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Monosaccharide composition of glycans based on Q-HSQC NMR

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ABSTRACT

Glycans have essential functions related to structural architecture and specific cell surface phenomena, such as differentiation, biosignalling, recognition and cell–cell interaction, with the carbohydrate structure determining main function in the cell. Due to the importance of the primary structure, the monosaccharide composition is crucial to show the glycan structure. We now present a method for complex carbohydrates based on NMR spectroscopy, which has shown to give similar results to those obtained by the classic GC–MS-carboxy-reduction/deuterium labeling approach. Quantitative HSQC, through J_{C-H} dependence showed 155 Hz as the best value for $^{1}H/^{13}C$ anomeric aldoses, allowing milli-microM detection using conventional inverse probe heads. Combining the quantification of native monosaccharide units of the glycan and those from the hydrolyzed product, a strong correlation occurs between the molecular mobility of the monosaccharide units, giving rise to some insights on the dynamic properties of the parent glycan.

1. Introduction

The biological properties of glycans have been studied extensively, with their carbohydrate moiety having essential roles, contributing to structural architecture, cell integrity, growth, and specific cell surface phenomena, such as differentiation, biosignalling, recognition and interaction with endogenous or exogenous substances (Pinto, Barreto-Bergter, & Taborda, 2008). Due to their related function, the chemical structure determination of native glycoconjugates in terms of their monosaccharide and oligosaccharide chains is essential (Dembitsky, 2004; Pinto et al., 2008; Varki, Freeze, & Manzi, 1995). The latter has been determined by several chromatographic methods, namely thin layer chromatography (TLC), high performance liquid chromatography (HPLC), high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD), capillary zone electrophoresis (CZE) and mainly by gas chromatography–mass spectrometry (GC–MS) (Carpita & Shea, 1989; Kakehi & Honda, 1989; Mariño, Bones, Kattla, & Rudd, 2010; Paulsen, Olasfsdóttir, & Ingólfsdóttir, 2002; Pinto et al., 2008; Sassaki et al., 2008; Zanetta, Timmerman, & Leroy, 1999). However, determining all the structural and functional properties of carbohydrates is difficult and laborious, and generally requires different techniques to achieve desirable results. The strategy depends on the problems to be answered and also the sample amount. Thus, mass spectrometry (MS) techniques for analysis of glycan have become very popular. Combining it with chromatography, chemical and enzyme reactions, allied to tandem MS, it is possible to cover the major information for structural purposes. However, determining conformation, anomeric configuration and sugar binding is quite complicated using MS alone. For these reasons, in order to characterize an unknown glycan, NMR spectroscopy provides all the experimental strategies for structural and conformational determination. Recently, (Lundborg, Fontana, & Widmalm 2011; Souza, Rietkerk, Selin, & Lankhorst, 2013) used 1D and 2D NMR experiments to determine the sugar components in many polysaccharides. Due to the recent developments of cryogenic technology, high field magnets and probe design, the sensitivity of NMR spectroscopy has widened to carry out sub-nanomolecular analyses, giving rise to new perspectives for NMR users in the carbohydrate field. Due to the particular characteristics and NMR versatility, it is now possible to identify glycoconjugates in complex biological

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Table 1
Mutarotation values and $^{3}J_{H1-H2}$ obtained by independent ^{1}H NMR experiments, $^{1}H/^{13}C$ chemical shifts and J_{C-H} by HSQC.

[a]Monosaccharide	Mutarotated mixture (%)		$^{3}J_{H1-H2}$ (Hz)		δ (ppm)	δ (ppm)
	α	β	α	β	α (J_{C-H} Hz)	β (J_{C-H} Hz)
Glc*A*p	48.4	51.6	3.7	8.0	5.255/95.12(172)	4.681/98.86(163)
Man*p*	66.2	33.8	1.7	1.1	5.163/96.88(170)	4.881/96.53(161)
ManNAc*p*	54.1	45.9	1.5	1.6	5.111/95.94(172)	5.011/95.82(162)
Glc*p*	37.5	62.5	3.8	7.9	5.214/94.90(171)	4.629/98.72(162)
ManHep	32.2	67.8	3.6	7.8	5.214/95.17(170)	4.548/99.06(160)
Ara*p*[b]	33.6	60.3	3.5	7.7	5.221/95.44(169)	4.498/99.60(161)
Ara*f*[b]	3.1	5.6	4.4	–	5.204/103.82(173)	5.285/100.44(173)
GlcNAc*p*	55.1	44.9	3.5	8.4	5.187/93.67(172)	4.700/97.76(162)
2-deoxy-Glc	48.3	51.7	3.6	9.8	5.362/94.14(171)	4.919/96.25(162)
GlcHep	11.2	88.8	4.0	8.4	5.127/95.90(171)	4.843/96.84(163)
Xyl*p*	35.0	65.0	3.7	7.8	5.180/95.07(170)	4.556/99.40(162)
$ManNH_2p$[b]	37.6	58.5	1.3	1.6	5.382/93.22(170)	5.191/93.87(164)
$ManNH_2f$[b]	1.4	2.4	5.5	5.6	5.541	5.528
$GlcNH_2$	62.5	37.5	3.6	8.4	5.431/91.99(171)	4.927/95.56(161)
$GalNH_2p$[b]	57.3	40.2	3.7	8.5	5.457/92.13(171)	4.859/95.99(162)
$GalNH_2f$[b]	1.6	0.8	4.9	2.5	5.543	5.489
Fuc*p*[b]	28.6	67.2	3.9	7.9	5.180/95.07(170)	4.533/99.00(160)
Fuc*f*[b]	1.7	2.3	4.2	2.9	5.251	5.204
Rib*p*[b]	20.5	58.9	2.1	6.5	4.914/96.60(167)	4.849/96.33(162)
Rib*f*[b]	13.3	7.4	1.8	3.9	5.231/103.76(173)	5.362/101.7(173)
Gal*p*[b]	32.2	62.4	3.7	7.8	5.246/95.12(172)	4.573/96.71(161)
Gal*f*[b]	2.0	3.2	4. 8	3.3	5.255/97.76(173)	5.194/101.23(173)
Rha*p*[b]	62.4	37.6	1.7	1.1	5.095/96.78(170)	4.849/96.33(162)
GalA*p*[b]	42.1	48.1	3.8	7.9	5.314/95.16(171)	4.603/99.00(161)
GalA*f*[b]	3.9	5.8	4.8	3.6	5.242	5.195

