Published in final edited form as:
Anal Chem. 2013 April 16; 85(8): 4074–4079. doi:10.1021/ac400106x.

Glycomic Profiling of Tissue Sections by LC-MS

Yunli Hu, **Shiyue Zhou**, **Sarah I. Khalil**, **Calvin L Renteria**, and **Yehia Mechref***
Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX 79409

Abstract

Because routine preparation of glycan samples involves multiple reaction and cleaning steps at which sample loss occurs, glycan analysis is typically performed using large tissue samples. This type of analysis yields no detailed molecular spatial information and require special care to maintain proper storage and shipping conditions. We describe here a new glycan sample preparation protocol using minimized sample preparation steps and optimized procedures. Tissue sections and spotted samples first undergo on-surface enzymatic digestion to release N-glycans. The released glycans are then reduced and permethylated prior to on-line purification and LC-ESI-MS analysis. The efficiency of this protocol was initially evaluated using model glycoproteins and human blood serum (HBS) spotted on glass or Teflon slides. The new protocol permitted the detection of permethylated N-glycans derived from 10 ng RNase B. On the other hand, 66 N-glycans were identified when injecting the equivalent of permethylated glycans derived from a 0.1-μl aliquot of HBS. On-tissue enzymatic digestion of nude mouse brain tissue permitted the detection of 43 N-glycans. The relative peak area of these 43 glycans were comparable to those from a C57BL/6 mouse reported by the Consortium for Functional Glycomics (CFG). However, the sample size analyzed in the protocol described here was substantially smaller than for the routine method (submicrogram *vs.* mg). The on-tissue N-glycan profiling method permits high sensitivity and reproducibility and can be widely applied to assess the spatial distribution of glycans associated with tissue sections, and may be correlated with immunoflourescence imaging when adjacent tissue sections are analyzed.

Keywords

Glycans; Mouse brain section; Permethylation; LC-MS

INTRODUCTION

Glycosylation is one of the most common posttranslational modifications of proteins and is widely involved in many biological processes.[1,2] Aberrant glycosylation occurs in many human diseases, including cancer,[3,4] autoimmune disease[5] and inflammation.[6] Thus, glycans are potentially useful as biomarkers for disease diagnosis and prognosis. Developing reliable quantitative methods are essential for biomarker discovery.

Various glycan analysis methods have been developed on cell lines,[7–11] blood serum[12–20] and large tissue samples.[21,22] Label free quantification strategies and isotopic labeling strategies are the most widely used quantitative methods. Many current preparation methods require relatively large fresh or frozen samples. Bulk tissue samples yield no detailed molecular spatial information and require special care to maintain proper storage and shipping conditions. Glycan analysis on tissue sections offers several advantages over bulk

*Corresponding author: Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX 79409-1061, yehia.mechref@ttu.edu, Tel: 806-742-3059, Fax: 806-742-1289.

material, including smaller sample size, easier storage and shipping conditions, and it provides histological scale information on pathology. Moreover, when several adjacent tissue sections are used, it is possible to correlate N-glycan characterization and immunofluorescence imaging.

Although there are obvious advantages of N-glycan profiling of tissue sections, approaches for extracting N-glycans from tissue histological slices await development. Current sample preparation methods first release glycans from glycoproteins in solution with PNGase F. To remove proteins and salts that might interfere with derivatization and detection, porous graphitized carbon,[19,23–25] Nafion® membrane,[26] cellulose membrane[27] were utilized. Various derivatization methods, such as hydrazone formation,[28,29] permethylation[30–32] and peracetylation,[33,34] are commonly applied for enhanced mass spectrometric (MS) ionization and detection. A clean up step, such as liquid-liquid extraction or solid-phase extraction, is then required to remove excess salts, which can interfere with MS analysis. Although these routine strategies have been shown to increase glycans recovery, they are labor-intensive and time-consuming. They are additionally not suitable for glycomic profiling of tissue sections since they might prompt sample loss and subsequently reduce sensitivity. Glycan analysis on-tissue sections can eliminate sample preparation steps, thus permitting efficient sample preparation method with a minimal number of steps.

Here, we describe a protocol that enables glycomic profiling of the surface of a tissue section. This protocol optimizes glycan sample preparation steps to minimize sample loss and analytical variability. On-plate digestion has previously been reported to yield analytically sufficient quantities of glycans for profiling.[35,36] Enzymatic cleavage of N-linked glycans from glycoproteins is performed on the glass or tissue surface, which is more efficient than digestion in solution. The crude digestion mixtures are then reduced and permethylated without prior purification. Lastly, permethylated N-glycans are on-line purified as we have previously described, thus allowing efficient and sensitive analysis of low abundance materials.[37]

EXPERIMENTAL

Chemicals

Model glycoproteins (including ribonuclease B (RNase B), porcine thyroglobulin (PTG), Fetuin) and pooled human blood serum (HBS) were purchased from Sigma-Aldrich (St. Louis, MO). Other chemicals such as borane-ammonia complex, sodium hydroxide beads, dimethyl sulfoxide (DMSO), iodomethane, trifluoroacetic acid, chloroform, and MS-grade formic acid were also obtained from Sigma-Aldrich. Microspin columns were purchased from Harvard Apparatus (Holliston, MA). Endoglycosidase purified from *Flavobacterium meningosepticum* (PNGase F, 500,000 units/mL) was obtained from New England Biolabs Inc. (Ipswich, MA). Acetic acid was procured from Fisher Scientific (Pittsburgh, PA) while acetonitrile (ACN) was obtained from Fisher Scientific (Fair Lawn, NJ). HPLC-grade water was acquired from Mallinckrodt Chemicals (Phillipsburg, NJ).

