IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 10, Issue 6 Series 1 (Nov. – Dec. 2024), PP 22-28
www.iosrjournals.org

Decoding Phosphorylated Glycans And Molecular Heterogeneity In N-Acetylgalactosamine-4-Sulfatase By High Resolution Mass Spectrometry

Nandeesha N. Puttaraju, Suryakant Kumar, Jothi Prabha S.,
Vinuth M, Saumen Saha*, Rajiv P. Bharadwaj, Sanjib Banerjee
Analytics And Characterization, Biopharma Division, Veeda Clinical Research Limited, P-3/3a, 1st Main Road, Peenya Industrial Area, 1st Stage, Bengaluru 560058, India

Abstract
Glycosylation is the most prevalent and varied covalent modification of recombinant therapeutic proteins. A wide spectrum of roles has been attributed to glycoproteins, reflecting this diversity.N-acetylgalactosamine-4-sulfatase (NAG4S) is a recombinant hu-man enzyme used as replacement enzyme therapy for the treatment of adults and children with mucopolysaccharidosis VI, a rare genetic disorder caused by a deficiency of a lysosomal enzyme. The presence of phosphorylated glycans in NAG4S contributes to the complexity in molecular characterization, attributed to its heterogeneity. This challenge was addressed by comprehensive methods based on mass spectrometry detailing the characterization of N-linked phosphorylated glycans and their associated heteroge-neity in molecular mass in NAG4S through glycopeptide, release N-glycan profiling, and molecu-lar mass analysis by electrospray-ion trap high-resolution mass spectrometry. Phosphorylated glycans were localized to glycopeptides by tryptic peptide mapping, con-firming the site of glycosylation in asparagine located at the 150, 241, 253, 327, 388 and 420 positions. N-glycans were released from NAG4S by glycosidase, labeled, selectively purified, and analyzed by hydrophilic inter-action chromatography coupled to fluorescence and mass spectrometric detection. Six abundant phosphorylated glycans were observed among the pool of standard N-glycans. A molecular mass of ~65-66 kilo Dalton was observed for NAG4S after augmented de-convolution of heterogeneous spectral data.
Keywords: *Galsulfase, Mass Spectroscopy, Glycosylation, Mannose*

Date of Submission: 17-11-2024 Date of Acceptance: 27-11-2024

I. Introduction

Glycosylation, a complex post-translational protein modification, contributes significantly to the increased diversity of the proteome. Glycoproteins encompass a diverse array of carbohydrate units coupled with a variety of glycan-protein linkages. Eukaryotic glycoproteins, in particular, play a pivotal role in numerous cellular functions such as the immunological response, intracellular targeting, intercellular recognition, and protein folding and stability [1-4]. Although the biological function of bacterial glycoproteins requires further investigation, it is evident that glycosylation is essential for pathogenicity and host invasion[5].

Despite the presence of N- and O-linked glycosylation, most glycoproteins predominantly exhibit N-linked oligosaccharides. These oligosaccharides typically adhere to the canonical sequence Asn-Xaa-Ser/Thr, where Xaa represents any amino acid other than proline[6-7]. The carbohydrate moiety shows considerable variability, and bacterial glycoproteins feature a large number of asparagine-linked monosaccharides, including rhamnose, α- and β-glucose, and β-N-acetylgalactosamine[8-9]. In contemporary drug development, the N-linked glycosylation approach in druggable proteins has gained prominence, as the removal of glycosylation sites enhances protein breakdown. Successful characterization of N-linked glycans in multiple monoclonal antibodies has been achieved through sensitive and rapid methodologies [10-12].

