Analytical Biochemistry 567 (2019) 82–84

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Glycosaminoglycans in human cerebrospinal fluid determined by LC-MS/MS MRM☆

Yanlei Yu[a], Fuming Zhang[b], Wilfredo Colón[a], Robert J. Linhardt[a,b,c,d], Ke Xia[a,*]

[a] Department of Chemistry and Chemical Biology, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY, 12180, USA
[b] Department of Chemical and Biological Engineering, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY, 12180, USA
[c] Department of Biology, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY, 12180, USA
[d] Department of Biomedical Engineering, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY, 12180, USA

ABSTRACT

Glycosaminoglycans (GAGs) were recovered from human cerebral spinal fluid (CSF) and after their conversion to disaccharides using polysaccharide lyases were analyzed by liquid chromatography tandem mass spectrometry using multiple reaction monitoring. CSF showed ng/mL levels of heparan sulfate, chondroitin sulfates and hyaluronan. The amounts and disaccharide composition of these GAGs differed from those found in human plasma. This approach may offer a new method for the discovery of biomarkers for diseases of the central nervous system.

Glycosaminoglycans (GAGs) are linear and anionic polysaccharides, populating the cell surface and extracellular matrix, which are biosynthesized in the Golgi or at the cell membrane [1]. GAGs perform a wide spectrum of important biological functions in development, homeostasis, and disease, ranging from controlling cell-cell interactions, cell signaling, cell proliferation and interact with many different proteins [2–5]. When classified by their core, GAGs can be divided into four major families: heparan sulfate/heparin (HS/HP), chondroitin sulfate/dermatan sulfate (CS/DS), keratan sulfate (KS) and hyaluronan (HA) based on their disaccharide (dp2) repeating units comprising their backbone structure. These units consist of an amino sugar, either *N*-acetyl-D-glucosamine (D-GlcNAc) or *N*-acetyl-D-galactosamine (D-GalNAc), and either an uronic acid, D-glucuronic (D-GlcA) or L-iduronic acid (L-IdoA), or galactose in the case of KS. The linkage positions and configurations vary in all GAGs with the exception of HA, which contain sulfo esters, and HS/HP can also contain sulfamide substituents [1].

The analysis of GAGs is less fully developed than the analysis of other biopolymers, proteins and nucleic acids. The most important structural determination of a GAG is its disaccharide composition analysis as this provides information on the family to which it belongs, and in the case of sulfated GAGs its level of substitution may serve as potential biomarkers for the detection of disease [6–8]. One of the most convenient methods to obtain this information relies on the enzymatic depolymerization of the GAG polysaccharide using polysaccharide lyases [9] followed by the separation and determination of the resulting disaccharides/oligosaccharides [10]. Recently a very sensitive analytical method relying on liquid chromatography tandem mass spectrometry (LC-MS/MS) with multiple reaction monitoring (MRM) has been introduced for the ultrasensitive determination of GAGs in biological fluids [11].

GAG-based biomarker discovery has primarily focused on biological fluids, such as urine and plasma due to their easy access [11,12]. Few studies have examined the presence and type of GAGs present in cerebral spinal fluid (CSF) and none of these have provided detail characterization of GAG families or substitution levels [13–17].

In the current study we investigated a pooled CSF sample (PrecisionMed, Inc., San Diego, CA) and CSF samples from three individuals collected locally following consensus guidelines for blood biobanking [18] (St. Peter's Hospital, Albany, NY, under informed consent and Institutional Review Board approval). All samples were immediately aliquoted and snap frozen and kept at −80 °C until further processed.

The overall work flow is illustrated in Fig. 1. Samples of CSF (100 μL) were defrosted at 4 °C and desalted by passing through a 3-kDa molecular weight cut-off spin column and washed twice with distilled water. Digestion buffer (300 μL of 50 mM ammonium acetate containing 2 mM calcium chloride, pH 7.0) was added to the filter unit and a mixture (10 mU each) of recombinant heparin lyase I, II, III, chondroitin lyase ABC were added to each sample. The samples were placed in a water bath at 37 °C and digested overnight. The reaction was terminated by removing the enzymes by centrifugation in a 3K Da MWCO

☆ Prepared for *Analytical Biochemistry* as a Notes & Tips, November 2018. Revised December 2018.