On surface enzymatic digestion of model glycoproteins and human blood serum

Several 0.5-μL aliquots of model glycoprotein mixture were deposited on a glass surface while 0.5 μL of HBS was deposited on a Teflon surface. Then, a 0.5-μL aliquot of PNGase F was added to each spot. Enzymatic digestion was performed either at room temperature or in a 37°C water bath. For these analyses, the glass slides were covered to decrease liquid evaporation. A 0.5-μL aliquot of water was added to each spot every 20 minutes to keep it wet. The digestion was allowed to proceed for 4 hours on the model glycoproteins and 8 hours on the HBS.

On-tissue enzymatic digestion of mouse brain section

A 0.5-μL aliquot of PNGase F (50 units) was deposited on the surface of each mouse brain section, spreading to form a spot *ca.* 1.5 mm in diameter. The enzymatic digestion was conducted in a 37 °C water bath for 4 hours. Water was added to each spot every 20 minutes.

Reduction of N-glycan

Released N-glycans were initially collected from the surfaces, and the spots were washed with 1 μL of water five times. The collected liquids were added to the same vial and dried under vacuum. Next, a 10-μl aliquot of an aqueous borane-ammonia complex solution (1 μg/μL) was added to each sample vial and incubated at 65°C for one hour. The incubated mixtures were then dried under vacuum. Methanol was then added into the sample and dried under vacuum. This process was repeated several times to ensure efficient removal of borate salts.

Permethylation of N-glycan

Permethylation was performed according to the previously reported procedure.[30–32] Briefly, an empty column was filled with sodium hydroxide beads. DMSO was added to the column to wash the sodium hydroxide beads. Then, dried sample was resuspended in a solution of 7.5 μL DMSO, 0.3 μL water and 20 μL iodomethane. The sample solution was then applied to the sodium hydroxide column and incubated at room temperature for 30 minutes. Another 20-μL aliquot of iodomethane was then added to the column and allowed to incubate for another 20 minutes. Next, the sodium hydroxide column was first centrifuged and then washed with a 100-μL aliquot of ACN to elute all permethylated glycans. The collected solution was then dried under vacuum.

LC-MS/MS analysis

Permethylated N-glycans were purified and separated using an ultimate 3000 nano-LC system (Dionex, Sunnyvale, CA) which consisted of a loading pump and a separation pump, autosampler, and a switching valve. Sample injection was performed in the microliter pick-up mode. Permethylated samples without additional purification were resuspended in a 20% ACN solution containing 0.1% formic acid and loaded onto an Acclaim® PepMap100 C_{18} nano-trap column (Dionex, Sunnyvale, CA) for on-line purification.[37] Mobile-phase A, which consisted of 2% ACN, 98% water, and 0.1% formic acid, was used to wash the nano-trap for 10 minutes at a flow rate of 3 μL/min. After sample loading, the 10-port valve was switched to separate the samples on an Acclaim® PepMap100 RSLC column (75cm × 15cm, C_{18}, 2μm, 100Å, Dionex, Sunnyvale, CA). The separation was attained using a gradient program starting at 20% mobile-phase B, which consisted of 98% ACN, 2% water, and 0.1% FA. Next, mobile-phase B was then increased to 38% over 11 min followed by an increase to 45% over 32 min. The column was then washed with 90% mobile-phase B for 5 min prior to preconditioning with 20% mobile-phase B for 5 min. The Dionex nano-LC system was interfaced to an LTQ Orbitrap Velos hybrid mass spectrometer (Thermo Scientific, San Jose, CA). MS was acquired in positive-ion mode alternating between an MS scan (mass range m/z 400–2000) and a data-dependent MS/MS scan of the 8 most abundant ions. For the MS/MS scan, the normalized collision energy was set to 30 with a 0.250 Q-value, 15 ms activation time and an isolation width of 3 Da. The dynamic exclusion was set at a repeat count of 2 and exclusion duration of 60 s.

Data Evaluation

Xcarlibur Qual Browser (Thermo Fisher Scientific) was used to generate extracted-ion chromatograms from the full MS scans using glycan isotopic masses with mass tolerance of

10 ppm. A seven-point boxcar smoothing was enabled to improve the peak quality. Peak areas of different charge states and ion adducts were added for quantification evaluation. Tandem mass spectra corresponding to glycan ions were manually annotated and diagnostic fragment ions were utilized to confirm the glycan structures.

