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Identification of sialic acid linkage isomers in glycans using coupled InfraRed Multiple Photon Dissociation (IRMPD) spectroscopy and mass spectrometry

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ABSTRACT

The gas phase IR spectra of α2,3 and α2,6 linkage isomers of sialic acid-containing human milk oligosaccharides and N-glycans epitopes are measured in the 3 μm spectral range using IRMPD spectroscopy in protonated and Na^+ charge states. Each linkage isomer has a unique IRMPD fingerprint, providing a convenient diagnostic of the sialic acid position. Finally, a generic trend is observed: 3-linked sialic acid results in a significant broadening of the spectroscopic signature regardless of the glycan core and the charge state which suggests a drastic influence of the sialic acid position on the glycan conformation.

1. Introduction

Sialic acids represent a large family of acidic monosaccharides sharing a nine carbon backbone. *N*-acetyl-neuraminic acid (Neu5Ac), often referred to as sialic acid, is the most common member of this class. They can be found free in nature and as α2,8-linked homopolymers, but are generally linked to glycans on the 3- or 6- hydroxyl group of galactose (Gal), the 6- hydroxyl group of *N*-acetyl-glucosamine (GlcNAc) or *N*-acetyl-galactosamine (GalNAc) residues [1–3]. This diversity enable a variety of roles in biological processes. In particular sialic acid can modulate the charge, hydrophilicity and binding affinity of glycans to diverse pathogens and toxins [4]. In the brain it participates in the ganglioside structure. In glycoproteins it modulates N-glycans and O-glycans epitopes. Sialic acid also represents a large fraction of functional modifications in HMO (Human Milk Oligosaccharides). They are attached to the terminal end of the free oligosaccharides, and prevent bacterial adhesion in guts [4–10].

MS-based strategies have been proposed since the 70's to analyze sialic acid-containing glycans and glycoconjugates [3,11]. MALDI-MS/MS was largely used in combination with various derivatizations to stabilize sialic acid and identify its linkage in N-glycans [12–14], glycopeptides [15], and gangliosides [16]. Pseudo MS [3] was also proposed to investigate linkage isomers of N-linked glycopeptides, O-linked glycopeptides and glycolipids [17]. Concurrently, ESI-MS/MS has been used in combination with liquid chromatography to identify isomers of N-glycans [18,19], O-glycans [20] and glycopeptides [21]. Recently the performance of capillary electrophoresis was also verified for the analyses of α2,3- and α2,6-syalilated glycans and glycopeptides [22,23]. Finally, ion mobility spectrometry was successfully applied to the separation of isomeric glycans, as well as sialylated MS/MS fragments of glycopeptides [24–26]

IRMPD (InfraRed Multiple Photon Dissociation) spectroscopy has recently shown great promise for the MS-based identification of carbohydrate isomers, including monosaccharide epimers [27,28], and diastereoisomers [29,30], regio- and stereoisomers of dissacharides [31]. The structural sensitivity of this approach has also been demonstrated for positional isomers of functional modifications in the case of sulfated glycans [32,33]. In this context, we propose to explore the analytical potential of IRMPD spectroscopy

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for the identification of positional isomers of sialic acid-containing glycans. In the present work we focus on α2,3 and α2,6 linkage isomers in two different classes of glycans: human milk oligosaccharides and N-glycan epitopes. We report IRMPD diagnostics for both protonated ions and sodium adducts.

2. Methods

2.1. Sample preparation

3’-Sialyllactose sodium salt (Neu5Ac-α-(2–3)-Gal-β-(1–4)-Glc), 6’-Sialyllactose sodium salt (Neu5Ac-α-(2–6)-Gal-β-(1–4)-Glc), 3’-Sialyl-*N*-acetyllactosamine (Neu5Ac-α-(2–3)-Gal-β-(1–4)-GlcNAc) and 6’-α-Sialyl-*N*-acetyllactosamine (Neu5Ac-α-(2–6)-Gal-β-(1–4)-GlcNAc) sodium salt were purchased from Carbosynth and used without further purification. They were prepared in water:methanol 50:50 solutions at a concentration of 50μM for direct infusion in an electrospray ionization source (ESI).

2.2. Mass spectrometry and IRMPD spectroscopy

MS analysis was performed using a commercial 3D ion trap mass spectrometer (Thermofinnigan LCQ classic) coupled with an ESI set in positive mode. Both protonated and sodium adducts were readily observed in the mass spectrometer. The conditions were optimised to promote the signal of protonated ions, resulting in a typical ratio of $I_{Na}/I_H \simeq 10$.

