Extensive Oxidative Protein Modifications Observed in Human Plasma and Candidate Biomarkers of Systemic Chronic Inflammatory and Oxidative Stress

Xu Zhang, Marina A. Gritsenko, Matthew E. Monroe, Ronald J. Moore, David G. Camp II, Diana J. Bigelow, Wei-Jun Qian, Jon M. Jacobs, Joel G. Pounds, and Richard D. Smith
Biological Sciences Division and Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA 99352

Pacific Northwest NATIONAL LABORATORY

Overview

- Human plasma samples from smokers (S) and non-smokers (NS); groups were further categorized with regard to BMI above 35 (HiBMI) or below 25 (LoBMI)
- Plasma from 6 HiBMI-NS, 7 HiBMI-S, 7 LoBMI-NS and 7 LoBMI-S were pooled
- 2D LC-MS/MS global profiling of protein oxidative modifications
- Extensive oxidative protein modifications were observed in plasma
- Dopaquinone (DQ) modification was investigated as candidate biomarker of systemic chronic inflammatory and oxidative stress

Introduction

- Smoking and obesity are two of the most important, yet preventable risk factors for human morbidity and mortality, each raising the risk of cancer, cardiovascular and respiratory disease.
- Chronic inflammation and oxidative stress appears to be the unifying mechanism underlying the interaction of these life-style-induced risk factors with the genome, resulting in a variety of chronic human diseases.
- We compared proteome profiles of human blood plasma generated from LC/LC-MS/MS analyses of samples obtained from smokers (S) or non-smokers (NS) with BMI above 35 (HiBMI) or below 25 (LoBMI). Protein abundance changes and changes in tyrosine oxidative modifications were evaluated.

CONTACT: Xu Zhang, Ph.D.
Biological Sciences Division, K8-98
Pacific Northwest National Laboratory
P.O. Box 999, Richland, WA 99352
E-mail: xu.zhang@pnl.gov

Methods

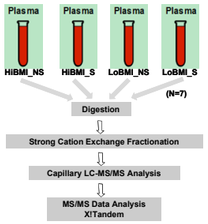


Figure 1. Experimental approach

Human Plasma: A subset of human plasma samples originated from representative participants in a cohort of 500 S and NS with HiBMI (above 35) and LoBMI (below 25).

Sample Preparation: Plasma from 6 HiBMI-NS, 7 HiBMI-S, 7 LoBMI-NS, and 7 LoBMI-S were pooled, and digested with trypsin under identical conditions, and then further separated into 24 fractions using strong cation exchange (SCX) chromatography (Polysulfoethyl A 200 mm x 4.6 mm (5 μm, 300 Å) column).

LC-MS/MS analysis: Each fraction was analyzed using an automated capillary HPLC system coupled on-line with an LTQ ion trap mass spectrometer. Column: 75 μm id x 65 cm capillary packed with 3 μm Jupiter C18.

Data Analysis: The MS/MS data were searched using X!Tandem against the Human International Protein Index (IPI) database with a false discovery rate (FDR) controlled at ~1% by decoy database searching.

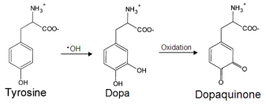
Results

2D LC-MS/MS proteome coverage

Table 1. Peptides and oxidative modified peptides

	HiBMI_NS	HiBMI_S	LoBMI_NS	LoBMI_S	Total
Total unique peptides	6804	6109	6458	6212	9908
Total unique proteins	807	660	718	720	1302
Unique Y-NO_2 peptides	5	3	3	3	11
Unique Y-NH_2 peptides	12	12	14	28	44
Unique YWP-OH peptides	636	589	642	576	1164
Unique Y-DQ peptides	167	315	241	278	502
Unique Y-Cl peptides	2	0	0	0	2
Unique Y-Br peptides	1	2	0	1	2
Unique C-SO_3H peptides	17	12	10	9	33
Unique C-SO_3H peptides	63	66	89	48	140