RESULTS AND DISCUSSION

N-glycan profiling of model glycoproteins and mouse brain tissue sections was performed according to the abovementioned experimental procedure. Enzymatic digestion was performed on the glass or Teflon surface for glycoproteins and on tissue sections for mouse brain samples. Enzymatic digestions at different incubation times and enzyme concentrations were initially investigated to attain efficient N-glycan enzymatic release (supplementary materials).

Profiling of Permethylated Glycans Derived from Glycoproteins

Optimum on-surface N-glycan release for model glycoproteins was achieved using 4 h incubation at 37°C and an enzyme concentration of 25 U (see supplementary Figures S1–S2). Different model glycoproteins were used to evaluate the efficiency of the sample preparation protocol. Three spots of the same concentration (10 ng) of RNase B glycoprotein were deposited on a glass surface and used to investigate the efficiency and reproducibility of enzymatic digestion. Reduced and permethylated N-glycans released from an 8.5-ng aliquot of RNase B were analyzed by LC-ESI-MS and all five glycans associated with RNase B were detected (Figure S3). Previously, on-plate enzymatic digestion allowed the detection of N-glycans derived from submicrogram amounts (5 μg) of RNase B.[35] The sensitivity of on-glass digestion is higher than that previously reported for solution digestion.[30,37–39] Permethylation increases the ionization efficiencies of glycans and may explain the higher sensitivity observed here.

The relative peak areas of the five identified N-glycans were also compared to previously reported results (Table S1).[39] The relative peak areas of N-glycans vary slightly among the different studies, and may reflect different sources of RNase B. Nevertheless, the glycomic profiles of RNase B N-glycans, in all cases, appear to be analytically comparable. By measuring the peak areas of N-glycans from different spots, we determined that the CV% was less than 10%. The high sensitivity of this protocol and its potential utility for glycomic profiling of small amounts of glycoproteins is evident in the data.

We further explored fetuin as another model glycoprotein to evaluate the efficiency of this protocol since fetuin N-glycans are sialylated complex-type. When 80 ng of fetuin were subjected to LC-MS/MS analysis, five peaks were successfully identified (Figure S4). The distribution of these five N-glycans was comparable to that yielded by in-solution digestion. The data demonstrated that the new protocol effectively released neutral and acidic N-glycans and can be applied to N-glycan profiling of very small samples.

On-surface PNGase F digestion was also very effective in releasing the N-glycans of a mixture of model glycoproteins, including RNase B, fetuin and PTG mixed at 1:2:5 ratios. The on-surface digestion was directly compared to in-solution digestion allowed to incubate for 4 h at 37°C. Figure 1 shows the peak areas of N-glycans derived from the glycoprotein mixture. In total, 14 N-glycans were detected via both methods. The relative peak areas of glycans released by the different digestion methods were highly comparable. On-glass enzymatic digestion was also found to be more efficient than in-solution digestion in releasing N-glycans. The data thus far described strongly suggest the effectiveness of on-surface digestion to release N-glycans from multiple glycoproteins in a mixture.

Profiling of Permethylated Glycans Derived from Human Blood Serum

Next, on-surface digestion was applied to 0.5 μL of HBS. To minimize sample loss from the surface, HBS solution was deposited on a Teflon surface rather than a glass surface. Using Teflon allowed the deposition of smaller sample spots, in order to limit the potential for loss of sample due to evaporation. N-glycans release was achieved at 8 hours incubation time at 37 °C and an enzyme concentration of 50 U. Incubation for 4 h with only 25 U of PNGase F did not produce an efficient release of HBS N-glycans (Figure S5). Again, released N-glycans were collected, reduced and permethylated as described above without any purification steps. To determine if a purification step was needed, the results of a sample purified with charcoal prior to reduction were compared to those of a sample not subjected to any purification. The comparison suggested that sample loss was associated with the purification step (Figure S6). Accordingly, the usual purification step is not required when analyzing small amounts of HBS. It may be that the salts and proteins associated with such a small sample (0.5 μL HBS) do not adversely influence permethylation. Moreover, attempts to purify such a small volume (0.5 μL) with charcoal will bring about substantial sample loss.

We believe that the efficient analysis of small sample volumes was aided by the online purification of permethylated glycans which we have recently described.[37] Injecting the equivalent of 0.1 μL HBS sample subjected to PNGase F allowed the detection of 66 glycan compositions (Figure 2). A 10-μL aliquot of HBS routinely yields 73 N-glycan compositions[37] and the smaller number of detected here is due to the much smaller amount of HBS analyzed (0.1 μL vs. 1 μL). The missing compositions were those of low abundances. The relative peak areas of common N-glycans among all studies, however, were comparable.[37,39]

