In-Depth Characterization of *N*-Linked Oligosaccharides Using Fluoride-Mediated Negative Ion Microfluidic Chip LC-MS

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SUPPLEMENTAL INFORMATION

Experimental Details:

Chemicals and reagents

1,3-β-Laminarihexaose and 1,4-β-D-cellohexaose were purchased from Megazyme (Bray, Ireland). Galactosylated triantennary (NA3) was purchased from Prozyme (Hayward, CA). The dextran ladder and malto-oligosaccharides, Dp3-7, and all other reagents, including the murine polyclonal antibody, were from Sigma-Aldrich (St. Louis, MO). Standard *N*-glycans were purchased from Prozyme (Hayward, CA), and peptide-*N*-glycosidase F (PNGase F) was from New England Biolabs (Ipswich, MA). LC-MS grade water was obtained from JT Baker (Phillipsburg, NJ) and LC-MS grade acetonitrile from Thermo Fisher Scientific (Fairlawn, NJ). Phytips, packed with 5 µL PGC, were a generous gift from PhyNexus (San Jose, CA). Microfluidic chips packed with PGC or for direct infusion were purchased from Agilent Technologies (Waldbronn, Germany).

Reduction of oligasaccharides

Maltoheptaose (Dp6) or galactosylated triantennary (NA3) was dissolved in 0.1 M sodium borohydride and incubated at 65^{o}C for 1 hour, followed by quenching of the reaction *via* gradual addition of acetic acid. The sample was then purified using PGC packed microextraction Phytips, washed extensively with water, and subsequently eluted with 40% v/v aqueous acetonitrile containing 0.1% trifluoroacetic acid.

Purification of polyclonal human and mouse IgG and N-glycan release

Polyclonal IgG from human and murine serum (Sigma) were purified by Protein G enrichment in a microplate format (Pierce, Rockford, IL). Following elution and buffer exchange into sodium bicarbonate, pH 7.0, the glycans were enzymatically liberated by PNGase F, using an enzyme to

protein ratio of 1:10 (v/v) at 37^{o}C overnight. Following incubation, the *N*-glycans were collected *via* centrifugation through a 10 kDa molecular weight cut-off filter, reduced to dryness *via* vacuum centrifugation and treated with 1% v/v formic acid to promote conversion of the reducing terminal glycosylamine to the corresponding reducing sugar.

Data analysis and spectral interpretation

Data analysis was performed on the Agilent MassHunter software B.02.00. Peaks were obtained using extracted ion chromatograms (EIC) generated with a 50 ppm mass accuracy window. The theoretical fragments from each oligosaccharide were automatically calculated by GlycoWorkBench Version 2.1 (Build 132) [1]. For automated annotation, MS/MS spectra were exported from MassHunter as Mascot generic format (.mgf). Mgf files were then loaded into Glycoworkbench as a peak list. Annotation of MS/MS spectra was performed using a combination of automatic searching with GlycoWorkBench in conjunction with manual verification. Annotation of fragment ions was as previously described by Domon and Costello [2].

Fig. SI-1: Annotated ESI negative ion CID-MS/MS analysis of $[M-H]^-$ ions of infused (A) cellohexaose (glucose-β-1-4-glucose), (B) laminarihexaose (glucose-β-1-3-glucose) and (C) glucose-α-1,6-glucose hexamer. The collision energy was set at 35 V. Additional details in the Experimental Section of the paper.