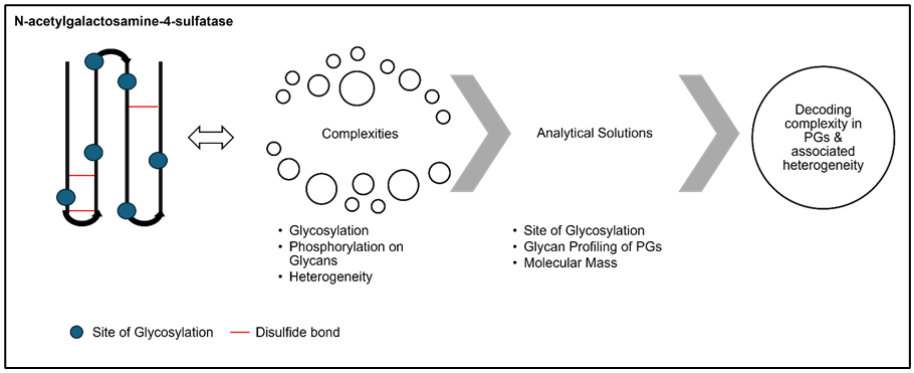

Figure 1. Manifestation of complexities in NAG4S due to presence of glycosylation, phosphorylated glycans and its contribution to molecular heterogeneity with analytical methodologies to characterize the molecule to comprehend the intricacies.

Furthermore, certain glycoproteins exhibit oligosaccharides attached to asparagine/serine/threonine via phosphodiesters, forming a distinct class of glycosylation known as phosphoglycosylation [2,13-15]. This type of glycosylation is observed in various unicellular parasites and slime molds. Mannose-phosphate residues, primarily mannose-6-phosphate, play a crucial role in the transport of proteins from the Golgi apparatus to lysosomes by serving as target signals. Phosphoglycans are essential for the lysosomal targeting of therapeutic enzymes used to treat lysosomal storage disorders, highlighting their importance in therapeutic contexts [16].

NAG4S (generic name: Galsulfase), a glycoprotein comprising 495 amino acids with a molecular weight of approximately 56 kDa, it has six asparagine-linked glycosylation sites, four of which feature bis mannose-6-phosphate (M-6-P) oligosaccharides crucial for specific cellular recognition[17–19]. The recombinant human enzyme NAG4S is used as a replacement enzyme therapy to treat mucopolysaccharidosis VI, a rare hereditary disorder resulting from deficiency of lysosomal enzymes. Most therapeutic enzymes utilized for diseases of lysosomal storage require M-6-P glycans to target the lysosome via the M-6-P receptor on the plasma membrane [16]. Understanding the diversity of phosphorylated glycans (PGs) in such proteins is vital for comprehending their structural integrity and the impact of heterogeneity on molecular characterization. Recent advances in glycan analysis, particularly for PGs using Rapiflour-MS, offer a promising method for analyzing phosphorylated glycans [20–22]. Mapping the site of glycosylation is imperative for continuous monitoring of product development to ensure consistency in glycosylation patterns. Glycosylation, as a heterogeneous factor, poses analytical challenges in characterizing glycoproteins, influencing their safety and efficacy [23]. The phosphorylation of glycans further complicates the determination of the molecular mass of NAG4S. Consequently, a series of mass spectrometry-based methods have been devised for the characterization of N-linked phosphorylated glycans, the determination of glycosylation sites, and the intact mass analysis of NAG4S.

II. Experimental Section

Materials: Recombinant human N-acetylgalactosamine-4-sulfatase in form of Naglazyme® (Biomarin®) was commercially procured for analysis and characterization purposes. Dithiothreitol (DTT), iodoacetamide (IAM), and formic acid were obtained from Sigma-Aldrich (Saint Louis, MO). Acetonitrile (ACN; LCMS grade) was purchased from Biosolve B.V. (Valkenswaard, The Netherlands). Glycoworks Rapifluor-MS glycan kit with phosphoglycan elution kit was purchased from Waters Corporation (Milford, MA).

Site of Glycosylation: NAG4S of 1 mg / ml was used for the analysis. 88 µL of primary stock was taken and denatured using 5% RapiGest at 80±1°C for 15 minutes. The sample was reduced by adding 5 µL of DTT to obtain a final concentration of 10 mM DTT in solution and incubated at 60±1°C for 30 minutes Post-incubation, the samples were allowed to cool and 5 µL of 400 mM IAM was added to obtain a final concentration of 20 mM. The sample was incubated at 25±1°C under dark with mixing for 60 minutes. The sample was subjected to protease digestion using trypsin in an enzyme-protein ratio of 1:20. After enzyme addition, the sample was incubated overnight at 37±1°C. Furthermore, digestion was terminated by adding 5 µL of 20% formic acid. The solution was transferred to vials for LCMS analysis.

