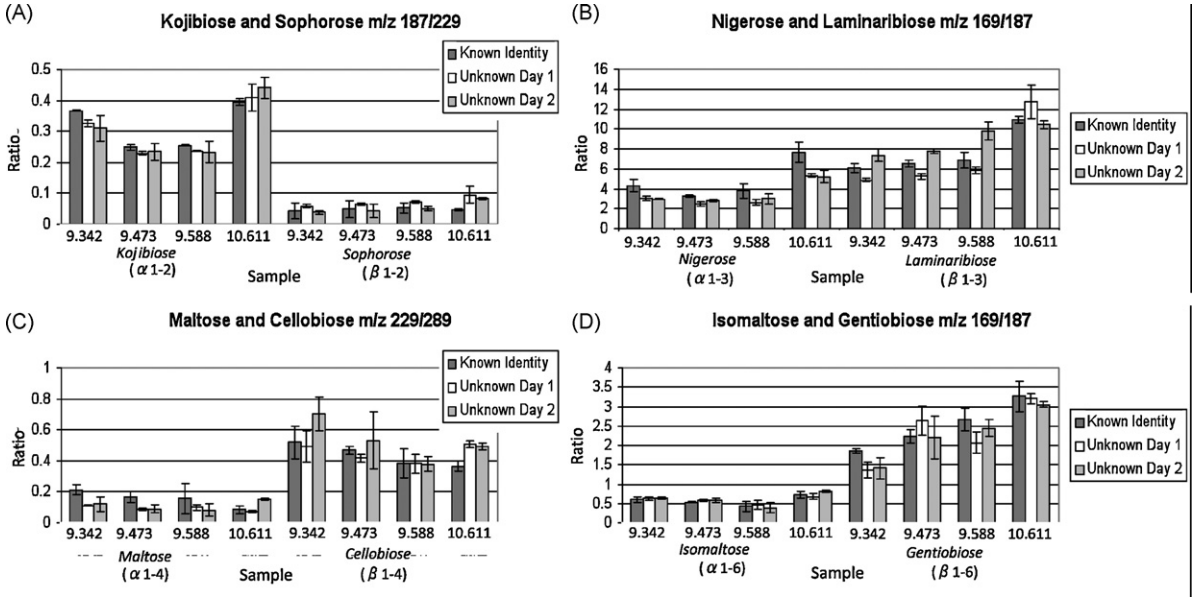


Fig. 3. Comparison of selected ratios of fragment ion peak heights at four different CO_2 laser wavelengths for known and unknown lithiated disaccharides obtained on two separate days.

laser power was adjusted to obtain a 1:2 ratio of the peak height of the m/z 349 precursor ion to that of specific fragments (m/z 229 for 1–2 linked, m/z 187 for 1–4 linked and m/z 169 for both 1–3 and 1–6 linked disaccharides), with the anomeric configuration determined based on the fragment ion ratios as detailed in Scheme 2. Fig. 3 shows the results of the two trials for determination of the unknown in comparison to results for the known samples that were obtained a few days before the unknown determination study. All bars show the 95% confidence interval of the mean of the results from three to six spectra (with each spectrum the result of Fourier transforming a transient response signal that was the average of 15 separate ion formation/isolation/irradiation/detection sequences). In all cases, the identity of the unknown was positively identified based on the fragment ion ratios and the use of Schemes 1 and 2. Some of the fluctuations and discrepancies between the peak height ratios for unknowns and known disaccharides are most likely caused by variation in the precursor ion abundance due to the electrospray ion formation process. Other uncertainties arise due to fluctuation in laser power and exact time of laser irradiation (determined by the mechanical mirror), even with adjustment several times daily to produce specific precursor/fragment ion peak height ratios. However, Fig. 3 shows that such fluctuations are not large enough to prevent unambiguous differentiation of the eight disaccharides. Fig. 3 also shows that both the 10.611 μm wavelength (similar to the output of non-tunable lasers) and the other various wavelengths accessible to a tunable CO_2 laser (9.342, 9.473 and 9.588 μm) provide comparable results for the 1–4 and 1–6 linked disaccharides. Although the 10.611 μm wavelength output provided the greatest difference between the ratios obtained from the fragmentation of the 1–2-linked anomers, this wavelength also gave the least difference in the ratios from the fragmentation of the 1–3 linked anomers. Fig. 3 also shows that the various anomers can be distinguished using only a single wavelength, but use of multiple wavelengths reaffirms the results.

4. Conclusion

Use of a tunable CO_2 laser to differentiate various disaccharide linkages with different anomeric configurations of Li^+-attached glucose-containing disaccharides is possible by comparing peak height ratios of selected fragment ions. While a similar approach was demonstrated earlier using a free electron laser [9], the possibility of using a much less expensive and complex line-tunable CO_2 laser makes this method more accessible to other research groups interested in determining disaccharide structures. Extension of this methodology to other disaccharides containing different monosaccharide units such as galactose and mannose will be explored. The method presented here is not easily adapted to mixture analysis. We are currently exploring more sophisticated mathematical data mining approaches to facilitate the use of IRMPD fragmentation data for such analyses.

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