Identification of growth factors in mouse plasma by immunoaffinity depletion, low molecular weight protein enrichment and LC-MS/MS analysis

Jian-Ying Zhou†, Janani I. Shutthanandan†, Brianne O. Petritis†, Karl K. Weitz†, Ronald J. Moore†, David G. Camp II†, Rohit N. Kulkarni‡, Richard D. Smith†, and Wei-Jun Qian†
†Biological Science Division and Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, Washington 99352, and ‡Division of Cell and Molecular Physiology, Joslin Diabetes Center, Department of Medicine, Harvard Medical School, Boston, Massachusetts 02215

Pacific Northwest NATIONAL LABORATORY

Overview

- IgY7 and SuperMix columns were applied online to separate mouse plasma proteins, which provided a nearly 2-fold improvement in the overall proteome coverage, as well as more than 2-fold increase in the coverage of cytokines, growth factors, and other low abundance proteins compared to single column depletion using IgY7.
- Mouse plasma was filtered by 10 kDa MWCO to collect the low molecular weight fraction, and analyzed by high resolution LC-MS/MS to identify plasma peptidome
- The IgY7-SuperMix and MWCO systems were combined to achieve deeper profiling of growth factors.

Introduction

- The discovery of novel circulatory growth factors in plasma has significant potential for advancing our understanding and therapeutic treatment of metabolic disorders such as obesity, insulin resistance, and diabetes.
- The relative low abundance of these factors and the enormous complexity of the plasma proteome make it challenging for proteomics identification and quantification.
- In this study, we explored multiple protein fractionation techniques to enrich the low molecular weight growth factors to facilitate the effective detection of these proteins by LC-MS/MS.

CONTACT: Jianying Zhou, Ph.D.
Biological Sciences Division, K8-98
Pacific Northwest National Laboratory
P.O. Box 999, Richland, WA 99352
E-mail: jianying.zhou@pnl.gov

Methods

Online IgY7-SuperMix separation

- Plasma proteins flowing through the IgY7 column were used as antigens to generate antibodies for the SuperMix column
- The IgY7 and SuperMix columns were applied online to separate mouse plasma proteins
- 125 µL mouse plasma per injection was used for tandem depletion

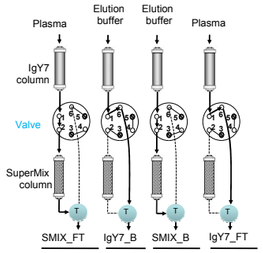

Figure 1. Online IgY7-SuperMix separation for mouse plasma. 4 different fractions corresponding to proteins of different abundance can be collected from the IgY7-Supermix system.

Low molecular weight (LMW) protein enrichment

- 50 µL mouse plasma was filtered using 10 kDa MW cutoff, and the flow-through (FT) fraction was collected for direct LC-MS/MS analysis.
- Protein samples were separated by SDS-PAGE and the low molecular weight fractions (<14 kDa) were digested by in-gel digestion and analyzed by LC-MS/MS

LC-MS/MS analysis

All samples were analyzed using an LTQ-Orbitrap instrument equipped with an automated column LC system.

Data analysis

- SEQUEST was used to search all the MS/MS spectra against the mouse International Protein Index (IPI) database (version 3.35, released October 24, 2007).
- A decoy-database was used to control the false discovery rate (FDR) to <1% at the peptide level.

Results

IgY7-SuperMix depletion

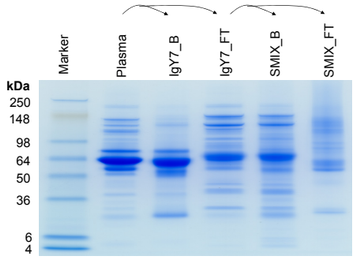

Figure 2. Extensive protein fractionation. Four different fractions—1) IgY7 bound, 2) IgY7 FT, 3) SuperMix bound, and 4) SuperMix FT corresponding to high, moderate plus low, moderate, and low abundance fractions, respectively—were collected from the IgY7-SuperMix system.

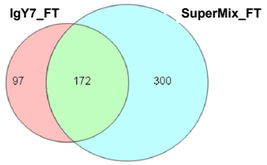

Figure 3. Improved proteome coverage by IgY7-SuperMix. 2D-LC-MS/MS analysis of IgY7_FT and SuperMix_FT revealed a nearly two-fold improvement in proteome coverage. Only proteins with ≥2 peptides are counted.