Release N-glycan analysis: The released glycan analysis was performed as outlined in the Waters Glycan analysis kit using fast-enzymatic release followed by rapid labeling of N-glycans. 15 µL of the initial stock was added to 7.8 µL of MS grade water. To this 6 µL of 5% RapiGest was added and incubated at 90° C for 3 minutes, followed by cooling at room temperature for 3 minutes. 1.2 µL of the Rapid PNGase F enzyme was added and incubated at 50° C for 5 minutes, followed by cooling for 3 minutes at room temperature. 12 µL of RapiFluor MS solution

was added and incubated at room temperature for 5 minutes. The reaction mixture was diluted with 358 µL of 100% ACN. The wells of the HILIC µelution plate were conditioned with 200 µL of water/well followed by vacuum. Furthermore, the wells were equilibrated with 200 µL 85% ACN followed by vacuum. Approximately 400 µL of samples were loaded into the wells followed by vacuum. The wells were washed 2 times with 600 µL of 1% formic acid : 9% water: 90% ACN solution. Finally, the released glycans were eluted 3 times with 30 µL of GlycoWorks Phosphoglycan (SPE) elution buffer. The obtained eluate was transferred to vials for LCMS analysis.

Intact mass analysis: NAG4S was diluted to 1 mg/mL concentration of acidified LCMS grade water and used for intact mass analysis. The sample was transferred to a vial for LCMS analysis.

LCMS analysis: For intact mass analysis and glycosylation site analysis of NAG4S, the Orbitrap QExactive Mass Spectrometer system from Thermo Scientific coupled with Shimadzu UFLC was used. For intact separation, a 15 min run was performed with the reverse phase method using BioResolve™ RP mAb polyphenyl column, 2.7 µm, 2.1 mm x 150 mm. The result was acquired with a UV detector at 280 nm wavelength with a gradient of 25% to 80% ACN for 15 minutes. For MS, mass spectra have been attained with the recommended method at a spray voltage of 3.8 kV with positive polarity for the mass range of 1800 to 4000 m/z. For the determination of the glycosylation site, the BEH C18 XP XBridge Peptide Column, 2.5 µm, 2.1 mm x 150 mm column, was used for the separation of digested peptides on a 50 minute gradient of 2% to 90% of ACN, with a column temperature of 80° C. Data acquisition was performed using XCalibur and processing on BiopharmaFinder. For intact mass analysis, Byos from Protein Metrics was utilized. Phosphoglycan release analysis was performed on the same system equipped with a fluorescence detector in place of a UV detector. The separation of the phosphoglycan was performed using the HILIC method with the help of Waters Acquity™ UPLC Glycan BEH amide column, 130, 1.7 µm, 2.1 mm x 150 mm column with a run time of 55 minutes. The data were acquired with FLR detector by excited the sample with a wavelength of 265 nm and monitored at a wavelength of 425 nm. Mass spectra have been acquired with the recommended method at a 3.5 kV spray voltage with positive polarity for the mass range 350 to 2500 m/z. After run, data was analyzed using Chromeleon software.

III. Results And Discussions

Site of glycosylation on NAG4S: The presence of glycosylation in NAG4S can be characterized by localizing the site of attachment. Mostly, N-linked glycosylation is known to covalently attach to asparagine residues through a glycosidic bond. The determination of these sites using a tryptic digestion-based approach is more appropriate. Figure 2 shows the base peak intensity chromatogram of the digested NAG4S LCMS run along with the phosphorylated glycan mark. Glycopeptides were identified on the basis of the peptide mapping processing algorithm from BiopharmaFinder software from Thermo Scientific. Asparagine sites were identified and correlated to the glycopeptides, which confirmed their mass-to-charge ratio and retention time. The glycopeptides contained the base glycan structure along with mannose ranging from 4 to 8 and phosphates from 1 to 2. A combination of these glycans diversified the glycopeptide pool.

