Sensitive and Specific Determination of N-Linked Oligomannoses in Mouse Liver and Brain Tissue Using LC-HRAM Method

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Introduction

Glycosylation is currently recognized as one of the most common posttranslational modifications of proteins and plays an important role in myriad of biological processes. Glycans and glycoproteins can be utilized as potential therapeutic and diagnosis assets. Therefore, qualitative and quantitative glycomic information has been deemed necessary to understand the biological roles of glycans and to control quality of glycosylated drugs. In this study, an LC-HRAM assay was developed and qualified to accurately and specifically quantify the total amount of different N-linked oligomannoses (Mannose-2a, Mannose-2b, mannose-3, mannose-4, mannose-5, mannose-6, mannose-7, mannose-8 and mannose-9) in mouse plasma and mouse brain tissues.

Methods

Sample Digestion and Labeling:
20 µL of plasma or tissue samples and 10 µL of working internal standard (2.0 µg/ml chitotriose and N,N',N"-Triacetyl-chitotriose combo solution in water) were digested with PNGase F in appropriate buffers. The samples were precipitated with 2.5X volumes of 50/50 MeOH/MeCN. The clear supernatant was dried and re-dissolved in 20 µL of freshly prepared labeling solution containing 400mM procainamide, 1M sodium cyanoborohydride in acetic acid:DMSO(3:7). The samples were incubated 3 hours at 65°C followed by addition of 500 uL of 95:5 MeCN:Water after cooling down. The samples were purified by SPE using Waters GlycoWorks HILIC µElution plate following the manufacture's instruction. 20 µL of the samples were injected for LC-HRAM analysis

UPLC-Fluorescene/HRAM:
HPLC System: Waters ACQUITY UPLC system
LC column: Waters Glycan 2.1 x 150 mm (1.7 µm)
Mobile Phases:
A) 50 mM ammonium formate solution, pH 4.4
B) MeCN
Column Temperature: 60°C
Flow Rate: 0.4 mL/min
Fluorescence Detector: ACQUITY UPLC FLR Detector
Mass Spectrometer: Bruker MicroTOFQ II Q-TOF

Results and Discussions

Figure 1, General reaction scheme for the enzymatic release of N-glycans by PNGases [Wang et. al., Bioscience Reports, 2014, 34 (6), e00149]. Waters GlycoWorks RapiFluo-MS N-Glaycan Kit labels the amino groups of the freshly released N-glycans while the current procainamide method labels the carbonyl groups (free reducing ends) of the stable hydrolyzed the N-glycans.

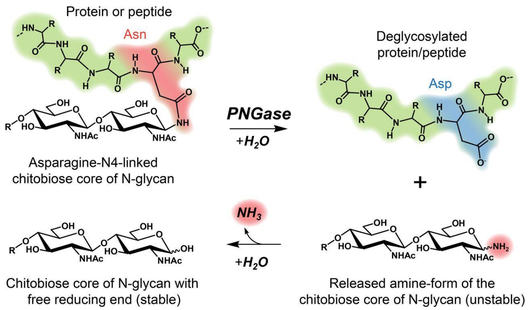
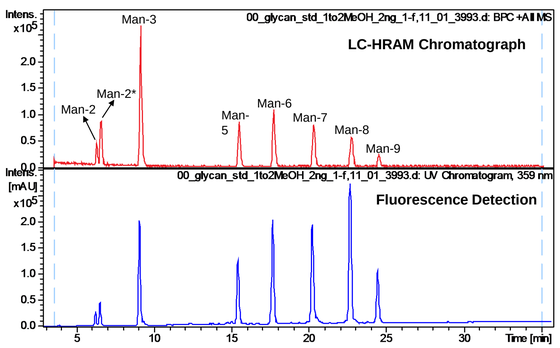


Figure 2, LC-MS/Fluorescence of derivatized neat standard oligomannose mixtures (2.0 ng/mL of Man-2, Man3, Man-5 (purchased from Chemily LLC) and Man 6-9 (purchased from ProZyme Inc) in water)



Conclusions

- A sensitive, specific and high throughput method developed to quantify N-linked oligomannoses in plasma or tissue samples using LC-HRAM assay.