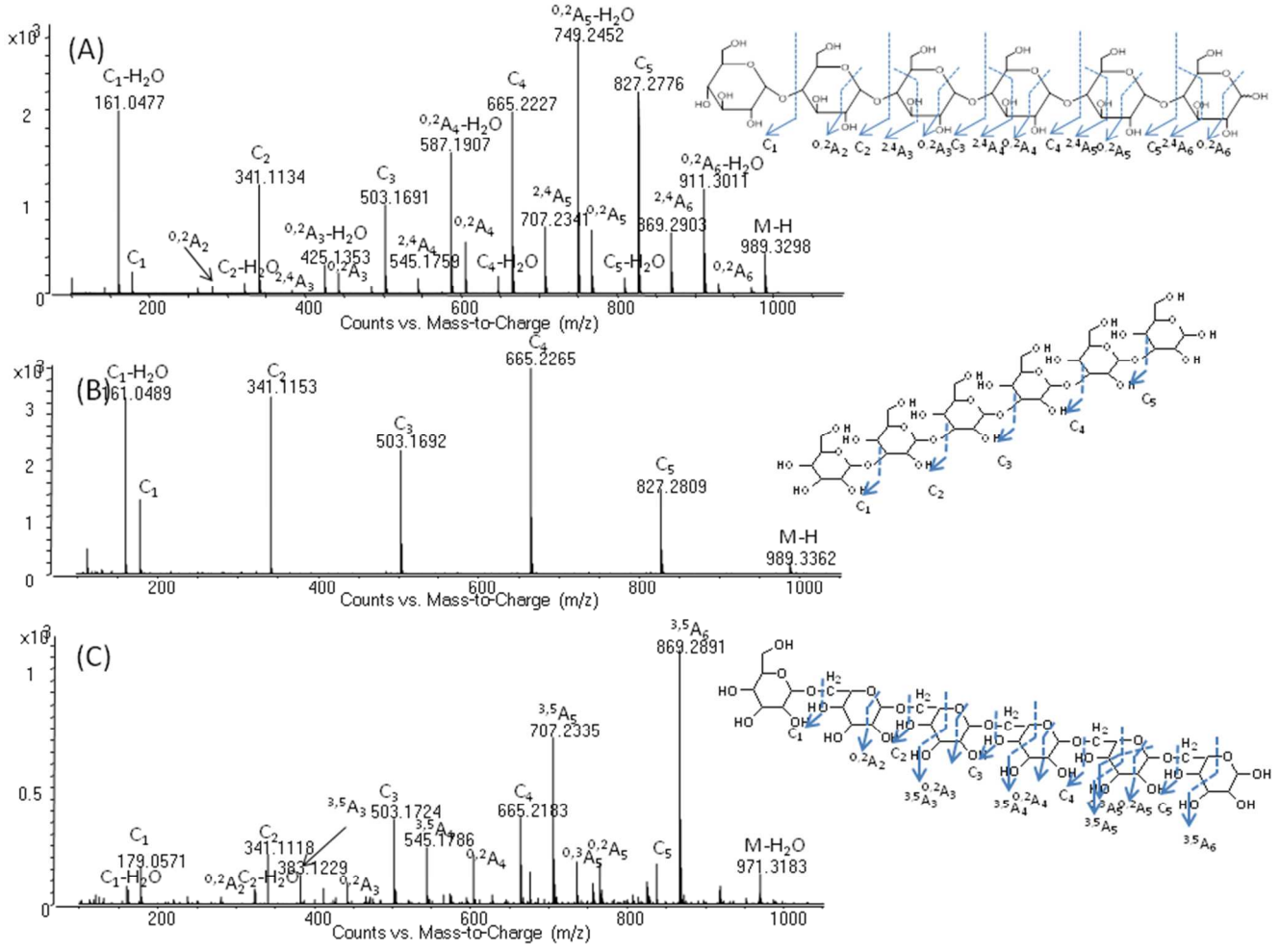

Fig. SI-2: Annotated ESI negative ion CID-MS/MS analysis of infused Dp6: (A) $[2M-H+F]^{2-}$ ion, and (B) $[M+F]^{-}$ ion. The collision energy was set at 35 V. Additional details in the Experimental Section of the paper.