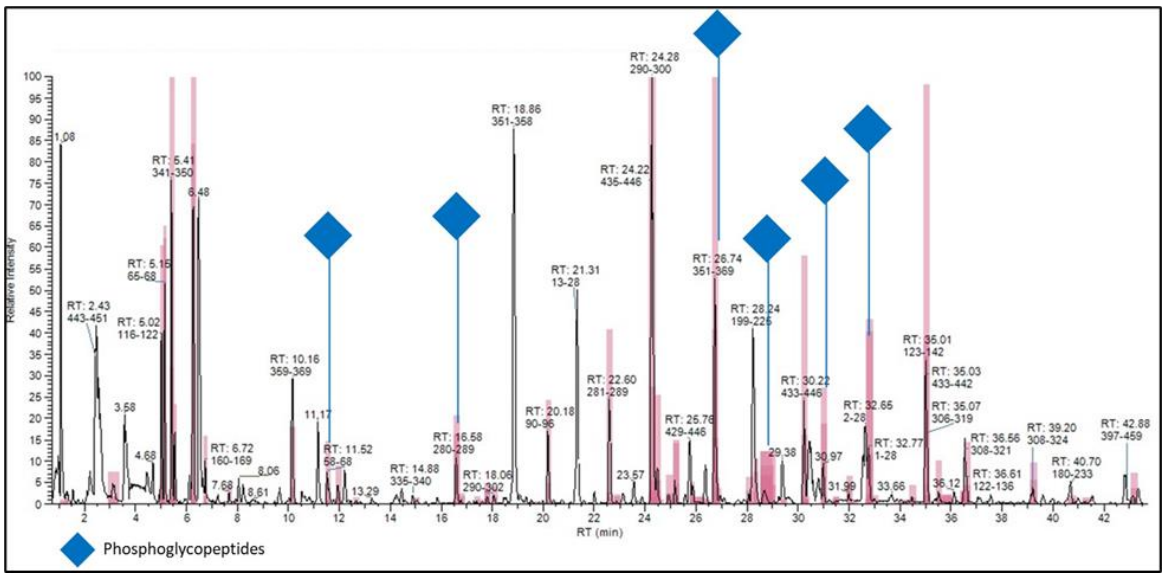

Figure 2. Base peak intensity chromatogram of trypsin-digested Galsulfase with annotations of phosphorylated glycopeptides.

N-linked phosphoglycan analysis in NAG4S: NAG4S harbors a diverse pool of glycans including few phosphorylated glycan species that are primarily N-linked glycans attached to asparagine at defined locations. Rapid deglycosylation, labelling, and selective purification have enabled extraction of all glycan groups, including PGs, into a single pool for chromatographic separation and mass spectrometric detection. Phosphoglycans are of special interest due to their biological properties and their role in the structural manifestation of therapeutic protein [24,25]. Specialized elution buffer from Glycoworks RapiFluor-MS kit ensures the release of PGs from HILIC plates along with non-phosphorylated glycans. The glycan pool is subjected to an amide-based chromatographic separation, fluorescence read-outs, and molecular mass of individual glycans. The equivalence established in both mass and fluorescence traces due to the presence of tertiary amine and quinolinyl fluorophore supports confidence annotations. Figure 3 shows the N-glycan profile of NAG4S. In this glycan profile, there are six major phosphorylated peaks corresponding to this glycan profile; 2P-Man4GlcNAc2-F, P-Man4GlcNAc2, P-Man5GlcNAc2, P-Man6GlcNAc2, P-Man7GlcNAc2, P-Man8GlcNAc2 glycans were identified and annotated. Along with these phosphoglycan peaks, few nonphosphorylated glycan peaks were also observed in processed N-glycan pool analyses. All the observed glycan species with their annotations, retention time (in minutes), mass to charge ratio, and relative percentage have been summarized in Table 1. The extracted ion chromatogram for phosphates is shown in Figure 4. This figure represents the peak 1 [2P-Man4GlcNAc2-F] with m/z 846.84 having two phosphates and one fucose attached. Figure 4 shows peak 2 [P-Man4GlcNAc2] with m/z 732.77 attached with a single phosphate. Similarly, peak 3 [P-Man5GlcNAc2], peak 4 [P-Man6GlcNAc2], peak 5 [P-Man7GlcNAc2], and peak 6 [P-Man8GlcNAc2] are attached to a single phosphate group with m/z 813.80, 894.82, 975.84 and 1056.87 respectively.