The reproducibility of this protocol was examined by processing N-glycans from three different spots. The standard deviations of 66 glycan compositions are shown in Figure 2. The average %CV for the protocol was *ca.* 20%. Part of this %CV is attributed to ESI. For peptide quantification, LC-ESI-MS run-to-run CV has been reported to be 13%.[40] This suggests that the reproducibility of on surface N-glycan digestion and sample preparation methods prior to LC-MS/MS is better than 10%. Lebrilla and coworkers[41] recently analyzed dried blood spots (~2 μL) and identified 44 native glycan compositions, 39 of which are detected by our new protocol. In another study, 19 native glycans were identified from 0.1 μL of HBS subjected to on-line PNGase F digestion prior to separation on an activated charcoal chip.[42] This relatively small number of glycans may reflect the limit of the loading capacity of charcoal chip used. The higher number we observed relative to others may be explained as a result of the permethylation of glycans prior to LC-MS/MS analysis. The addition of methyl groups increases the hydrophobicity of glycans and permits higher MS ionization efficiency. Alley *et al.*[13] have reported the identification of 18 permethylated N-glycans from 0.25 μL of HBS. This low number of detected glycans might be partially attributed to the use of a chip with a limited sample loading capacity. Here, we detect more glycans from very small samples with the new protocol because enzymatic digestion on the sample surface is highly efficient, permethylation increases ionization efficiency in ESI, and eliminating charcoal purification steps conserves sample material.

Profiling of Permethylated Glycans Derived from Mouse Brain Sections

The data described above indicate the high efficiency of on-surface release of N-glycans from glycoproteins associated with simple and complex biological samples (i.e., HBS). The protocol was then employed to enzymatically release N-glycans from the surface of mouse brain tissue sections. A 0.5-μL aliquot of PNGase F was deposited on the surface of tissue spreading to cover a spot approximately 1.5 mm in diameter (Figure 3 insert). N-glycan

profiling of mouse brain sections utilizing this method is depicted in Figure 3. We identified a total of 43 glycan compositions, or four more than the 39 glycan compositions identified from the analysis of 100 mg to 400 mg of mouse brain tissue reported by the Consortium for Functional Glycomics (CFG) (Table S2, Table S3 and Figure S7). The relative peak areas of 32 common glycan compositions in our study and CFG studies were comparable. The differences between the two set of results may be related to the use of different mouse species. CFG used C57BL/6 mice, while nude mouse brain tissue was analyzed in this study. Despite using minute amounts of sample (submicrogram amounts of a tissue section) as compared to 100's of mg of brain tissue used by CFG, the number of detected N-glycans was found to be highly comparable. In a recent study, only neutral N-glycans, including five high-mannose-type and one complex-type, were detected from mouse brain tissue section using hydrazinolysis method.[43] The released N-glycans were purified using a graphitic carbon cartridge to remove hydrazine. The procedure also involved purification on a cellulose column after pyridylamine derivatization. These purification steps may have reduced the number of glycans detected.

To evaluate the repeatability of the sample preparation approach, twelve spots from 3 brain tissue sections were collected and analyzed. All of the 43 glycans were detected in the 12 spots, including 5 high mannose and 38 complex type glycans. The average %CV of peak areas of the glycans in the twelve spots was *ca.* 35%. We believe this high %CV indicates variation in the spatial distribution of glycans across a brain tissue section. A high %CV is common among biological replicates, which is what the spots represent. The much lower %CV observed in the case of the technical replicates described in earlier sections are also worth noting in this context.

The new protocol was further compared with in solution digestion, by scratching a mouse brain tissue section from glass for analysis. Fewer N-glycans detected under this procedure, and the peak areas of detected N-glycans were 2-fold lower (data not shown). The molecular spatial information available from on-tissue digestion was lost when analyzing this homogenized tissue.

CONCLUSION

A highly efficient sample preparation method developed for N-glycan profiling of tissue sections, permitting the detection of 43 N-glycans from mouse brain sections. The new protocol permits the detection of more glycans from very small samples not only because enzymatic digestion on the sample surface is highly efficient, but also because eliminating charcoal purification conserves sample material and the permethylation step increases MS ionization efficiency. It also offers practical advantages in terms of submicrogram scale sample size and consequent ease of sample storage and shipping and in terms of increased throughput since sample preparation time is reduced to several hours. Enzymatic digestion time is reduced to 4–8 h and the charcoal purification step is eliminated. Finally, and most promising in terms of discovering glycan biomarkers of human disease, this new protocol for N-glycans analysis can obtain molecular spatial information of tissue pathology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by an NIH grant (1R01 GM093322-01). We thank Dr. Paul Lockman of Texas Tech University, Health Sciences Center, School of Pharmacy, Amarillo, for providing the mouse brain tissue sections.