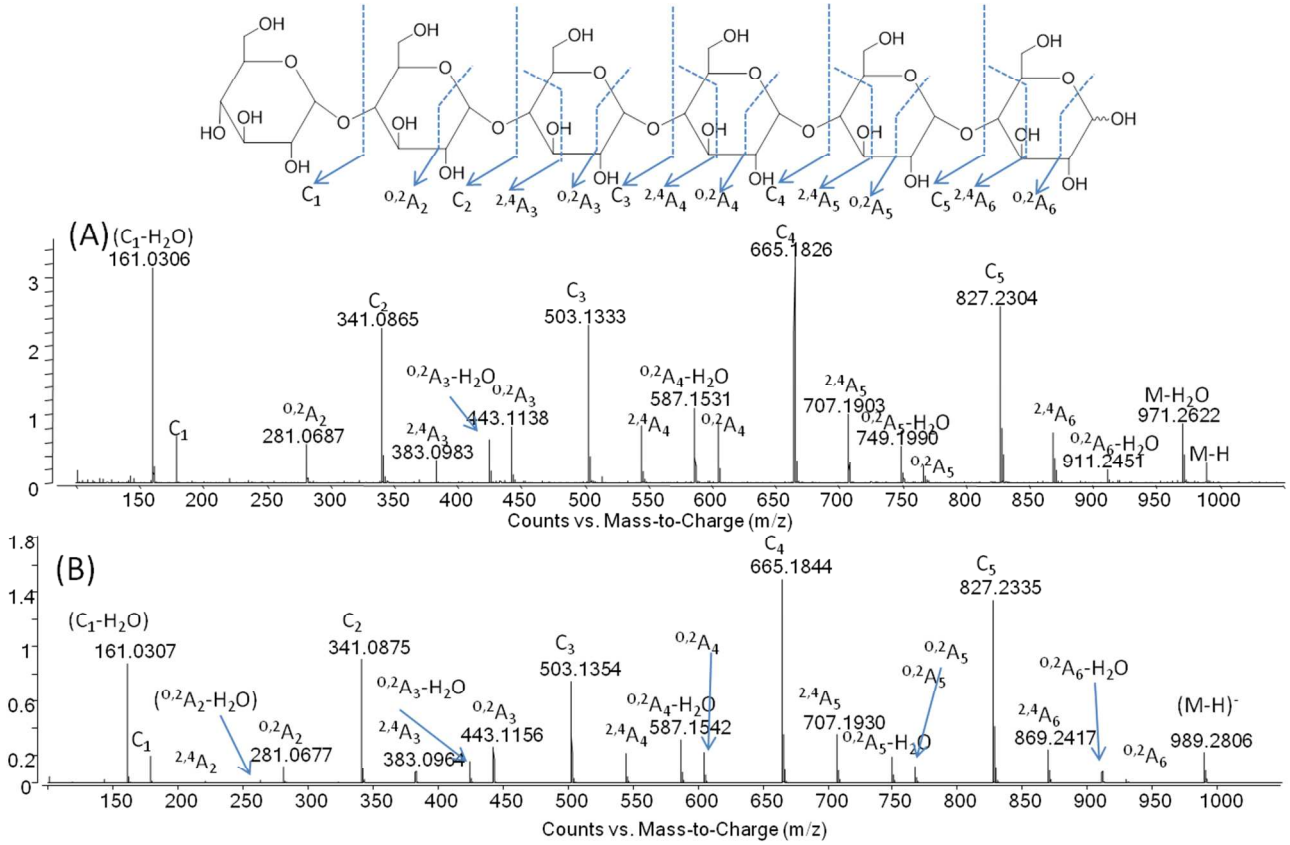

Table SI-1: *N*-linked oligosaccharides identified by fluoride-mediated negative ion chip-LC-MS/MS in the human polyclonal IgG oligosaccharide pool.

Glycans	Theoretical m/z[a]	Observed m/z[a]	Retention time (min)[b]
FA2	730.2649	730.2677	17.6, 18.9
A2G[6]1	738.2624	738.2668	16.2, 16.6
FA2G[6]1 FA2G[3]1	811.2914	811.2956	18.3, 19.6, 19.0, 20.3
A2G2	819.2888	819.2951	17.2[c]
FA2G2	892.3178	892.3225	19.0, 20.2
FA2BG2	993.8575	993.8601	16.4, 17.0
FA2G2S1	1037.8655	1037.8697	20.1, 21.2
FA2BG2S1	1139.4052	1139.4047	18.2, 18.4
FA2G1S1	956.8391	956.8428	19.6, 20.8
FA2BG1S1	1058.3787	1058.3720	17.8[c]
FA2G2S2	1183.4132	1183.4143	20.8, 21.6
FA2BG2S2	1284.9529	1284.9534	18.8, 19.2

a: The charge in each case was -2.
b: The two retention times represent the anomers.
c: Anomer separation was not observed.

Table SI-2: *N*-linked oligosaccharides identified by fluoride-mediated negative ion chip-LC-MS/MS in the murine polyclonal IgG oligosaccharide pool.

Glycans	Theoretical m/z[a]	Observed m/z[a]	Retention time (min)[b]
FA2G[3]1S(Neu5Gc)1	964.8635	964.8633	19.1, 19.6
FA2G2Gal [6]1	973.3442	973.3735	20.7, 21.5
FA2G2S[6]1 (Neu5Gc)	1045.8629	1045.8921	20.1, 21.3
FA2G2S[3]1 (Neu5Gc)	1045.8629	1055.8898	23.8[c]
FA1G[3]1S1 (Neu5Gc)	863.2968	863.3217	19.7, 21.1
FA2G2S1	1037.8655	1037.8898	20.1, 21.4
FA2G2S2 (Neu5Gc)	1199.4081	1199.4412	20.7, 22.0
FA2G2S2	1183.4132	1183.4475	21.1, 22.2
FA2	730.2649	730.2851	17.5, 18.8
FA2G[6]1, FA2G[3]1	811.2914	811.3140	18.1, 19.4
FA2G2	892.3178	892.3423	18.9, 20.2
FA2G2Gal2	1054.3706	1054.3965	22.8[c]
FA2G2Gal[6]1S[3]1 (Neu5Gc)	1126.8893	1126.8860	21.3, 22.4

a: The charge in each case was -2.
b: The two retention times represent the anomers.
c: Anomer separation was not observed.

References:

1 Ceroni, A.; Maass, K.; Geyer, H.; Geyer, R.; Dell, A.; Haslam, S. M. *J Proteome Res.* **2008**, *7*, 1650-1659.
2 Domon, B.; Costello, C. E. *Glycoconjugate Journal.* **1988**, *5*, 397-409.