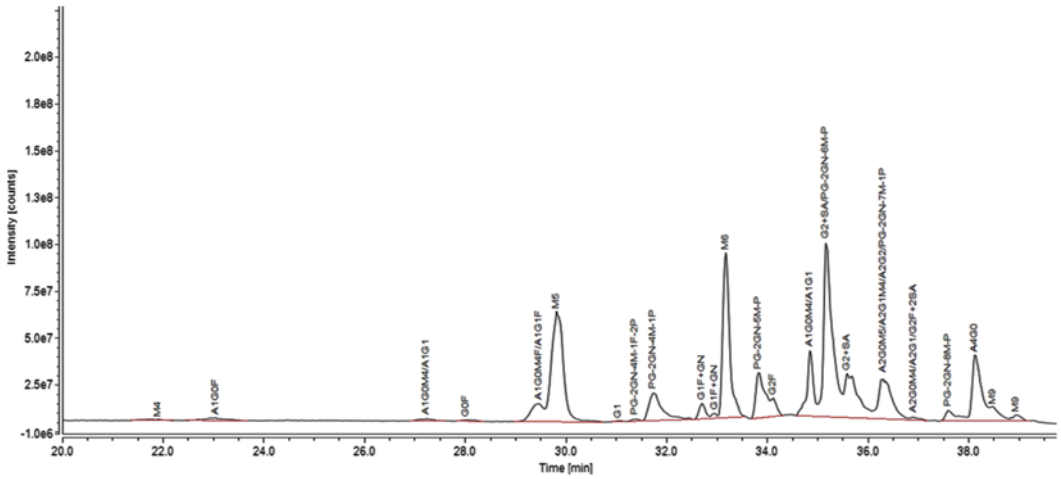

Figure 3. HILIC-UPLC-FLR-ESI-IT-HRMS based mass spectrometry trace of N-glycans released from NAG4S using Glycoworks RapiFluor MS kit containing annotated peaks for both phosphorylated and non-phosphorylated glycans.

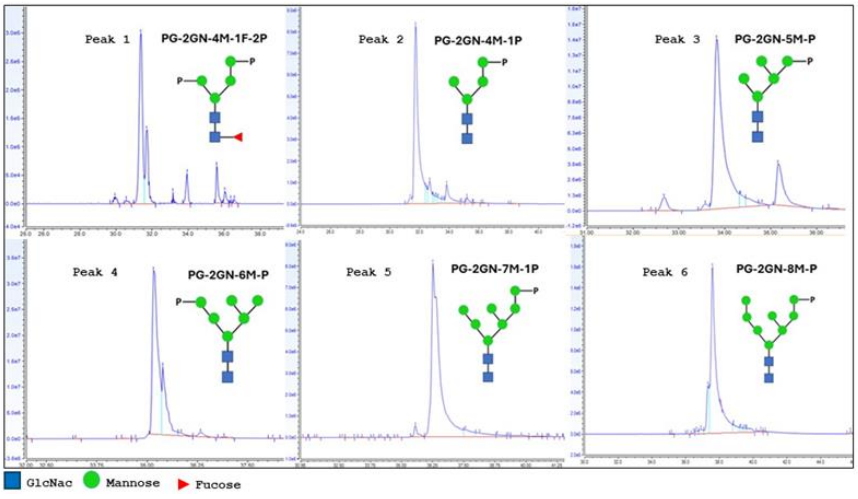

Figure 4. Extracted ion chromatogram of phosphorylated glycans, highlighting the structures of the abundant species observed in the release N-glycan analysis.

Tables

Table 1. List of annotated glycans (including phosphorylated glycans) observed in the N-glycan release analysis of NAG4S.

Glycan Annotation	RT (min)	m/z (M+2H)	%
M4	21.90	692.79	0.25
A1G0F	23.05	786.33	0.76
A1G0M4/A1G1	27.22	794.32	0.36
G0F	28.01	887.86	0.22
A1G0M4F/A1G1F	29.47	867.36	2.78
M5	29.81	773.81	16.77
G1	31.04	895.87	0.06
PG-2GN-4M-1F-2P	31.37	846.84	0.21
PG-2GN-4M-1P	31.73	732.77	4.66
G1F+GN	32.70	1012.90	1.25
G1F+GN	32.96	854.84	0.32
M6	33.17	854.84	14.46
PG-2GN-5M-P	33.82	813.80	5.08
G2F	34.12	1049.92	1.50
A1G0M4/A1G1	34.85	935.86	5.44
G2+SA/PG-2GN-6M-P	35.16	894.82	19.17
G2+SA	35.58	1195.96	7.87
A2G0M5/A2G1M4/A2G2/PG-2GN-7M-1P	36.30	975.84	6.97
A2G0M4/A2G1/G2F+2SA	36.91	894.68	0.34
PG-2GN-8M-P	37.60	1056.87	0.78
A4G0	38.12	1015.83	7.56
M9	38.48	1096.85	1.77
M9	38.94	1096.85	0.64