Figure 3, LC-fluorescence (left) and LC-HRAM (right) analysis of extracted and derivatized blank mouse plasma

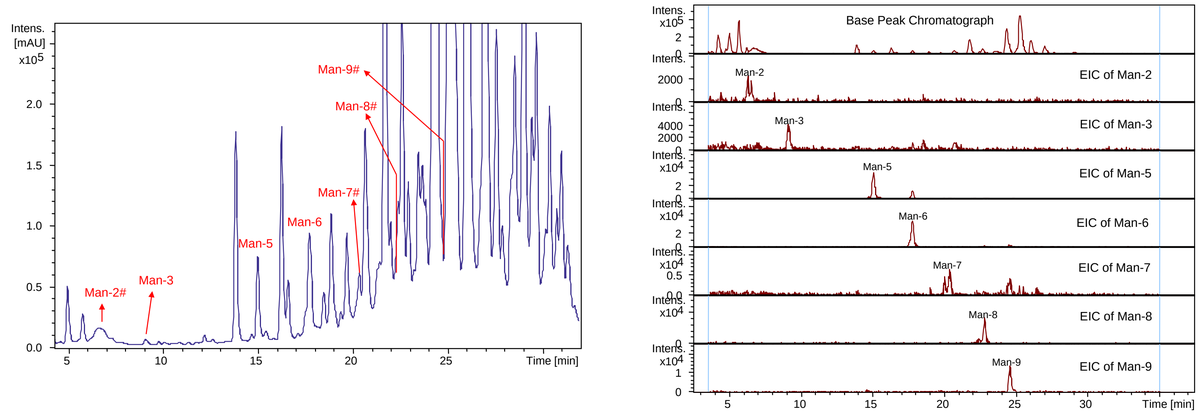


Table 1, Statistics of two sets of calibration standard curves prepared in surrogate matrix (water)

	Target C (ng/mL)	Measured C (ng/mL)/Accuracy															
		Mannose-2a		Mannose-2b		Mannose-3		Mannose-5		Mannose-6		Mannose-7		Mannose-8		Mannose-9	
Std 1-1	9.77	8.40	86%	8.75	90%	8.27	85%	10.7	110%	7.82	80%	8.85	91%	8.83	90%	10.0	102%
Std 2-1	39.1	32	82%	32.3	83%	36.5	94%	36.6	94%	36.1	92%	33.6	86%	36.5	93%	50.6	129%
Std 3-1	156	133	85%	142	91%	145	93%	129	83%	147	94%	141	90%	161	103%	199	127%
Std 4-1	625	784	125%	623	100%	694	111%	531	85%	627	100%	617	99%	594	95%	825	132%
Std 5-1	2500	2454	98%	2615	105%	2302	92%	2273	91%	2239	90%	2565	103%	2561	102%	3314	133%
Std 6-1	10000	10171	102%	9008	90%	8951	90%	9466	95%	9034	90%	9257	93%	10484	105%	N/A	N/A
Std 1-2	9.77	11.8	121%	11.3	116%	10.3	106%	N/A	N/A	10.6	109%	11.3	116%	8.84	91%	6.63	68%
Std 2-2	39.1	36.6	94%	38.5	99%	38.1	97%	45.2	116%	45.9	118%	43.4	111%	44.0	113%	33.5	86%
Std 3-2	156	142	91%	154	99%	179	115%	151	97%	168	108%	166	107%	174	111%	129	83%
Std 4-2	625	699	112%	704	113%	666	107%	631	101%	625	100%	629	101%	656	105%	478	77%
Std 5-2	2500	2770	111%	2868	115%	2528	101%	2899	116%	2756	110%	2451	98%	2401	96%	1634	65%
Std 6-2	10000	9336	93%	10156	102%	11881	119%	10501	105%	11372	114%	10966	110%	9695	97%	6052	61%

Table 2, Measured total concentrations of each oligomannose in selected mouse liver and brain tissues (ng/g)

	Mannose-2a		Mannose-2b		Mannose-3		Mannose-5		Mannose-6		Mannose-7		Mannose-8		Mannose-9	
	Liver	Brain	Liver	Brain	Liver	Brain	Liver	Brain	Liver	Brain	Liver	Brain	Liver	Brain	Liver	Brain
Mouse 1	2,314	487	155	38	587	210	9,720	34,861	4,080	6,801	5,044	4,749	4,518	2,627	14,097	8,809
Mouse 2	3,400	449	124	34	741	209	9,432	34,220	6,319	7,180	6,461	4,666	8,290	2,641	18,159	8,857
Mouse 3	4,620	540	199	32	926	246	12,070	35,246	8,828	7,397	9,161	5,204	11,099	3,095	22,062	9,986
Mouse 4	4,111	541	263	32	941	227	10,127	45,631	6,204	8,416	6,629	5,875	8,379	3,127	16,492	11,676
Mouse 65	4,434	576	354	26	1,072	224	12,902	42,114	7,339	8,672	8,199	6,234	10,014	3,375	18,681	8,470