Consistent quantitation of APTS-labeled glycans from a wide input range of glycoproteins

Abstract

This application note illustrates the capability of the Applied Biosystems™ 3500xL Genetic Analyzer for Protein Quality Analysis to consistently detect and quantitate 1-aminopyrene-3,6,8-trisulfonate (APTS)–labeled N-glycans from varying levels of glycoprotein input. The results reinforce the robustness of the Applied Biosystems™ GlycanAssure™ glycan analysis system's workflow at low and high glycoprotein input. The GlycanAssure™ platform performs quantitative, high-resolution glycan analysis with high sensitivity and reproducibility, and offers an N-glycan analysis platform for customers working with microbioreactors.

Introduction

Glycosylation plays many important roles in biological processes affecting function, pharmacokinetics, stability, and immunogenicity [1-3]. Consequently, it is critical to monitor glycosylation patterns in recombinant proteins to ensure safety and efficacy as well as consistency in the manufacturing of biopharmaceuticals [4-5]. The most widely used methods for glycan analysis in laboratories today have several disadvantages: a very time-consuming sample preparation procedure with overnight N-glycan release steps, purification steps to remove excess salts and labeling reagents, requirement for a large amount of glycoprotein input, and inconsistent quantitation of highly sialylated glycans. Here we report an integrated solution for glycan analysis that can generate data from 96 samples in a work day (7–9 hr), consisting of easy magnetic bead–based sample preparation, a 24-capillary array–based capillary electrophoresis (CE) instrument, and assay-specific software for analysis (Figure 1).

GlycanAssure glycan analysis system

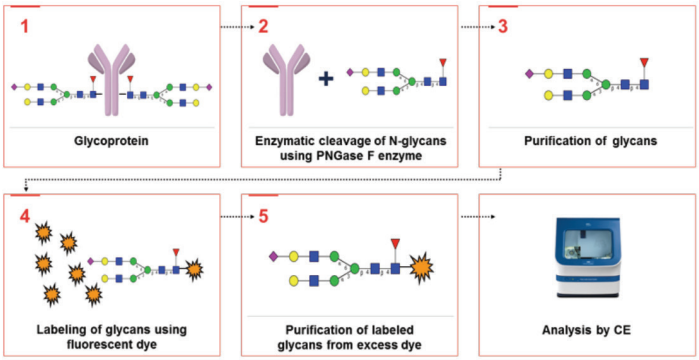

Figure 1. GlycanAssure glycan analysis workflow. A typical glycan analysis requires 3 hours of hands-on time, including sample incubation, and a total of 7–9 hours to process and analyze 96 samples without the need for vacuum centrifugation steps.

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Materials and methods

Varying input amounts (1–100 µg) of purified human serum IgG (Cat. No. 27102) were processed through the GlycanAssure workflow (Figures 2 and 3) and labeled with APTS dye as described in the "N-Linked Glycan Analysis" user guide (Pub. No. 100033998). For each input amount, three independent samples were prepared. Each sample was injected in triplicate for CE separation. All CE separations were performed using the 3500xL Genetic Analyzer (Cat. No. A30556) configured with a 505 nm solid-state laser and laser-induced fluorescence detection. Results were analyzed with Applied Biosystems™ GlycanAssure™ Data Analysis Software v1.0 (Cat. No. A30751).

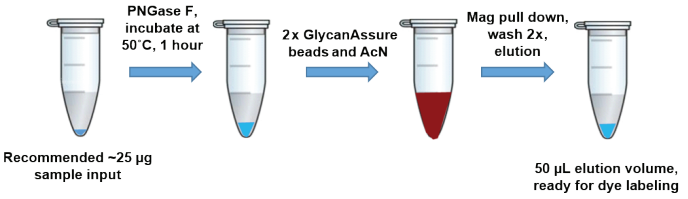

Figure 2. Deglycosylation and purification workflow. The PNGase F enzyme cleaves glycans from proteins, and the glycans are then purified using the magnetic bead–based standard workflow for APTS labeling.

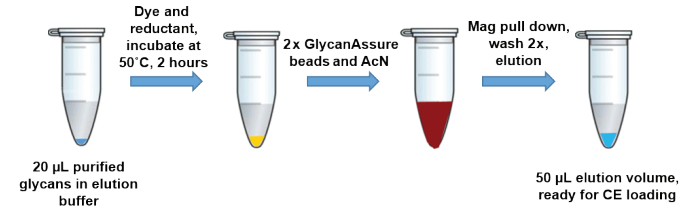

Figure 3. APTS dye labeling and excess dye removal using magnetic beads.

Supplies and run conditions were as follows:

- Applied Biosystems™ POP-7™ Polymer for Protein Quality Analysis
- Applied Biosystems™ Anode Buffer Container (Cat. No. A31278) and Cathode Buffer Container (Cat. No. A31279)
- Applied Biosystems™ 3500xL Genetic Analyzer Capillary Array, 50 cm (Cat. No. 4404689)
- Applied Biosystems™ GlycanAssure™ APTS Kit (Cat. No. A28676)
- Applied Biosystems™ GeneScan LIZ Size Standard (Cat. No. 4408399) used in every injection
- Capillary length: total length = 61 cm, length to detector = 50 cm
- Capillary diameter: 50 µm I.D.
- Injection conditions: 1.6 kV for 24 sec
- Run voltage: 19.5 kV
- Capillary oven temperature: 60°C
- APTS dye Ex/Em 450/515 nm

Results and discussion

The goal of the project was to test the robustness of the GlycanAssure workflow combined with a high-throughput separation method to quantitate complex glycan species associated with therapeutic glycoproteins. Cleaved glycans were purified using magnetic beads and labeled with APTS using optimized labeling conditions. Excess dye was removed using the same magnetic beads used for glycan purification. CE separation was performed using optimized conditions (Figure 4).