Resolving Heterogeneity in Molecular Mass of Phosphoglycosylated Protein: The determination of the molecular mass of the therapeutic protein is essential to identify and recognize its primary structural integrity. The mass of glycosylated proteins such as NAG4S confirms the contribution of protein and carbohydrate content to the manifestation of the molecule. The presence of complex glycosylation is known to cause heterogeneity during the mass spectrometry ionization process and generation of non-deconvoluted spectral traces [26]. The availability of intact masses of such complex glycosylated proteins is critical analytical information that supplants the attribute of therapeutic applications. A well-developed method, right from sample preparation to LCMS analysis, defines the data quality, which is further complemented by data processing features. This distribution appeared heterogeneous because of the presence of various phosphoglycan sites that experienced difficulties during sample ionizations. The deconvoluted mass spectrum of NAG4S is shown in Figure 5 which is a result of optimized deconvolution to differentiate baseline and glycoprotein signals. The flexibility offered by Byos software to optimize the processing algorithm to deconvolute these heterogeneous data to produce a clear and measurable spectrum is noteworthy. The molecular mass of the molecule was observed to be between ~65-66 kDa where the protein contribution is ~56 kDa. The rest of ~9-10 kDa is contributed by the glycans present in NAG4S. This observed range of molecular weight is attributed to the multiple glycoforms present in the molecule and results in multiple masses of glycoproteoforms.

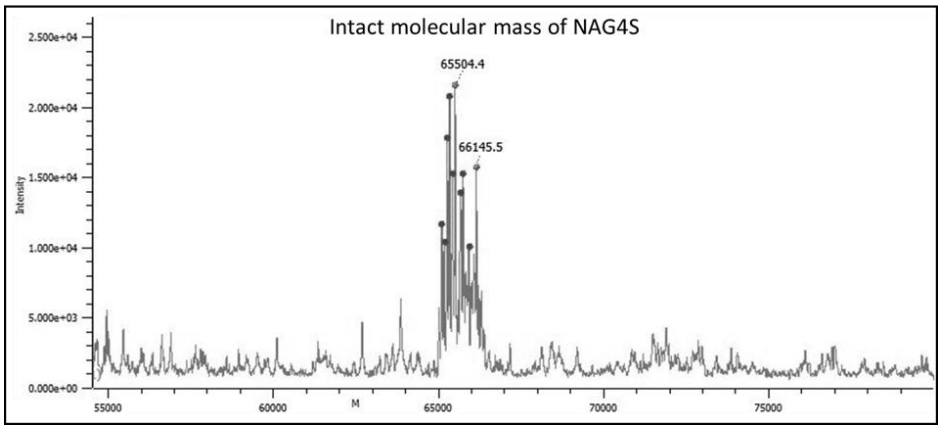

Figure 5. Deconvoluted mass spectra of intact NAG4S with molecular weight ranging from ~65000 to ~66000 Da containing multiple species of glycoproteoforms.

IV. Conclusions

Glycosylation is a pivotal post-translational modification that imparts significant functional diversity and structural heterogeneity to therapeutic proteins. These modifications, involving complex glycan attachments, present formidable analytical challenges because of their intricate nature. Biopharmaceuticals such as etanercept, abatacept, and aflibercept are prime examples of such glycosylated proteins, which exhibit a myriad of N-linked and O-linked glycan structures. Understanding the molecular intricacies of these glycoproteins requires advanced analytical techniques, and high-resolution mass spectrometry is emerging as a powerful tool for unraveling their complexities. Within this landscape, NAG4S stands out as a glycosylated protein adorned with phosphorylated glycans, which requires a thorough and reliable analytical solution for comprehensive characterization. The current suite of analytical methodologies encompasses a multifaceted approach, including the identification of glycosylation sites, profiling of the glycan pool (encompassing both phosphorylated and non-phosphorylated forms), and decoding of intact molecular masses. These analytical techniques collectively provide an in-depth structural evaluation of NAG4S, shedding light on its intricate architecture and functional attributes. Furthermore, these advanced mass spectrometry methods serve as reliable assays for the systematic evaluation of NAG4S in multiple batches. By meticulously assessing critical attributes such as phosphoglycans and intact masses, these techniques contribute significantly to the analytical aspects of chemistry, manufacturing, and controls (CMC). Ensuring the integrity and quality of NAG4S through rigorous analytical validation is paramount for its safe and effective use in therapeutic applications. In conclusion, the complete characterization of glycosylated therapeutic proteins such as NAG4S underscores the importance of advanced analytical techniques in modern biopharmaceutical research and development. By elucidating the complex interplay between glycan structures and protein function, these analytical approaches pave the way for the advancement of precision medicine and targeted therapeutics.