* Corresponding author.
E-mail address: Xiak@rpi.edu (K. Xia).

https://doi.org/10.1016/j.ab.2018.12.013
Received 12 November 2018; Received in revised form 14 December 2018; Accepted 14 December 2018
Available online 18 December 2018
0003-2697/ © 2018 Published by Elsevier Inc.

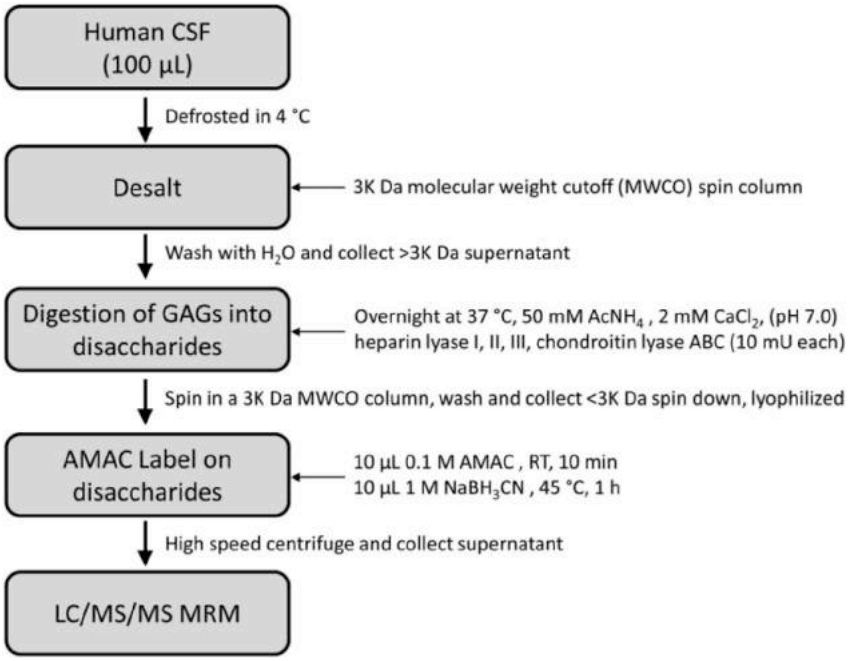

Fig. 1. Analytical workflow for quantitative measurement GAG content and composition in human CSF. Excess AMAC is removed as a precipitate in the final step and residual AMAC (eluting as an early peak) is diverted from the mass spectrometer.

