Temporal Proteomic Analysis of Systemic Inflammation upon Lipopolysaccharide Administration

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Overview

- Analysis of temporal changes in plasma protein abundances within 24 h upon lipopolysaccharide (LPS) administration.
- A combined strategy of immunoaffinity top-12 protein depletion, ^{18}O labeling, chemical peptide fractionation, and the accurate mass and time (AMT) tag approach.
- 3201 peptides were identified and quantified from 6 time point samples, covering 446 proteins.
- A number of proteins showed significant changes in abundance within the 24 h period.

Introduction

Inflammation:

- A hallmark of many human diseases
- Previous studies have relied heavily on gene expression analysis of blood leukocytes and other tissues (from critically injured patients)
- High-throughput quantitative proteomics may provide new insights

Advantages of current approach:

- Increased dynamic range of detection: top-12 protein depletion, cysteinyl peptide enrichment
- Greater proteome coverage: combined results from both Cys- and non-Cys-peptides.
- Accurate quantitation: post-digestion ^{18}O labeling (with incorporation of two ^{18}O atoms)
- Increased throughput: LC-FTICR and AMT tag approach.

Previously established AMT tag database:

- Through various studies involving human plasma
- Cys database: 9145 peptides; 2342 proteins
- Non-Cys database: 13,534 peptides, 2603 proteins

Methods

Sample:

- Prepared from a healthy subject using intravenous administration of LPS: T=0, 2, 4, 6, 9, and 24 h.

Top-12 protein depletion:

- IgY-12 LC column (Beckman Coulter)

^{18}O labeling:

- Post-digestion labeling using immobilized trypsin[1]
- Control sample (T=0): ^{16}O; all other time point samples: ^{18}O
- Each time point sample was mixed with the control in equal amounts

Fractionation via Cys-peptide enrichment:

- Each peptide mixtures: individually separated into Cys- and non-Cys-peptides using a thiol affinity resin[2]

AMT tag approach[3]:

- Peptide identification: paired mass-elution time features observed in LC-FTICR analyses were matched against corresponding databases using a error tolerance window of 2 ppm and 1.5% normalized elution time (NET)
- A new LCMSWARP algorithm was applied[4]

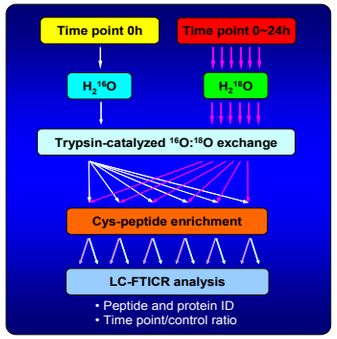


Fig. 1. The combined strategy using the AMT tag approach.

Data analysis:

- $^{18}O/^{16}O$ ratios: calculated as previously described[5]
- Results from Cys- and non-Cys-peptides were combined for final protein identification and quantification
- Protein ID redundancy: Protein Prophet
- Clustering: TIGR MultiExperiment Viewer[6]

Results

False discovery rate (FDR)

- A random database was created by shifting the masses of all peptides in the AMT tag database by 11 Da, and merged with the original database
- FDR = (# of matches from shifted DB)/(# of matches from normal DB)
- FDR is estimated to be ~4%

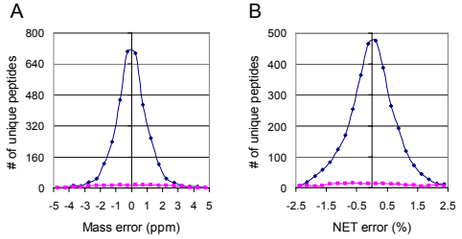
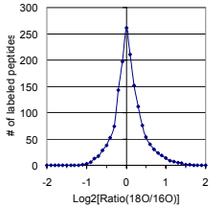


Fig. 2. Error distribution histograms for (A) mass and (B) NET of unique peptides matching to the merged AMT tag database. Blue diamonds: matches from the normal part of the merged database; magenta squares: matches from the 11-Da shifted part of the merged database.



Quantitation accuracy

- Two identical aliquots of T_0 samples were labeled in either $H_2{}^{16}O$ or $H_2{}^{18}O$
- A total of 1499 peptides were identified and quantified from this 1:1 labeled sample.
- Average Log_2 ratio: 0.02±0.37

Fig. 3. Ratio distribution of all peptides from the 1:1 labeled plasma sample.