Authors Contributions

NNP, SK, VM and JP performed the experiments and analyzed the data. NNP, SK, and JP interpreted the results. JP, SS and R.P.B wrote the manuscript. RB and SB supervised the project. All authors have approved the final version of the manuscript.

Acknowledgment

The authors acknowledge the support extended by Dr. Dipak Thakur and the team from ACRNS Analytical Technologies Private Limited by providing access to Byos software for deconvolution of mass spectrometry data.

Conflict Of Interest Statement

The authors declare no conflicts of interest related to this study.

Abbreviations

HILIC: Hydrophilic Interaction Chromatography
kDa: Kilo Dalton
LCMS: Liquid Chromatography Mass Spectrometry
NAG4S: N-acetylgalactosamine-4-sulfatase
PG: Phosphorylated Glycan
UPLC: Ultra Performance Liquid Chromatography
UV: Ultraviolet
m/z: Mass to Charge Ratio
RP: Reverse Phase
ACN: Acetonitrile
M-6-P: Mannose-6-Phopshate

References

[1] Varki, A. Biological Roles Of Oligosaccharides: All Of The Theories Are Correct. Glycobiology, 1993, 3(2), 97-130.
[2] Haynes P. A. Phosphoglycosylation: A New Structural Class Of Glycosylation? Glycobiology, 1998, 8, 1–5.
[3] Springer, T.A. Adhesion Receptors Of The Immune System. Nature, 1990, 346, 425–433.
[4] Lasky, L. Selectins: Interpreters Of Cell-Specific Carbohydrate Information During Inflammation. Science, 1992, 258, 964–969.
[5] Upreti, R.K.; Kumar, M.; Shankar, V. Bacterial Glycoproteins: Functions, Biosynthesis And Applications. Proteomics, 2003, 3(4), 363-379.
[6] Marshall, J.J. Application Of Enzymic Methods To The Structural Analysis Of Polysaccharides: Part I. Adv, Carbohydr. Chem. Biochem., 1974, 1(30), 257-370.
[7] Prime, S.; Dearnley, J.; Ventom, A.M.; Parekh, R.B.; Edge, C.J. Oligosaccharide Sequencing Based On Exo- And Endoglycosidase Digestion And Liquid Chromatographic Analysis Of The Products. J. Chromatogr. A., 1996, 720, 263-274.
[8] Lis, H.; Sharon, N. Protein Glycosylation: Structural And Functional Aspects. Eur. J. Biochem., 1993, 218(1), 1-27.
[9] Shibata,S.; Takeda,T.; Natori,Y. The Structure Of Nephritogenoside. J. Biol. Chem., 1988, 263, 12483–12485.