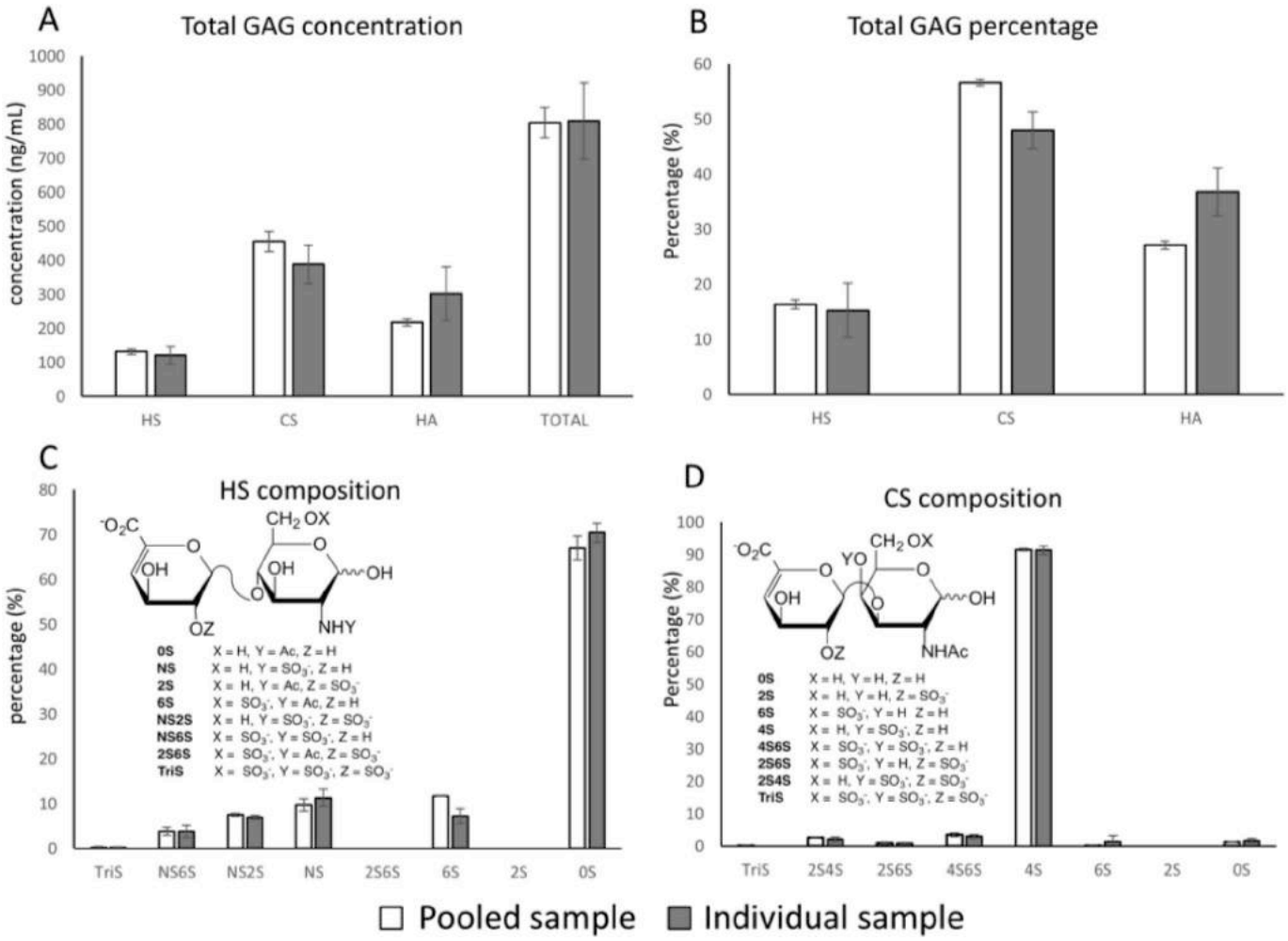

Fig. 2. CSF disaccharide analysis results in the pooled (white bars) and individual (gray bars) samples. A. Total concentration of CSF GAGs (the sum of the GAG disaccharides observed in lyase-digested CSF based on a standard curve prepared from the 17 disaccharide standards); B. percentage of CSF HS, CS and HA; C. CSF HS composition; D. CSF CS composition. Result of pooled sample shows the analytical error bar from triplicate analysis and the result from three individual sample shows the biological error bar.

column at 13000 × *g* for 20 min. The filter unit was washed with 300 μL of distilled water and the filter through was collected and lyophilized.

The dried samples were labeled with 2-aminoacridone (AMAC) by adding 10 μL of 0.1 M AMAC in dimethyl sulfoxide (DMSO)/acetic acid (17:3, v/v), and incubated at room temperature for 10 min, followed by adding 10 μL of 1 M aqueous sodium cyanoborohydride ($NaBH_3CN$). The mixture was incubated at 45 °C for 1 h and centrifuged at 10000 × *g* for 10 min. Supernatant was collected and analyzed using LC-MS/MS MRM performed on an Agilent 1200 LC system connected to a triple quadrupole mass spectrometry system equipped with an ESI source (Thermo Fisher Scientific, San Jose, CA). Mobile phase A was 50 mM ammonium acetate, mobile phase B was methanol. An Agilent Poroshell 120 ECC18 (2.7 μm, 3.0 × 50 mm) column was used at a flow rate of 300 μL/min. The gradient was 0–10 min, 5–45% B; 10–10.2 min, 45–100% B; 10.2–14 min, 100% B; 14–22 min 100-5% B. MRM mode was used in MS experiments with the following parameters: negative ionization mode with a spray voltage of 3000 V, a vaporizer temperature of 300 °C and a capillary temperature of 270 °C. MRM parameters of are provided in Table S1.

The average raw total GAG content was 804 ng/mL in pooled sample and 809 ng/mL in individual samples. CS, at 455 ng/ml pool and 387 ng/mL individual, was the most abundant GAG (avg. 52%), followed by HA, at 218 ng/mL pool and 301 ng/mL individual (avg. 32%), and HS, at 132 ng/mL pool, 120 ng/mL individual (avg. 16%) (Fig. 2 A and B).