REFERENCES

1. Drake PM, Cho W, Li B, Prakobphol A, Johansen E, Anderson NL, Regnier FE, Gibson BW, Fisher SJ. Clin. Chem. 2010; 56:223–236. [PubMed: 19959616]
2. Hart GW, Copeland RJ. Cell. 2010; 143:672–676. [PubMed: 21111227]
3. Mechref Y, Hu Y, Garcia A, Hussein A. Electrophoresis. 2012; 33:1755–1767. [PubMed: 22740464]
4. Mechref Y, Hu Y, Garcia A, Zhou S, Desantos-Garcia JL, Hussein A. Bioanalysis. 2012; 4:2457–2469. [PubMed: 23157355]
5. van Kooyk Y, Rabinovich GA. Nature Immunology. 2008; 9:593–601. [PubMed: 18490910]
6. Kreisman LS, Cobb BA. Glycobiology. 2012; 22:1019–1030. [PubMed: 22492234]
7. Machado E, Kandzia S, Carilho R, Altevogt P, Conradt HS, Costa J. Glycobiology. 2011; 21:376–386. [PubMed: 21030537]
8. de Leoz MLA, An HJ, Kronewitter S, Kim J, Beecroft S, Vinall R, Miyamoto S, White RV, Lam KS, Lebrilla C. Dis. Markers. 2008; 25:243–258. [PubMed: 19126968]
9. Li B, An HJ, Kirmiz C, Lebrilla CB, Lam KS, Miyamoto S. J. Proteome Res. 2008; 7:3776–3788. [PubMed: 18642944]
10. Vercoutter-Edouart A-S, Slomianny M-C, Dekeyzer-Beseme O, Haeuw J-F, Michalski J-C. Proteomics. 2008; 8:3236–3256. [PubMed: 18651673]
11. Goetz JA, Mechref Y, Kang P, Jeng M-H, Novotny MV. Glycoconj. J. 2009; 26:117–131. [PubMed: 18752066]
12. Kyselova Z, Mechref Y, Kang P, Goetz JA, Dobrolecki LE, Sledge GW, Schnaper L, Hickey RJ, Malkas LH, Novotny MV. Clin. Chem. 2008; 54:1166–1175. [PubMed: 18487288]
13. Alley WRJ, Madera M, Mechref Y, Novotny MV. Anal. Chem. 2010; 82:5095–5106. [PubMed: 20491449]
14. Alley WRJ, Novotny MV. J. Proteome Res. 2010; 9:3062–3072. [PubMed: 20345175]
15. Mechref Y, Hussein A, Bekesova S, Pungpapong V, Zhang M, Dobrolecki LE, Hickey RJ, Hammoud ZT, Novotny MV. J. Proteome Res. 2009; 8:2656–2666. [PubMed: 19441788]
16. Isailovic D, Kurulugama RT, Plasencia MD, Stokes ST, Kyselova Z, Goldman R, Mechref Y, Novotny MV, Clemmer DE. J. Proteome Res. 2008; 7:1109–1117. [PubMed: 18237112]
17. An HJ, Miyamoto S, Lancaster KS, Kirmiz C, Li B, Lam KS, Leiserowitz GS, Lebrilla CB. J. Proteome Res. 2006; 5:1626–1635. [PubMed: 16823970]
18. Zhao J, Patwa TH, Qiu W, Shedden K, Hinderer R, Misek DE, Anderson MA, Simeone DM, Lubman DM. J. Proteome Res. 2007; 6:1126–1138. [PubMed: 17249709]
19. Kyselova Z, Mechref Y, Bataineh MM, Dobrolecki ALE, Hickey RJ, Vinson J, Sweeney CJ, Novotny MV. J. Proteome Res. 2007; 6:1126–1138. [PubMed: 17249709]
20. Hua S, An HJ, Ozcan S, Ro GS, Soares S, DeVere-White R, Lebrilla CB. Analyst. 2011; 136:3663–3671. [PubMed: 21776491]
21. Haslam SM, North SJ, Dell A. Curr. Opin. Struct. Biol. 2006; 16:584–591. [PubMed: 16938453]
22. Chen Y-J, Wing DR, Guile GR, Dwek RA, Harvey DJ, Zamze S. Eur. J. Biochem. 1998; 251:691–703. [PubMed: 9490042]
23. Packer NH, Lawson MA, Jardine DR, Redmond JW. Glycoconjugate J. 1998; 15:737–747.
24. Liu X, McNally DJ, Nothaft H, Szymansk CM, Brisson J-R, Li J. Anal. Chem. 2006; 78:6081–6087. [PubMed: 16944887]
25. Kronewitter SR, de Leoz MLA, Peacock KS, McBride KR, An HJ, Miyamoto S, Leiserowitz GS, Lebrilla CB. J. Proteome Res. 2010; 9:4952–4959. [PubMed: 20698584]
26. Börnsen KO, Mohr MD, Widmer HM. Rapid Commun. Mass Spectrom. 1995; 9:1031–1034.
27. Mechref Y, Muzikar J, Novotny MV. Electrophoresis. 2005; 26:2034–2046. [PubMed: 15841499]
28. Walker SH, Budhathoki-Uprety J, Novak BM, Muddiman DC. Anal. Chem. 2011; 83:6738–6745. [PubMed: 21774516]
29. Walker SH, Lilley LM, Enamorado MF, Comins DL, Muddiman DC. J. Am. Soc. Mass Spectrom. 2011; 22:1309–1317. [PubMed: 21953184]