[10] Lauber, M.A.; Yu, Y.Q.; Brousmiche, D.W.; Hua, Z.; Koza, S.M.; Magnelli, P.; Guthrie, E.; Taron, C.H.; Fountain, K.J. Rapid Preparation Of Released N-Glycans For Hilic Analysis Using A Labeling Reagent That Facilitates Sensitive Fluorescence And Esi-Ms Detection. Anal. Chem., 2015, 1987(10), 5401-5409.
[11] Kaur, H. Characterization Of Glycosylation In Monoclonal Antibodies And Its Importance In Therapeutic Antibody Development. Crit. Rev. Biotechnol., 2021, 41, 300-315.
[12] Liu L. Antibody Glycosylation And Its Impact On The Pharmacokinetics And Pharmacodynamics Of Monoclonal Antibodies And Fc-Fusion Proteins. J Pharm Sci., 2015, 104, 1866-1884.
[13] Mehta, D.P.; Ichikawa, M.; Salimath, P.V.; Etchison, J.R.; Haak, R.; Manzi, A.; Freeze, H.H. A Lysosomal Cysteine Proteinase From Dictyostelium Discoideum Contains N-Acetylglucosamine-1-Phosphate Bound To Serine But Not Mannose-6-Phosphate On N-Linked Oligosaccharides. J. Biol. Chem., 1996, 271(18), 10897-10903.
[14] Guha-Niyogi, A.; Sullivan, D.R.; Turco, S.J. Glycoconjugate Structures Of Parasitic Protozoa. Glycobiology, 2001, 11, 45r-59r.
[15] Srikrishna, G.; Wang, L.; Freeze, H.H. Fucose B-1-P-Ser Is A New Type Of Glycosylation: Using Antibodies To Identify A Novel Structure In Dictyostelium Discoideum And Study Multiple Types Of Fucosylation During Growth And Development. Glycobiology, 1998, 8, 799–811.
[16] Kang, J-Y.; Kwon, O.; Gil, J.Y.; Oh, D-B. Data Foranalysisofmannose-6-Phosphate Glycans Labeled With Fluorescent Tags. Data Brief, 2016, 7, 1531–1537.
[17] Tifft, C.; Proud, V.; Levy, P.; Demarco, K.; Nicely, H.; Turbeville, S. Enzyme Replacement Therapy In The Home Setting For Mucopolysaccharidosis Vi: A Survey Of Patient Characteristics And Physicians' Early Findings In The United States. J Infus. Nurs., 2009, 32, 45-52.
[18] Dogan, M.; Cesur, Y.; Peker, E.; Oner, A.F; Dogan, S.Z. Thrombocytopenia Associated With Galsulfase Treatment. Hum. Exp. Toxicol., 2011, 30, 768-771.
[19] White, J.T.; Argento M.L.; Prince, W.S.; Boyer, R.; Crockett, L.; Cox, C.; Van, T.A.; Aguilera, A.; Foehr, E. Comparison Of Neutralizing Antibody Assays For Receptor Binding And Enzyme Activity Of The Enzyme Replacement Therapeutic Naglazyme (Galsulfase). Aaps J., 2008, 10, 439-449.
[20] Liu, X.; Wang, Q.; Lauber, M.A. High Sensitivity Acidic N-Glycan Profiling With Ms-Enhancing Derivatization And Mixed Mode Chromatography. J. Chromatogr. B., 2022, 1191, 123120.
[21] Lauber, M.A.; Brousmiche, D.W.; Hua, Z.; Koza, S.M.; Guthrie, E; Magnelli, P.; Taron, C.H.; Fountain, K.J. Rapid Preparation Of Released N-Glycans For Hilic Analysis Using A Novel Fluorescence And Ms-Active Labeling Reagent, Poster Note, Waters Corporation, 2015.Www. Waters.Com/Posters.
[22] Lauber, M.A.; Morris, M.F.; Brousmiche, D.W.; Koza. S.M. Robustness Of Rapifluor-Ms N-Glycan Sample Preparations And Glycan Beh Amide Hilic Chromatographic Separations, Application Note. Waters Corporation, 2015.
[23] Schachner, L.F.; Mullen, C.; Phung, W.; Hinkle, J.D.; Beardsley, M.I.; Bentley, T.; Day, P.; Tsai, C.; Sukumaran, S.; Baginski, T.; Dicara, D. Exposing The Molecular Heterogeneity Of Glycosylated Biotherapeutics. Nat. Commun., 2024, 15(1), 3259.
[24] Muthana, S.M.; Campbell, C.T.; Gildersleeve, J.C. Modifications Of Glycans: Biological Significance And Therapeutic Opportunities. Acs Chem. Biol., 2012, 7(1), 31-43.
[25] Szabo, Z.; Guttman, A.; Karger, K.L. Rapid Release Of N-Linked Glycans From Glycoproteins By Pressure-Cycling Technology. Anal. Chem., 2010, 82(6), 2588–2593.
[26] Wu, D.; Robinson, C.V. Understanding Glycoprotein Structural Heterogeneity And Interactions: Insights From Native Mass Spectrometry. Curr. Opin. Struct. Biol., 2022, 74, 102351.