Protein oxidative modifications



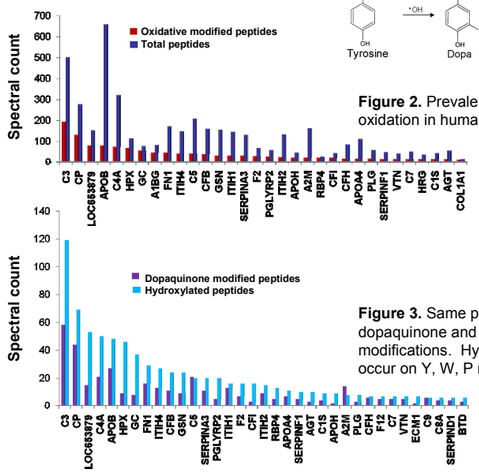


Figure 2. Prevalence of protein oxidation in human plasma

Figure 3. Same proteins with both dopaquinone and hydroxylation modifications. Hydroxylation can occur on Y, W, P residues.

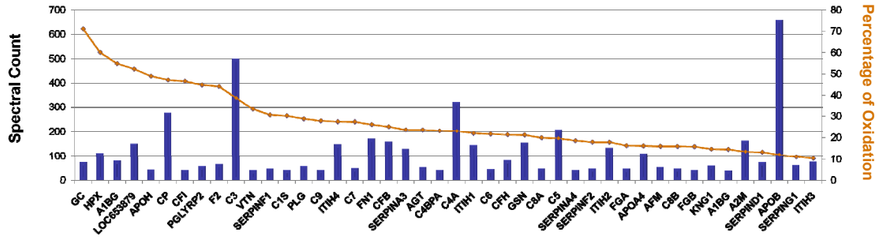


Figure 4. Protein abundance and percentage of oxidative modifications

Abundance changes of tyrosine dopaquinone and hydroxylation modifications

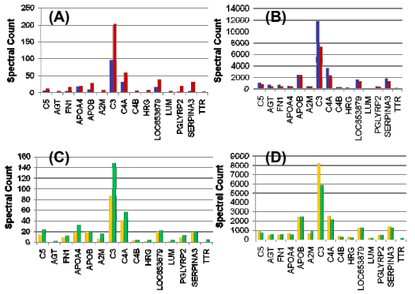


Figure 5. Abundance changes for both modified **(A, C)** and unmodified **(B, D)** peptides of dopaquinone modified proteins.

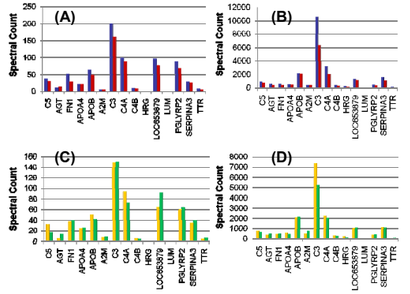


Figure 6. Abundance changes for both modified **(A, C)** and unmodified **(B, D)** peptides of hydroxylated proteins