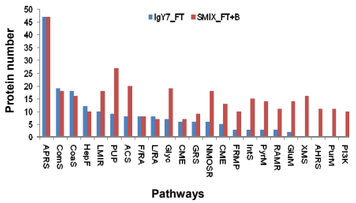

Figure 4. Improved detection of low abundance pathways. After combining SuperMix_FT and SuperMix_B data, the coverage of a number of top mapped canonical pathways based on Ingenuity Pathways Analysis was significantly improved.

Table 1. Improved detection of low abundance proteins

Description	Gene	IPA category	Peptide number	spectral count	
				IgY7_FT	SMIX_FT
Osteopontin precursor	Spp1	cytokine	8	2	10
Uteroglobin precursor	Scgb1a1	cytokine	2		5
Transitional endoplasmic reticulum ATPase	Vcp	enzyme	32		56
Guanine deaminase	Gda	enzyme	19		52
Isoform IGF-IA of Insulin-like growth factor I precursor	Igf1	growth factor	2	10	16
Regenerating islet-derived protein 3 beta precursor	Pap	growth factor	4		16
Regenerating islet-derived protein 3 gamma precursor	Reg3g	growth factor	4		7
Phosphoglycerate kinase 1	Pgk1	kinase	16	1	59
Transforming growth factor-beta-induced protein ig-h3 precursor	Tgfbi	other	24	6	60
Protein DJ-1	Park7	other	9		16
Epithelial cadherin precursor	Cdh1	other	4	1	9
Isoform 1 of Alpha-synuclein	Snca	other	5	1	8
Neuropeptide Y precursor	Npy	other	3		3
C-reactive protein precursor	Crp	other	18	6	160
Proteasome subunit beta type-1 precursor	Psmb1	peptidase	17	1	39
Insulin-degrading enzyme	Ide	peptidase	7		8

Detection of LMW proteins

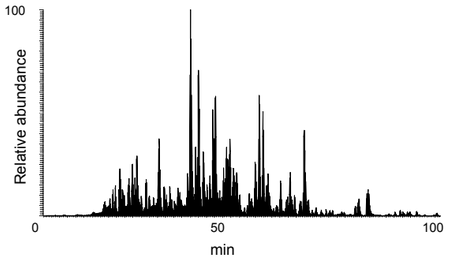

Figure 5. LC-MS/MS analysis of LMW fraction without digestion. After 10 kDa MW cut off filtering of 50 µL original plasma, the LMW fraction was directly analyzed by LC-MS/MS. A combination of Xcorr, ΔCn and mass accuracy was used to control the identification FDR. >900 unique peptides with FDR<1% at the peptide level were identified from duplicate LC-MS/MS analyses. ~80% of identified peptides belonged to the top 5 proteins.