30. Kang P, Mechref Y, Klouckova I, Novotny MV. Rapid Commun. Mass Spectrom. 2005; 19:3421–3428. [PubMed: 16252310]
31. Kang P, Mechref Y, Novotny MV. Rapid Commun. Mass Spectrom. 2008; 22:721–734. [PubMed: 18265433]
32. Mechref Y, Kang P, Novotny MV. Methods Mol. Biol. 2009; 534:53–64. [PubMed: 19277536]
33. Chao CS, Chen MC, Lin SC, Mong KK. Carbohydr. Res. 2008; 343:957–964. [PubMed: 18258219]
34. Dell A. Methods Enzymol. 1990; 193:647–660. [PubMed: 2074840]
35. Mechref Y, Novotny MV. Anal. Chem. 1998; 70:455–463. [PubMed: 9470483]
36. Orlando R, Colangelo J. Anal. Chem. 1999; 71:1479–1482. [PubMed: 10204046]
37. Desantos-Garcia JL, Khalil SI, Hussein A, Hu Y, Mechref Y. Electrophoresis. 2011; 32:3516–3525. [PubMed: 22120947]
38. Gennaro LA, Harvey DJ, Vouros P. Rapid Commun. Mass Spectrom. 2003; 17:1528–1534. [PubMed: 12845576]
39. Hu Y, Mechref Y. Electrophoresis. 2012; 33:1768–1777. [PubMed: 22740465]
40. Barnidge DR, Goodmanson MK, Klee GG, Muddiman DC. J. Proteome Res. 2004; 3:664–652.
41. Ruhaak LR, Miyamoto S, Kelly K, Lebrilla CB. Anal. Chem. 2012; 84:396–402. [PubMed: 22128873]
42. Jmeian Y, Hammad LA, Mechref Y. Anal. Chem. 2012; 84:8790–8796. [PubMed: 22978794]
43. Yoshimuraa T, Yamadaa G, Narumia M, Koikea T, Ishiia A, Selac I, Mitrani-Rosenbaumc S, Ikenaka K. Anal. Biochem. 2012; 423:253–260. [PubMed: 22369894]