There were two previous LC-MS/MS studies, one reported average HS levels of 160 ng/mL [16], while the other reported average levels of HS of 140 ng/mL and CS of 670 ng/mL [15], respectively. Our results of 126 ng/mL HS (average of 132 ng/mL and 120 ng/mL) and 421 ng/mL CS (average of 455 ng/mL and 387 ng/mL) compares favorably with these reported values. Another study reported 50 ng/mL HS in CFS using a thrombin activity assay, which has a limit of detection of 30 ng/mL [13]. The current study is the first report looking at the disaccharide composition of HS and CS in CSF (Fig. 2C and D). HS was comprised principally of the 0S disaccharide (68.7%), followed by the NS (10.5%), 6S (9.5%), NS2S (7.2%), NS6S (3.8%) and TriS (0.2%) disaccharides. No significant amounts of 2S or 2S6S disaccharides were observed in any samples. CS was comprised principally of the 4S disaccharide (91.4%), followed by 4S6S (3.2%), 2S4S (2.3%), 0S (1.5%), 6S (0.9%), 2S6S (0.8%), and TriS (0.1%) disaccharides. Compared with human plasma, another well-studied bio-fluid used in biomarker discovery, human CSF contains 5-fold more HS (126 ng/mL in CSF vs. 20 ng/mL in plasma), 3-fold less HA (260 ng/mL in CSF vs. 800 ng/mL in plasma), and 6-fold less CS (421 ng/mL in CSF vs. 2400 ng/mL in plasma) [12]. Furthermore, the compositions of HS and CS in CSF and plasma were very different with HS in plasma comprised of ~90% 0S and ~10% NS and CS comprised primarily of ~58% 0S and ~41% 4S with trace amounts (0.2%) of 6S disaccharides.

MRM detection facilitated sensitive detection of the high-resolution LC separation of all 17 labeled CS, HS, and HA disaccharides, making it essential for the analysis in biological samples by avoiding interference from other substances co-extracted from such complex biological samples. The sensitivity of MRM afforded an LOD of < 2 ng/mL, and was robust showing only 5% variability between analytical replicates. CSF from different individuals, biological replicates, showed 15% variability. This very sensitive and robust MRM method is necessary since certain HS and CS disaccharides are found in the low ng/mL range in normal CSF. Previous MRM analysis has been successfully applied to other tissues or bio-fluids [8,11,12]. Future studies are planned to examine the GAG composition of CSF in various disease states.

Acknowledgement

This work was supported by grants from the National Institutes of Health (Grants HL125371, GM38060, GM090127, HL096972, and HL10172 to R.J.L.)

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ab.2018.12.013.

References

[1] U. Lindahl, J. Couchman, K. Kimata, J.D. Esko, Proteoglycans and sulfated glycosaminoglycans, in: A. Varki, R.D. Cummings, J.D. Esko, P. Stanley, G.W. Hart, M. Aebi, A.G. Darvill, T. Kinoshita, N.H. Packer, J.H. Prestegard, R.L. Schnaar, P.H. Seeberger (Eds.), Essentials of Glycobiology, third ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor (NY), 20172015-2017. Chapter 17.

[2] S. Mizumoto, S. Yamada, K. Sugahara, Molecular interactions between chondroitin-dermatan sulfate and growth factors/receptors/matrix proteins, Curr. Opin. Struct. Biol. 34 (2015 Oct) 35–42.

[3] D. Xu, J.D. Esko, Demystifying heparan sulfate-protein interactions, Annu. Rev. Biochem. 83 (2014) 129–157.

[4] R.J. Linhardt, T. Toida, Role of glycosaminoglycans in cellular communication, Acc. Chem. Res. 37 (7) (2004) 431–438.

[5] I. Capila, R.J. Linhardt, Heparin-Protein interactions, Angew. Chem. Int. Ed. 41 (2002) 390–412. Angewandte Chemie, 114, 426-450, 2002.

