Charge Profiling of 2AB-labelled N-linked Glycans

The Agilent 1260 Infinity Bio-inert Quaternary LC with Fluorescence Detection

Application Note

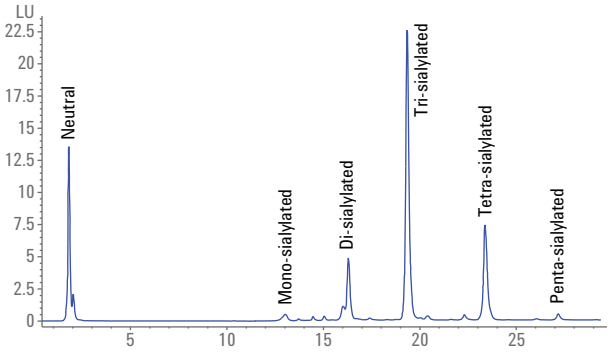
Biologics and Biosimilars

Author

Sonja Schneider
Agilent Technologies, Inc.
Waldbronn, Germany

Abstract

This Application Note describes the analysis of charged N-linked glycans from bovine fetuin using the Agilent 1260 Infinity Bio-inert Quaternary LC with fluorescence detection. Excellent resolution and precision were achieved from zero to five sialic acids attached to the N-glycans using strong anion-exchange chromatography with an Agilent Bio SAX column.



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Introduction

The glycosylation profile of recombinant therapeutic glycoproteins is an important parameter affecting the safety, efficacy, and consistency of the produced glycoproteins[1]. Besides the glycosylation pattern analyzed using HILIC[2], the glycan charge profile can be analyzed using anion-exchange chromatography. The charge profile of N-glycans shows the distribution of N-glycans containing different numbers of anionic sugars, which may include neutral, mono-, di-, tri-, tetra-, and higher-charged glycan structures[3]. Glycan charges are usually due to sialic acids, though in rarer cases they can result from phosphorylation or sulfation of monosaccharide units within the glycan structure. Two types of sialic acid residues are found within the N-glycans attached to proteins expressed in mammalian cells: N-acetyl-neuraminic acid (NeuAc) and N-glycolylneuraminic acid (NeuGc) (Figure 1A). Those structures are usually attached to galactose (Gal) residues at the non-reducing termini of both N- and O-linked glycans (Figure 1B). NeuAc is the normal sialylation that occurs in human proteins, whereas NeuGc is found in non-human mammalian proteins and is undesirable in therapeutic glycoproteins. In addition to a detailed HILIC profile, the glycan charge profile is also an important parameter for biotherapeutic protein monitoring.

A

◆ N-acetylneuramic acid

◇ N-glycolylneuraminic acid

B FA2G2S1, A1F

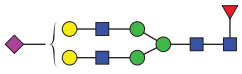

Figure 1. Sialic acids occurring in proteins expressed in mammalian cells. A) Monosaccharide description after the Consortium for Functional Glycomics (CFG). B) An example of the predominant glycan structure of human IgG.

Experimental

The Agilent 1260 Infinity Bio-inert Quaternary LC System consisted of the following modules:

- Agilent 1260 Infinity Bio-inert Quaternary Pump (G5611A)
- Agilent 1260 Infinity High performance Bio-inert Autosampler (G5667A)
- Agilent 1290 Infinity Thermostat (G1330B)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C) with bio-inert solvent heat exchangers (G5616-81000)
- Agilent 1260 Infinity Fluorescence Detector (G1321B), equipped with a bio-inert standard FLD flow cell

Column

Agilent Bio SAX, 2.1 × 250 mm, 5 µm (p/n 5190-2471)

Software

Agilent OpenLAB CDS ChemStation Edition for LC & LC/MS Systems Rev. C.01.06 [61]

Solvents and samples

Glycan samples were 2-AB N-glycans from fetuin, dissolved in 100 mM ammonium formate, pH 4.5. All solvents used were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak). Bovine fetuin, ammonium formate, PNGase F from Elizabethkingia miricola, GlycoProfil 2-AB Labeling Kit and GlycoProfil Glycan Cleanup Cartridges were purchased from Sigma-Aldrich Corp., St. Louis, USA.

Instrumental conditions

Parameter	Value
Mobile phase	A) 20 % acetonitrile: 80 % water B) 20 % acetonitrile: 250 mM ammonium formate in water, pH 4.5
Flow rate	0.2 mL/min
Gradient	Time (min) % B 0 0 5 0 35 100 45 100 41 0
Stop time	41 minutes
Post time	20 minutes
Injection volume	0.5 µL
Thermostat autosampler	5 °C
Column temperature	Ambient
Fluorescence detection	Ex. 260 nm, Em. 430 nm
Peak width	> 0.025 minutes, 0.5 seconds response time, 18.52 Hz

Results and Discussion

N-glycans from fetuin were enzymatically cleaved from the proteins and subsequently labeled with 2-aminobenzamide (2-AB) following cleanup using HILIC cartridges. The glycan charge profile was determined using an Agilent Bio SAX column. Figure 2 shows the charge profile from bovine fetuin, separating neutral (peak 1), mono- (peak 2), di- (peak 3), tri- (peak 4), tetra- (peak 5), and penta-sialylated (peak 6) N-glycans. The structures next to the peaks represent differently charged glycans with zero to five sialic acids that typically occur in bovine fetuin. The peaks were very well resolved and the precision of the analysis was excellent, with relative standard deviations of < 0.002 % for retention time and < 10 % for area precision.

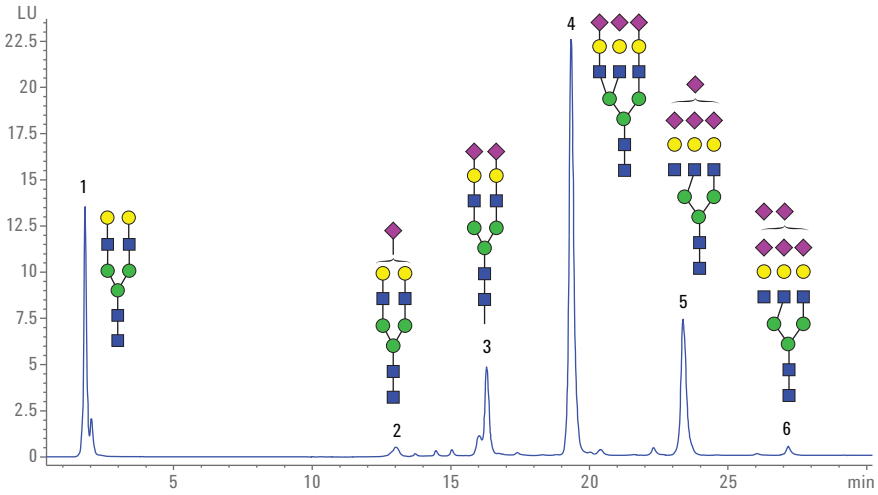

Figure 2. Charge profile of bovine fetuin. The structures are examples of differently charged/sialylated N-glycans, typically occurring in fetuin.

Conclusions

This Application Note demonstrates the charge profiling of N-glycans present on fetuin using anion exchange chromatography on the Agilent 1260 Infinity Bio-inert Quaternary LC System with fluorescence detection. The analysis revealed excellent resolution and precision, separating five differently charged glycans from zero to five sialic acids attached to the glycans.

References

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