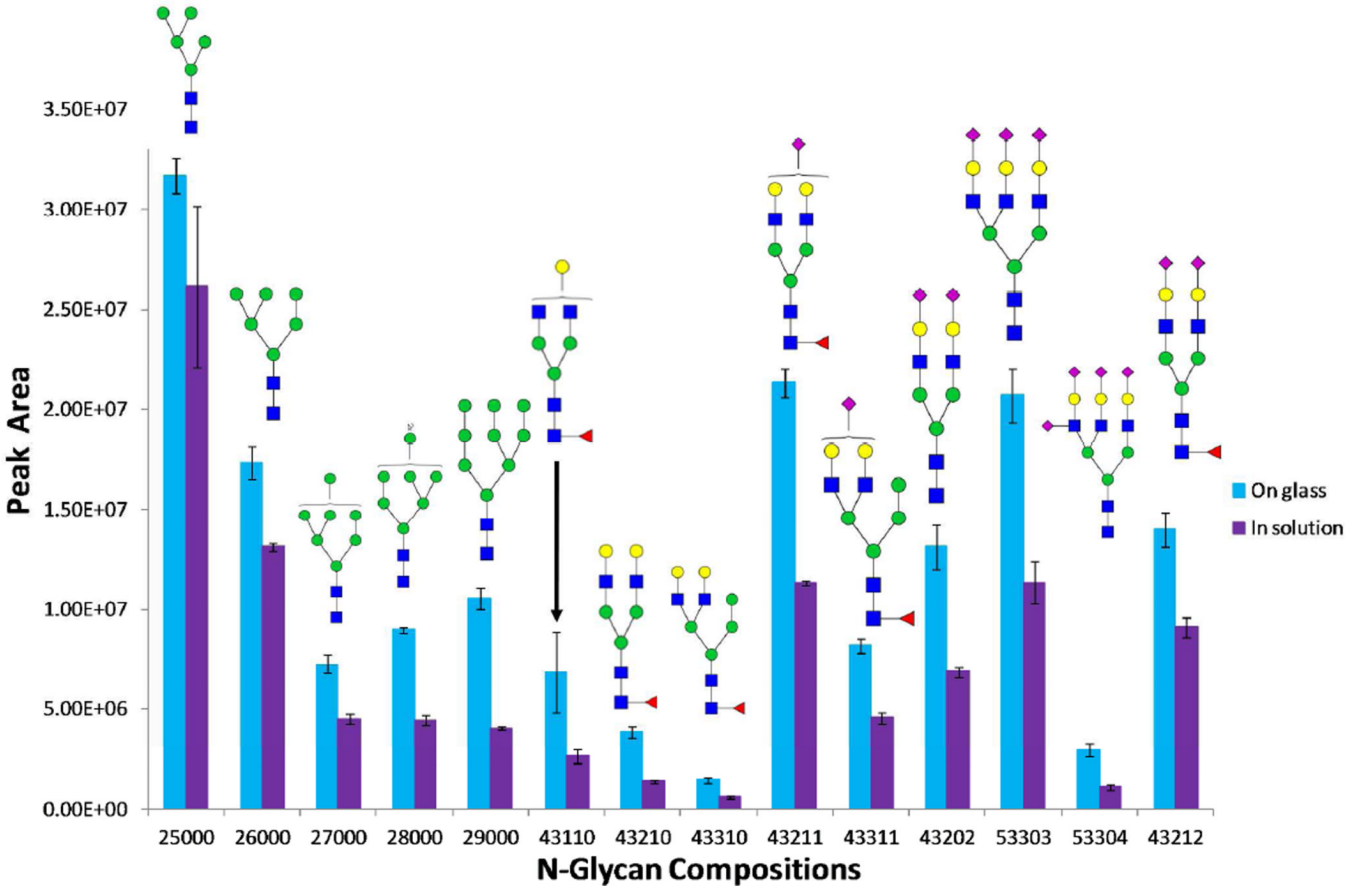

Figure 1.

Bar graphs of the LC-ESI-MS peak areas of permethylated N-glycans derived from model glycoprotein mixtures through on-glass (blue) and in-solution (purple) digestions. Error bars represent the standard deviations (N=3 technical replicated). Model glycoproteins include RNase B, fetuin and PTG. The x-axis represents glycan compositions (GlcNAc, Man, Gal, Fuc, NeuNAc). Symbols; GlcNAc, blue squares; Man, green circles, Gal, yellow circles, NeuNAc, magenta diamonds.

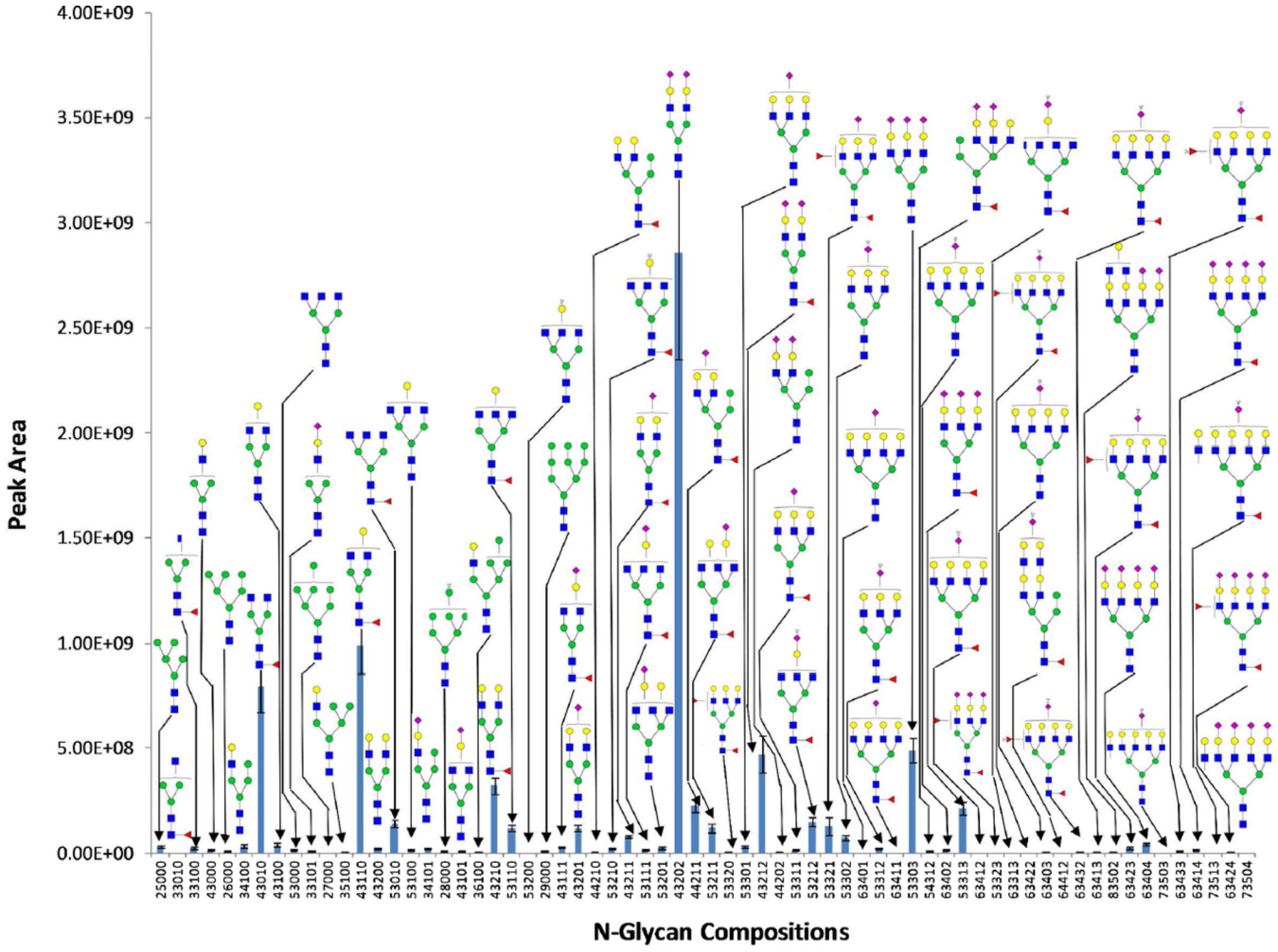

Figure 2.