We tested low to high glycoprotein inputs of human IgG for deglycosylation and dye labeling. Table 1 evaluates the variation of human IgG input from as low as 1 µg up to 100 µg. The coefficient of variation (CV) of relative peak areas of APTS-labeled glycans was <15% in all cases for peaks above 0.5%. While relative peak intensities were low for the lower inputs, as expected (data not shown), there were no significant differences in the relative peak ratios for APTS-labeled IgG glycans (Figure 5).

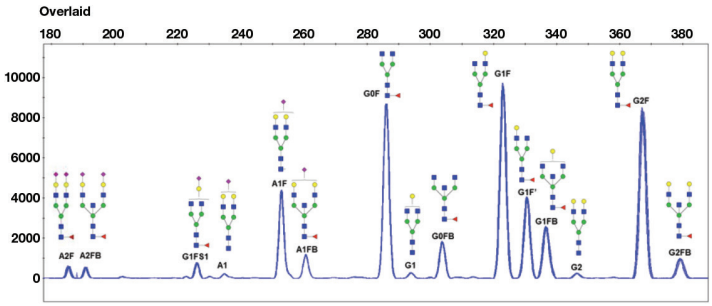

Figure 4. Separation of APTS-labeled N-glycans from human serum IgG.

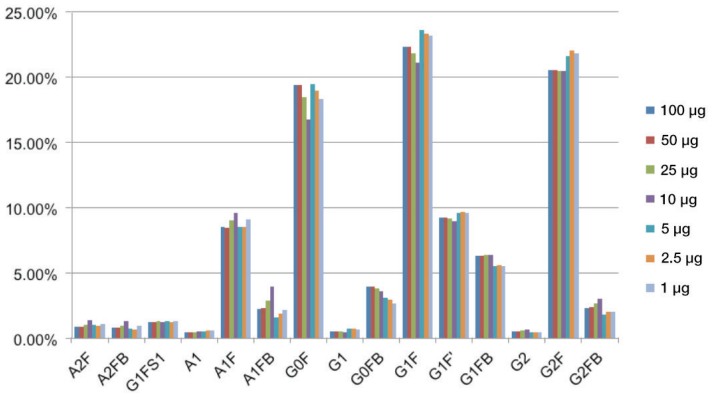

Figure 5. Average APTS-labeled glycan peak areas from 3 independent sample preparations and triplicate injections on the 3500xL analyzer.

Table 1. Consistent relative quantities of glycans from varying inputs of IgG. Three independent sample preparations were performed for every input amount. Each sample was injected in triplicate in the 3500xL analyzer.

	1 µg		2.5 µg		5 µg		25 µg		50 µg		100 µg	
Peak no.	Area (%)	CV (%)	Area (%)	CV (%)	Area (%)	CV (%)	Area (%)	CV (%)	Area (%)	CV (%)	Area (%)	CV (%)
A2F	1.11	12.27	0.96	5.23	1.03	3.60	1.08	4.48	0.93	4.99	0.89	8.25
A2FB	0.96	18.10	0.72	6.87	0.78	11.15	1.01	5.31	0.86	4.28	0.83	8.62
G1FS1	1.32	7.04	1.25	2.83	1.32	4.67	1.32	5.17	1.29	4.49	1.28	3.95
A1	0.61	14.19	0.59	14.17	0.54	13.6	0.47	11.45	0.48	5.16	0.45	3.67
A1F	9.14	4.32	8.53	2.76	8.55	3.97	9.04	1.89	8.51	3.54	8.53	3.64
A1FB	2.20	3.95	1.90	5.78	1.65	3.69	2.92	13.97	2.36	3.60	2.26	7.28
G0F	18.34	6.21	19.03	3.18	19.52	2.83	18.51	0.67	19.39	0.92	19.45	2.00
G1	0.73	12.69	0.77	3.73	0.80	2.86	0.53	2.81	0.56	4.86	0.57	6.40
G0FB	2.73	4.15	2.99	2.25	3.10	1.22	3.86	1.52	3.98	1.39	3.97	1.17
G1F	23.24	1.28	23.35	0.98	23.61	0.40	21.88	1.06	22.39	1.43	22.38	1.07
G1F'	9.66	1.19	9.71	1.82	9.66	1.41	9.21	1.62	9.30	0.83	9.29	1.10
G1FB	5.59	4.89	5.65	2.13	5.56	1.30	6.39	0.58	6.36	0.65	6.36	1.27
G2	0.49	10.45	0.49	5.55	0.46	1.54	0.60	6.16	0.57	3.34	0.57	3.50
G2F	21.83	6.3	22.03	2.02	21.62	1.77	20.49	1.00	20.60	1.19	20.59	1.37
G2FB	2.05	14.73	2.04	4.91	1.82	1.92	2.67	7.78	2.42	2.12	2.37	2.78

Conclusions

The GlycanAssure glycan analysis system offers an integrated N-glycan analysis platform for a range of input levels of glycoproteins. Customers working with limited-volume samples for analytical characterization can expect to get high-quality N-glycan data from their limited samples. The automatable, easy, magnetic bead–based workflow combined with the capability to simultaneously analyze 24 samples offers an integrated glycan analysis platform that can save time and cost in analysis.

References

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