[a] Monosaccharide standards belong to the D-series relative to D-glyceraldehyde. NMR measurements were performed after 24 h of mutarotation to reach the equilibrium at 27 °C.
[b] For the same monosaccharide the pyranoside and furanoside ring forms. The latter is present at least 2% in the equilibrium mixture.

samples, detect contaminants in pharmaceutical products, perform carbohydrate interaction with proteins and develop quantitative analysis of complex carbohydrates (Guerrini et al., 2008; Mayer & Meyer, 1999; Sassaki et al., 2011; Torri & Guerrini, 2008). We now present a method in which monosaccharide composition of complex carbohydrates was determined by quantitative-HSQC through J_{C-H} dependence for the anomeric carbons solely by NMR spectroscopy, which showed similar results to those obtained from the GC–MS-carboxy reduction approaches.

2. Materials and methods

2.1. Monosaccharide analysis

Polysaccharide samples (5 mg) were hydrolyzed with 2 M TFA at 100 °C for 8 h. The solution was then evaporated, and the residue dissolved in D_2O for NMR analysis (400 μL) and adjusted to pH 3.5 using a diluted D_2O-H_2SO_4 solution. After ^{1}H and Q-HSQC experiments, the monosaccharides were reduced with

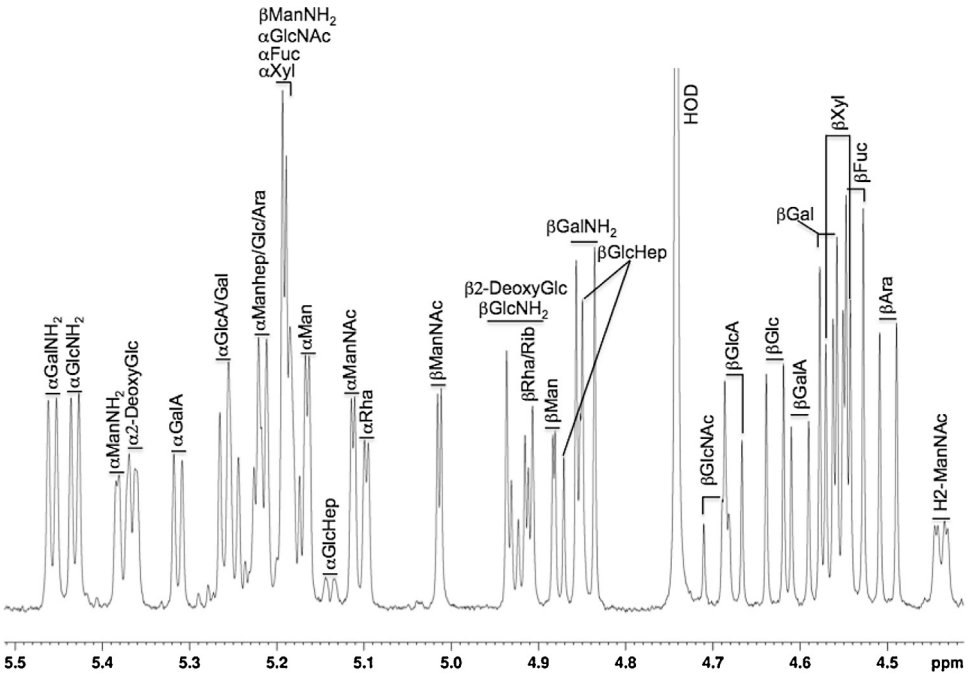

Fig. 1. Partial ^{1}H NMR spectrum of the anomeric region of 18 monosaccharides recorded at 400 MHz in D_2O at 27 °C, using the pulse program noesygppr1d.2: chemical shifts referenced to TMSP-d_4 ($\delta = 0$).