Bar graphs of the LC-ESI-MS peak areas of permethylated N-glycans derived from analyzing a 0.1 μL aliquot of HBS (a 0.5 μl aliquot of HBS was subjected to sample preparation procedure as described in Methods). Error bars represent the standard deviations (N=3 technical replicates). The x-axis represents glycan compositions (GlcNAc, Man, Gal, Fuc, NeuNAc). Symbols as in Figure 1.

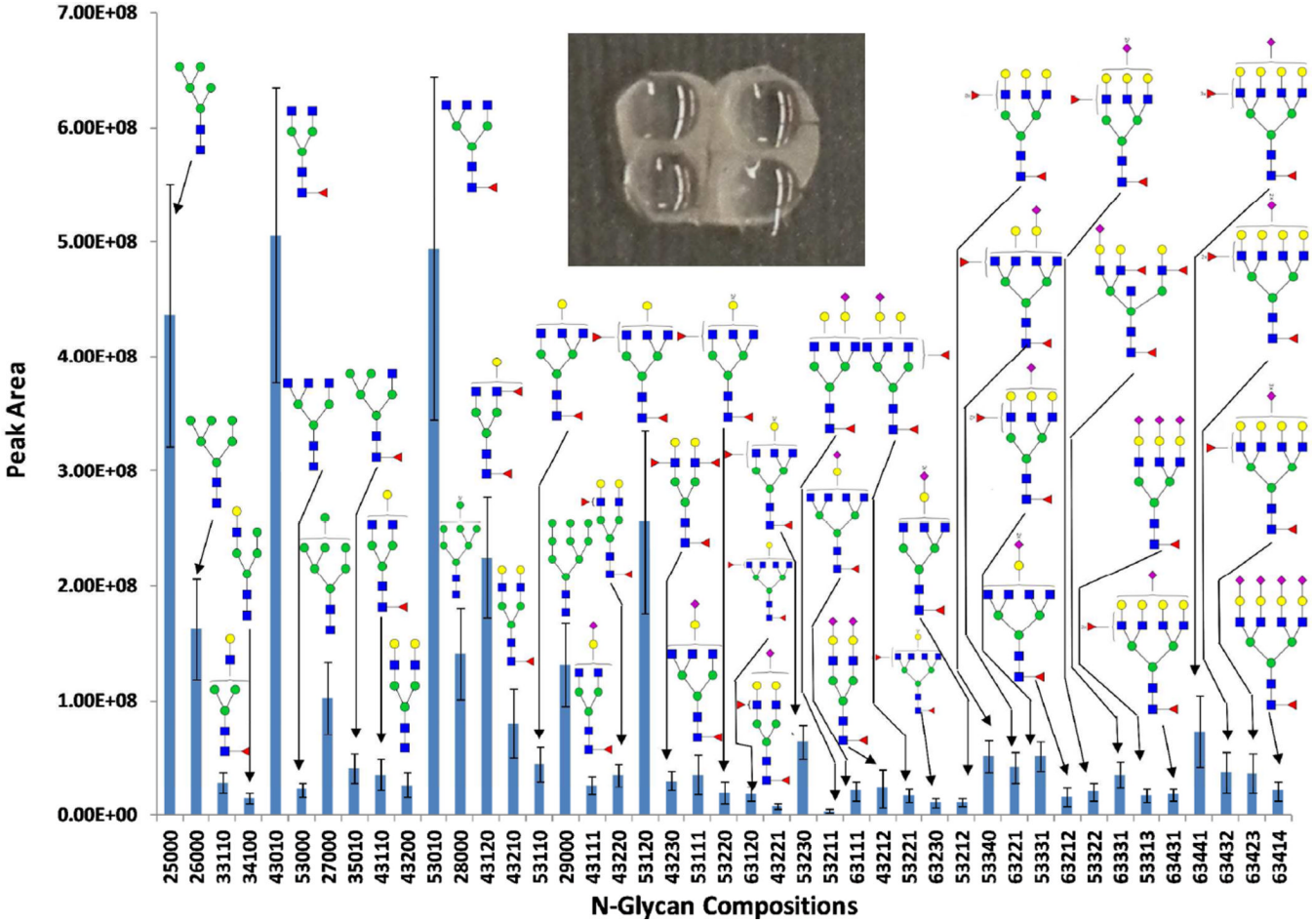

Figure 3.

Bar graphs of the LC-ESI-MS peak areas of permethylated N-glycan derived from mouse brain sections. Error bars represent the standard deviations (N=12 biological replicates). Inset depicts an image of a tissue section with 4 spots of PNGase F. The x-axis represents glycan compositions (GlcNAc, Man, Gal, Fuc, NeuNAc). Symbols as in Figure 1.