Quantification of relatively low-abundance proteins*

IPI	Description	Conc. (ng/mL)
IPI00297646.1	Collagen alpha 1(I) chain.	0.3
IPI00024825.1	Megakaryocyte stimulating factor.	1
IPI00022296.1	Mast/stem cell growth factor receptor (CD117).	4.8
IPI00027780.1	72 kDa type IV collagenase (MMP2).	8.8
IPI00011229.1	Cathepsin D.	8.9
IPI00022446.1	Platelet factor 4.	9.7
IPI00018274.1	Epidermal growth factor (EGF) receptor.	11
IPI00293276.7	Macrophage migration inhibitory factor (MIF).	11.1
IPI00032292.1	Metalloproteinase inhibitor 1 (TIMP-1).	14
IPI00297284.1	Insulin-like growth factor (IGF) binding protein 2.	15
IPI00218795.1	L-selectin.	17
IPI00292218.3	Hepatocyte growth factor-like protein.	20
IPI00011218.1	Macrophage colony stimulating factor (M-CSF) receptor.	26
IPI00008580.1	Antileukoproteinase 1.	32
IPI00005721.1	Neutrophil defensin 1.	42
IPI00008494.3	Intercellular adhesion molecule-1 (ICAM1).	43

*Reference concentrations are from healthy individuals.

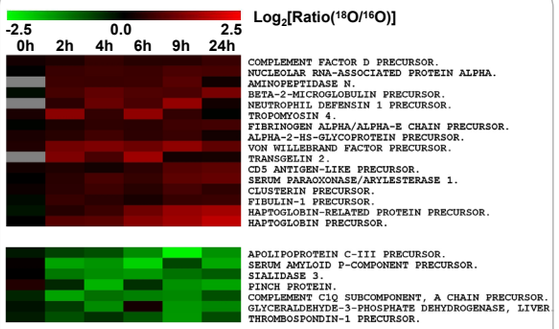


Fig. 4. K-means clustering analysis. Heat maps are shown for typical up-regulated proteins (upper panel) and down-regulated proteins (lower panel) that were quantified from the time point samples.

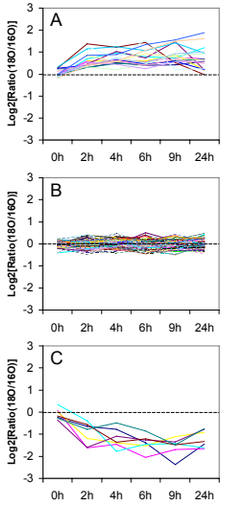


Fig. 5. K-means clustering analysis. Expression graphs are shown for typical up-regulated (A), unchanged (B), and down-regulated (C) proteins.

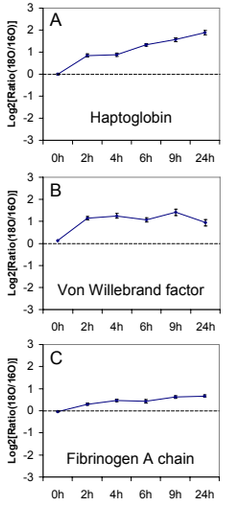


Fig. 6. Increased abundances are shown for selected proteins: (A) haptoglobin; (B) Von Willebrand factor; and (C) fibrinogen A chain.

Conclusions

- The combination of immunoaffinity depletion, isotopic labeling, and fractionation with the AMT tag approach enables effective proteomic studies of systemic inflammation in humans. The validity of the current strategy is supported by the fact that a number of proteins having known positive acute phase expression (e.g., haptoglobin, fibrinogen) were determined to have increased abundance.
- The behavior of false positive matches arising from random matching is similar for both the normal and the "11-Da shifted" data analyses. This approach can be used to effectively estimate the FDR for LC-MS-type analyses.
- Matching of the experimental data to the AMT tag database can be significantly enhanced by applying the new LCMSWARP algorithm (corrects both variation in LC elution time and drift in mass measurement accuracies).
- While abundances of the majority of proteins quantified in this study remained unchanged, a number of proteins were determined to have significantly altered abundances within 24 h upon LPS administration, providing new candidates for further validation experiments.

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References

1. Liu T, Qian WJ, Strittmatter EF, et al. *Anal. Chem.* **2004**, 76, 5345-5353.
2. Liu T, Qian WJ, Chen WN, et al. *Proteomics.* **2005**, 5, 1263-1273.
3. Smith RD, Anderson GA, Lipton MS, et al. *Proteomics.* **2002**, 2, 513-523.
4. Jaitly N, Monroe ME, Petyuk, VA, et al. *Anal. Chem.* **In press**.
5. Qian WJ, Monroe ME, Liu T, et al. *Mol. Cell. Proteomics.* **2005**, 4, 700-709.
6. Saeed AI, Sharov V, White J, et al. *Biotechniques.* **2003**, 34, 374-378.

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