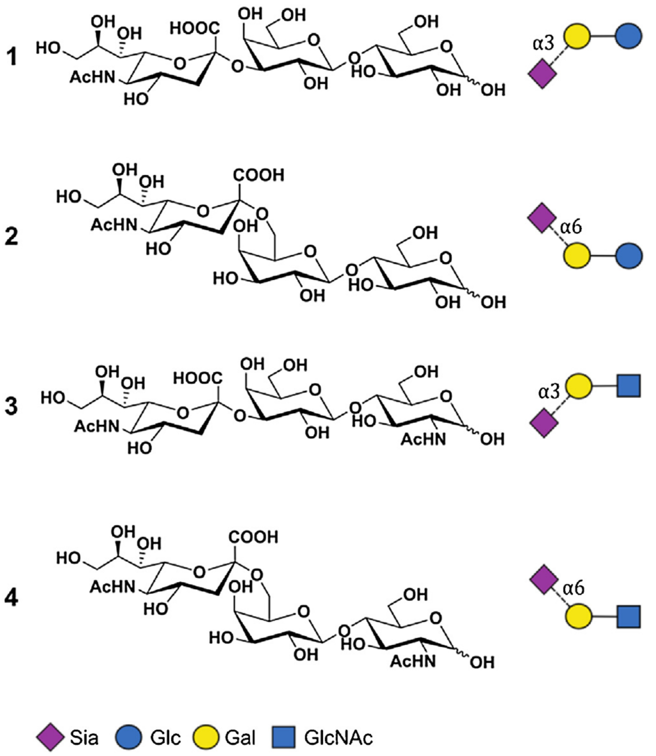
The mass spectrometer was modified as described previously [34] in order to perform IRMPD spectroscopy. Briefly, the ring electrode was drilled and an IR transparent windows was installed to allow irradiation of the ion cloud by the IR laser beam produced by a YAG-pumped IR OPO/OPA system (LaserVision) tunable in the 3 μm range. Mass-selected ions are isolated and irradiated for 700 ms and the resulting photofragmentation mass spectrum is averaged three times. The photofragmentation yield is calculated using the following variation of the Beer–Lambert formula: $-log\left(\frac{I_p}{I_p+\sum I_f}\right)$ where I_p is the intensity of the precursor ion and I_f is the total intensity of fragments detected after laser irradiation. The fragmentation yield is then monitored as a function of the wavenumber in the 2700–3700 cm^{-1} spectral range to retrieve the absorption spectrum of the mass selected precursor ions. All data points shown through this report were obtained using the same procedure. Finally, a trend line: 5-points Fourier transform (FT) rolling averaging is added to guide the eye.

3. Results and discussion

The four sialylated glycans chosen for this study are shown in Scheme 1. **1** and **2** are linkage isomers of milk oligosaccharides, featuring a lactose core (Gal-β-(1–4)-Glc) and a α2,6 or α2,3 sialic acid, respectively. **3** and **4** are model trisaccharides of N-glycan epitopes. They feature a lactosamine (Gal-β-(1–4)-GlcNAc) core with a α2,6 or α2,3 sialic acid, respectively.

3.1. IRMPD spectroscopy of protonated ions

Fig. 1a shows the MS/MS spectra of the milk oligosaccharides in protonated charge state (634 *m/z*) obtained by collision induced dissociation (CID). For both linkage isomers the main fragmentation product corresponds to protonated sialylated moiety, after loss of water (B_3 fragment) or glycosidic cleavage (B_2 and B_1 with traces of C_2 and C1). More surprisingly we observe a strong signal of the Y_2 fragment with traces of Z_2, which correspond to the protonated lactose moiety. None of these fragments are isomer specific.



Scheme 1. Chemical structures of the investigated trisaccharides using the SNFG nomenclature [35].

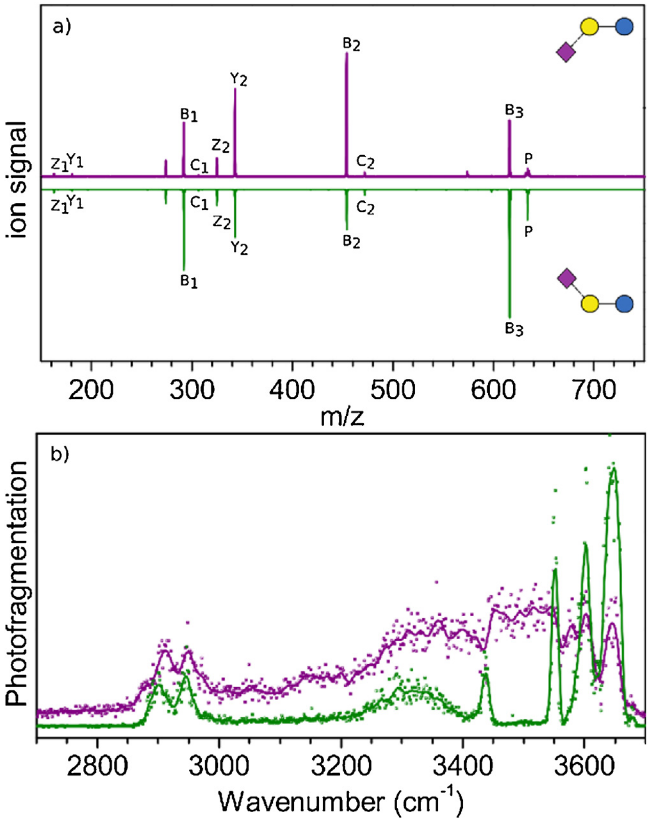


Fig. 1. (a) CID spectra (30 ms of activation time at 15% normalized collision energy) of protonated **1** (purple trace) and **2** (green trace). Precursors are labelled P and fragments are labelled according to the Domon and Costello nomesnclature [36]. (b) IRMPD spectra of protonated **1** (purple trace) and **2** (green trace): photofragmentation yield (dots) and 5 pts FT rolling averaging (line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).