[6] E. Makino, H. Klodnitsky, J. Leonard, J. Lillie, T.C. Lund, J. Marshall, J. Nietupski, P.J. Orchard, W.P. Miller, C. Phaneuf, D. Tietz, M.L. Varban, M. Donovan, A. Belenki, Fast, sensitive method for trisaccharide biomarker detection in mucopolysaccharidosis type 1, Sci. Rep. 8 (2018) 3681.

[7] C. Auray-Blais, P. Lavoie, S. Tomatsu, V. Valayannopoulos, J.J. Mitchell, J. Raiman, M. Beaudoin, B. Maranda, J.T. Clarke, UPLC-MS/MS detection of disaccharides derived from glycosaminoglycans as biomarkers of mucopolysaccharidoses, Anal. Chim. Acta 936 (2016 Sep 14) 139–148.

[8] G. Li, L. Li, E.J. Joo, J.W. Son, Y.J. Kim, J.K. Kang, K. Bok Lee, F. Zhang, R.J. Linhardt, Glycosaminoglycans and glycolipids as potential biomarkers in lung cancer, Glycoconj. J. 34 (2017) 661–669.

[9] R.J. Linhardt, P.M. Galliher, C.L. Cooney, Polysaccharide lyases, Appl. Biochem. Biotechnol. 12 (1986) 135–177.

[10] K.G. Rice, Y.S. Kim, A.C. Grant, Z.M. Merchant, R.J. Linhardt, High-Performance liquid chromatographic separation of heparin derived oligosaccharides, Anal. Biochem. 150 (1985) 325–331.

[11] X. Sun, L. Li, K.H. Overdier, L. Anne Ammons, I.S. Douglas, C. Cothren Burlew, F. Zhang, E.P. Schmidt, L. Chi, R.J. Linhardt, Analysis of total human urinary glycosaminoglycan disaccharides by liquid chromatography tandem mass spectrometry, Anal. Chem. 87 (2015) 6220–6227.

[12] E.P. Schmidt, G. Li, L. Li, L. Fu, Y. Yang, K.H. Overdier, I.S. Douglas, R.J. Linhardt, The circulating glycosaminoglycan signature of respiratory failure in critically ill adults, J. Biol. Chem. 289 (2014) 8194–8202.

[13] C.J. Hendriksz, J. Muenzer, A. Vanderver, J.M. Davis, B.K. Burton, N.J. Mendelsohn, N. Wang, L. Pan, A. Pano, A.J. Barbier, Levels of glycosaminoglycans in the cerebrospinal fluid of healthy young adults, surrogate-normal children, and Hunter syndrome patients with and without cognitive impairment, Mol. Genet. Metabol. 5 (2015) 103–106.

[14] N. Tanaka, S. Kida, M. Kinoshita, H. Morimoto, T. Shibasaki, K. Tachibana, R. Yamamoto, Evaluation of cerebrospinal fluid heparan sulfate as a biomarker of neuropathology in a murine model of mucopolysaccharidosis type II using high-sensitivity LC/MS/MS, Mol. Genet. Metabol. 125 (2018) 53–58.

[15] H. Zhang, S.P. Young, C. Auray-Blais, P.J. Orchard, J. Tolar, D.S. Millinngton, Analysis of glycosaminoglycans in cerebrospinal fluid from patients with mucopolysaccharidoses by isotope-dilution ultra-performance liquid chromatography-tandem mass spectrometry, Clin. Chem. 57 (2011) 1005–1012.

[16] H. Naimy, K.D. Powell, J.R. Moriarity, J. Wu, T.G. McCauley, P.A.J. Haslett, A.J. Barbier, Y.C. Qiu, A novel LC-MS/MS assay for heparan sulfate screening in the cerebrospinal fluid of mucopolysaccharidosis IIIA patients, Bioanalysis 8 (2016) 285–295.

[17] P. Pan, M. Chen, Z.L. Zhang, A. Dalla Corte, C. Souza, R. Giugliani, L.Y. Pan, Y.C. Qiu, L. Amaravadi, J. Wu, A novel LC-MS/MS assay to quantify dermatan sulfate in cerebrospinal fluid as a biomarker for mucopolysaccharidosis II, Bioanalysis 10 (2018) 825–838.

[18] C.E. Teunissen, A. Petzold, J.L. Bennett, et al., A consensus protocol for the standardization of cerebrospinal fluid collection and biobanking, Neurology 73 (2009) 1914–1922.