Table 2. Significantly changed DQ modified peptides

Peptides	Peptide Sequence	Protein Name	Description
C3-1	K.QLY#NVEATSY#ALLALLQLK.D	IPI:IPI00783987.2	COMPLEMENT C3
C3-2	K.QLY#NVEATSYALLALLQLK.D	IPI:IPI00783987.2	COMPLEMENT C3
C3-3	K.QLYNVEATSY#ALLALLQLK.D	IPI:IPI00783987.2	COMPLEMENT C3
C3-4	K.Y#FKPGMPFDLMVFVTNPDGSPAYR.V	IPI:IPI00783987.2	COMPLEMENT C3
C3-5	R.SGIPIVTSPY#QIHFTK.T	IPI:IPI00783987.2	COMPLEMENT C3
C3-6	R.SY#TVAIAGYALAQMGR.L	IPI:IPI00783987.2	COMPLEMENT C3
C3-7	R.TMQALPY#STVGNSNNYLHLSVLR.T	IPI:IPI00783987.2	COMPLEMENT C3
C3-8	R.Y#YGGGYGSTQATFMVFQALAQYQK.D	IPI:IPI00783987.2	COMPLEMENT C3
C3-9	R.YYGGGYGSTQATFMVFQALAQYQK.D	IPI:IPI00783987.2	COMPLEMENT C3
C3-10	R.YYGGGY#GSTQATFMVFQALAQYQK.D	IPI:IPI00783987.2	COMPLEMENT C3
C3-11	R.YYGGGYM#GSTQATFMVFQALAQYQK.D	IPI:IPI00783987.2	COMPLEMENT C3
C4A-1	K.LHLETDSLALVALGALDTALY#AAGSK.S	IPI:IPI00032258.4	COMPLEMENT C4-A
C4A-2	R.RGHLFLQTDQPIY#NPGQR.V	IPI:IPI00032258.4	COMPLEMENT C4-A
C4A-3	R.STQDTVIALDALSAY#WIASHTTEER.G	IPI:IPI00032258.4	COMPLEMENT C4-A
C4B-1	K.ASAGLLGAHAAAITAY#ALTLTK.A	IPI:IPI00418163.3	COMPLEMENT COMPONENT 4B PREPROPROTEIN
LOC653879-1	K.VQLSNDFDEY#IM*AIEQTIK.S	IPI:IPI00739237.1	SIMILAR TO COMPLEMENT C3
LOC653879-2	K.VQLSNDFDEY#IMAIEQTIK.S	IPI:IPI00739237.1	SIMILAR TO COMPLEMENT C3
PGLYRP2-1	K.ASLLTM*AFLNGALDGVILGDY#LSR.T	IPI:IPI00163207.1	ISOFORM 1 OF N-ACETYLMURAMOYL-L-ALANINE AMIDASE
SERPINA3-1	R.DY#NLNDILLQLGIEEAFTSK.A	IPI:IPI00550991.3	ALPHA-1- ANTICHYMOTRYPSIN

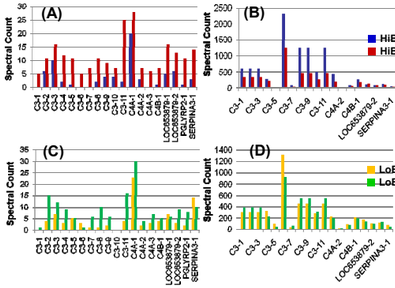


Figure 7. Abundance changes for selected modified **(A,C)** and unmodified **(B, D)** dopaquinone peptides.

Conclusions

- 9908 unique peptides covering 1302 proteins were confidently identified.
- Six different oxidative modifications on tyrosine residues and two on cysteine residues were explored, leading to identification of >1500 unique modified peptides.
- Multiple proteins of interest were detected with a diversity of modifications
- Dopaquinone modifications were revealed as candidate biomarker of systemic chronic inflammation and oxidative stress.
- The identified oxidative modifications provide insights into systemic chronic inflammatory responses, and many other observed proteins may constitute potential biomarkers of environmental stress.

Acknowledgements

Portions of this research were supported by the NIH National Center for Research Resources (RR18522), the NIEHS Exposure Biology Program U54ES16015, and internal Battelle Science and Technology funds. Work was performed in the Environmental Molecular Sciences Laboratory, a U.S. Department of Energy (DOE)/BER national scientific user facility at Pacific Northwest National Laboratory (PNNL) in Richland, Washington. PNNL is a multiprogram national laboratory operated by Battelle for the DOE under Contract DE-AC05-76RL01830.

References

1. Van der Vliet A, Eiservich JP, Marelich GP, Halliwell B, Cross CE. Myeloperoxidase and protein oxidation in cystic fibrosis, *Adv Pharmacol* 38:491-513 (1997).
2. MacPherson JC, Comhair SA, Erzurum SC, Klein DF, Lipscomb MF, Kavuru MS, Samoszuk MK, Hazen SL. Eosinophils Are a Major Source of Nitric Oxide-Derived Oxidants in Severe Asthma: Characterization of Pathways Available to Eosinophils for Generating Reactive Nitrogen Species, *J Immunol* 166:5763-5772 (2001).
3. Zhang X, Chen BW, Sacksteder CA, Chin MH, Heibeck TH, Schepmoes AA, Camp DG, Pounds JG, Jacobs JM, Smith DJ, Bigelow DJ, Smith RD, Qian WJ. Endogenous 3,4-Dihydroxyphenylalanine and Dopaquinone Modifications on Protein Tyrosine – Links to mitochondrially derived oxidative stress via hydroxyl radical, *Mol Cell Proteomics,* Feb 2, 2010, 10.1074/mcp.M900321-MCP200.