Table 2. List of selected peptide identifications

Peptide Sequence	Description	Gene	Xcorr	ΔCn	ppm
Top 10 abundant peptides					
R.SEETKQNEAFSLTAK.G	ISOFORM LONG OF COMPLEMENT C3 (FRAGMENT).	C3	5.01	0.40	-0.12
R.SEETKQNEAFSLTAKGKGR.G	ISOFORM LONG OF COMPLEMENT C3 (FRAGMENT).	C3	5.80	0.41	-0.06
R.SAGTSLVNFFSSLM*NLEEKPA PAA.K	APOLIPOPROTEIN A-II.	Apoa2	6.23	0.49	-1.38
R.SEETKQNEAFSLTAKGK.G	ISOFORM LONG OF COMPLEMENT C3 (FRAGMENT).	C3	5.86	0.34	0.50
A.GTSLVNFFSSLM*NLEEKPAPA A.K	APOLIPOPROTEIN A-II.	Apoa2	5.95	0.43	-1.06
T.TDTEDKGEFLSEGGGVR.G	FIBRINOGEN, ALPHA POLYPEPTIDE ISOFORM 2.	Fga	5.09	0.44	0.88
R.RKEEPPSLRPAPPPISGGGYR. A	FIBRINOGEN BETA CHAIN.	Fgb	4.72	0.42	0.27
R.SAGTSLVNFFSSLMNLEEKPA PAA.K	APOLIPOPROTEIN A-II.	Apoa2	5.85	0.45	-0.99
A.GTSLVNFFSSLMNLEEKPAPA A.K	APOLIPOPROTEIN A-II.	Apoa2	5.97	0.43	-1.06
K.DGGSRGDSPGDSR.G	FIBRINOGEN, ALPHA POLYPEPTIDE ISOFORM 2.	Fga	3.69	0.24	-1.13
Selected interesting peptides from low abundance proteins					
P.SKPDNPGEDAPAEDMA.R	NEUROPEPTIDE Y.	Npy	3.11	0.37	-0.44
Y.PSKPDNPGEDAPAEDMA.R	NEUROPEPTIDE Y.	Npy	4.23	0.39	-0.36
K.GKYVVFFFYPLDFTFVCPTEIIA FSDR.A	PEROXIREDOXIN-1.	Prdx1	6.57	0.46	-2.20
R.ILGWGVENGVPYWLAANSWN LDWGDNGFFK.I	CATHEPSIN B.	Ctsb	4.75	0.35	-1.79
K.DTTEKELLDSYIDGR.I	PROTHROMBIN (FRAGMENT).	F2	3.49	0.25	0.34
R.NAEENSAIQKVAKDIAYLGITD VR.V	MANNOSE-BINDING PROTEIN C.	Mbl2	3.52	0.08	-1.29
K.SLKDTTEKELLDSYIDGR.I	PROTHROMBIN (FRAGMENT).	F2	3.72	0.18	1.44
K.NTLPTKETIEQE.K	THYMOSIN BETA-10.	Tmsb10	3.02	0.18	0.67
K.IFYYIDSLSYTVDAFDYDLQTG QISNR.R	REGUCALCIN.	Rgn	3.83	0.23	-1.37
R.GVDLDQLLDMSYEQLMQLYS AR.Q	40S RIBOSOMAL PROTEIN S15.	Rps15	3.38	0.20	-2.73

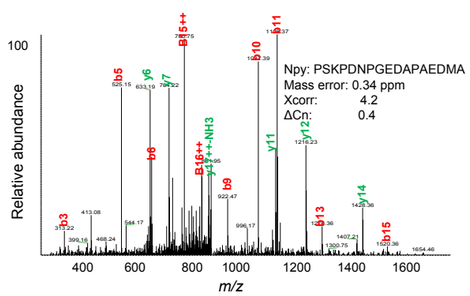

Figure 6. An example MS/MS spectrum of Neuropeptide Y, a 36-amino acid peptide neurotransmitter associated with a number of physiologic processes in the brain.

Conclusions

- Our initial global characterization of the mouse plasma proteome using SuperMix depletion and LC-MS/MS resulted in identification of ~1000 unique proteins, with 472 proteins identified by more than two unique peptides.
- More than 30 known cytokines and growth factors and many other putative factors in mouse plasma, such as insulin, leptin, adiponectin, insulin growth factor 1, granulin, and prosaposin, were identified.
- Different protocols for further enriching the LMW fractions are being explored and are anticipated to significantly expand the coverage of putative growth factors.
- Currently we are integrating the IgY7-SuperMix depletion and LMW fractionation methods to achieve deeper profiling of low abundance plasma actors.
- We anticipate this integrated methodology will greatly facilitate the discovery of novel growth factors related to different disease conditions.

Acknowledgements

This work was funded by he National Institutes of Health grants R01 DK074795 and RR018522. Samples were analyzed using capabilities developed under the support of the NIH National Center for Research Resources (RR18522) and the U.S. Department of Energy Biological and Environmental Research (DOE/BER).

Significant portions of the work were performed in the Environmental Molecular Science Laboratory, a DOE/BER national scientific user facility at Pacific Northwest National Laboratory (PNNL) in Richland, Washington. PNNL is operated for the DOE by Battelle under contract DE-AC05-76RLO-1830.

References

1. Qian WJ, et al. Enhanced detection of low abundance human plasma proteins using a tandem IgY12-SuperMix immunoaffinity separation strategy, *Mol Cell Proteomics* 7:1963-1973 (2008).
2. Zhou JY, et al. Mouse-specific tandem IgY7-SuperMix immunoaffinity separations for improved LC-MS/MS coverage of the plasma proteome. *J Proteome Res* 8:5387-5395 (2009).