Differentiation of Lithium Cation-Attached Mono- and Disaccharide Isomers by Wavelength-Dependent CO₂ Laser Photofragmentation and FTICR Mass Spectrometry

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Overview

- The purpose of this research was to differentiate various initial mono- and disaccharides using diagnostic fragmentation patterns produced by a nanosecond, line-tunable CO₂ laser over the wavelength range of 9.2 to 9.7 µm.
- A line-tunable CO₂ laser in conjunction with a Fourier transform ion cyclotron resonance (FTICR) mass spectrometer with an electrospray ionization (ESI) source was used to study the fragmentation patterns of lithiated mono- and disaccharides.
- Isomers and anomeric configurations were determined using line-tunable CO₂ laser photofragmentation.

Introduction

- Carbohydrates not only act as targets for microorganisms, viruses, and antibodies, but they also play a crucial role in many biological functions such as cell-cell interaction, fertilization, cell growth and post-translational protein modifications.
- The ability to distinguish isomers of mono- and disaccharides aids in the treatment and synthesis of carbohydrates.
- Multiple possible stereochemistries and anomeric conformations of saccharides, all having the same size, make the analysis of carbohydrates by mass spectrometry challenging.
- Use of FTICR-MS allows for unambiguous mass-selective ion isolation and mass accuracy.
- Infrared multiple-photon dissociation (IRMPD) is a fragmentation technique that can provide diagnostic fragmentation patterns from the line-tunable CO₂ laser allows for selectivity of the wavelengths used for fragmentation.

Method

- Positive ion analysis was done on a Bruker FTICR mass spectrometer with a 4.7 T superconducting magnet and linear ion trap.
- An Analytik Jena external electrospray ionization (ESI) source with a user-built heated metal capillary was used to ionize the lithium-attached mono- and disaccharides. Samples were injected at a rate of 15 µL/hr.
- An A-20G (Coherent) CO₂ laser (Innova 860 CO₂) was used to provide irradiation with variable wavelength of 9.2 to 9.7 µm. Wavelengths were selected based on the stability and laser output power used in the laser manual.
- The focusing condenser was moved from the far end of the laser beam to pass into the back of the mass spectrometer and then into the cell where the beam traveled in the ion source and ion optics.
- Solutions of 10⁻² M lithium mono- and disaccharides were prepared in H₂O/MeOH (50:50) under vacuum. Samples included lithium β-D-glucopyranosides and disaccharides composed of two glucose units, lipopolysaccharide (1-3), sophorose (1-2), rhamnose (1-6), gentiobiose (1-4), melibiose (1-4), cellulbiose (1-4), isomaltose (1-4) and gentiobiose (1-6).
- At least two sets of twenty scans of 512 K data points were collected and averaged at each wavelength used in analyses. An experiment was performed days apart. Statistical significance of results reported here is based on the 95% confidence interval of the mean.

Results - Monosaccharides

- Use of a line-tunable CO₂ laser produced unique fragmentation patterns for anomers of O-methyl glucopyranosides and O-methyl galactopyranoside over the wavelength range of 9.2 to 9.7 µm (Figure 1 and 2).
- The fragmentation patterns could be used to distinguish the isomers (gluco- vs. galacto-) of the precursors (Figure 3).
- The relative percent abundances of the specific fragment ions (m/z 187/229 for 1-2 linked, m/z 169/151 for 1-3 linked) were predicted based on the fragmentation patterns and ratios for the various disaccharides.
- A) Ratio of lithiated disaccharides in the range of 9.2 to 9.7 µm.
- B) Ratio of lithiated disaccharides in the range of 9.2 to 9.7 µm.
- C) Ratio of lithiated disaccharides in the range of 9.2 to 9.7 µm.

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