

ESI QToF MS/MS Analysis of the Rat Cardiac Mitochondrial Proteome

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OVERVIEW

Purpose

> Identify key proteins and biochemical pathways that cause, or contribute to, mitochondrial dysfunction in the aging heart.

Methods

> Subsarcolemmal and interfilamentary mitochondria from three young- and three aged rat hearts were isolated by centrifugation and protease treatment.

> Multi-dimensional liquid chromatography and mass spectrometry was used to identify mitochondrial proteins by searching MSMS data against the Swissprot protein database.

> Peptide sequence information from over 200 Mascot output files was combined and condensed with DBParser software¹.

Results

> Between- and within group comparisons of protein identifications are shown.

> Common proteins from between-group comparisons are listed in context of their respective Mascot scores.

Introduction

A significant challenge for those attempting to use quantitative proteomics is to identify physiologically normal levels of variability and distinguish these from variances that are pathological. One aspect of this challenge is the difficulty involved in processing the copious volumes of data needed to describe "normal" variability of results between- and within groups.

The ability to process these data sets has allowed the development of specialized software. One such tool, currently under development, is the open source software DBParser¹. DBParser software was used in this experiment to condense and compare results from multiple shotgun proteomics experiments.

Introduction Cont.

Our aim is to use MS-based quantitation to dissect mechanisms linked to the imbalance of mitochondrial pro- and anti-oxidant systems in the aging heart. This work provides preliminary data for this aim by: 1) determining what proteins are regularly detectable, and 2) evaluating within- and between group variation of results from multiple MS-based shotgun proteomics experiments.

Methods

Mitochondrial preparation
Mitochondria from 3-, 3-month-old and 3-, 14-month-old Fisher rat hearts (Table 1) were enriched and subdivided into subsarcolemmal and interfilamentary fractions (SSM and IFM respectively) by centrifugation and protease treatment¹. Protein concentration was measured by spectrophotometric assay. Protein fractions (1.5 mg/each) were suspended in 12 ml 8M urea, 50mM Tris, 50 mM octyl-β-D-glucopyranoside pH 7.4, in order to denature and solubilize mitochondrial proteins. Disulfides were reduced with dithiothreitol (5mM) and blocked with iodoacetamide (10mM) for 20 minutes each at room temperature. The concentration of urea was reduced to 0.95M by addition of 50mM NH₄HCO₃ pH 7.8 prior to tryptic digestion (E:S, 1:50, overnight at 37°C). Digestion was stopped by addition of TFA to pH 2.5. Digests were separated by cation exchange and nanoflow C₁₈ HPLC, prior to analysis on a QToF mass spectrometer.

Table 1. Sample numbers and groups. Detailed results from comparisons between and within groups can be viewed at <http://ais2.114b-pc1.science.oregonstate.edu/dbparser/> click on view reports and mitochondrial proteomics. Comparisons are indexed according to 3-digit numbers in table.

	IFM	SSM
Young	037	038
	043	044
	047	048
Old	039	040
	042	041
	045	046

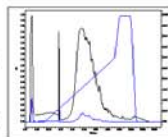
IFM: interfilamentary mitochondria, SSM: subsarcolemmal mitochondria. IFM and SSM fractions from each animal labeled consecutively thus 037 and 038 came from same animal.

Methods Cont.

Strong Cation-Exchange Chromatography
Trypic digests were suspended 1:1 in 5% ACN, 0.1% TFA, loaded on syringe-operated C₁₈ desalting cartridges, eluted in 50% ACN, 0.1% TFA and concentrated to a volume of 100 µl by vacuum centrifugation. Desalted and concentrated samples were diluted 1:1 in SCX solvent "A", KH2PO4, 25% ACN, pH 3, and loaded onto a 2.1x150.0 mm, 5 µm strong cation exchange column. Peptides were eluted by salt gradient (SCX "A" + 0.5M KC) over 20 min (Figure 1) and eluant was collected in 1-min fractions.

Fig. 1. Strong Cation Exchange

Injection = 1.5 mg of protein digest
Thermo BioBasic SCX column, flow 0.2 ml/min.
Fractions collected at one-minute intervals (1-21, 35-40 combined)

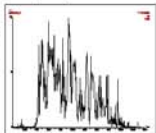


Nano-HPLC and Mass Spectrometry

SCX fractions (~18/sample) were desalted on C₁₈ cartridges, vacuum centrifuged to dryness and resuspended in 20 µl 5% ACN, 0.1% TFA, 1-3 µl of each of these was injected onto a 0.75 µm X15 cm nano-flow C₁₈ column and analyzed by QToF mass spectrometry.

Fig. 2. QToF Base-Peak Chromatogram

Injection = 1µl from processed SCX fraction (see above). In-house packed C₁₈, 0.075 X, 150.0 mm column, flow 0.3 µl/min.



Data Processing

Smoothed and centroided MSMS data were searched against the SwissProt database using Mascot version 2.1 with carbamidomethyl as a fixed modification, oxidized M, and formylkynurenine were allowed as variable modifications. Parent and fragment ion tolerances were 0.2 Da.

Results

There was considerable within-group variability in this data set. Among the 126 non-redundant proteins found in young SSM samples, only 70% were found in all three fractions (Figure 3). These values were 46%, 52% and 67% for young IFM, old IFM and old SSM respectively (data not shown).

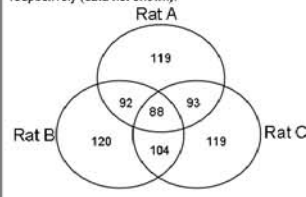


Fig. 3. Venn diagram of within-group comparisons between the 3 samples from young SSM.

Between-group variability was similar with 64% and 72% commonality between proteins from young and old IFM (Figure 4A) and SSM groups respectively (Figure 4B).

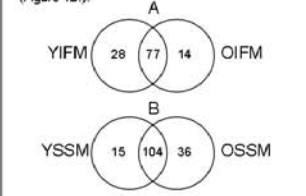


Fig. 4. Venn diagram of between-group comparisons from young and old animals.

Results cont.

Proteins that were common in between-group comparisons of IFM samples are shown in Table 1. Among these 77 proteins, 20 were detected in samples from all animals in both young and old groups (marked with *).

Table 1. Proteins that were common between young and old IFM (SSM comparisons can be seen on the website or in supplementary material).

Accession Name*	Mascot Score	Young	Mascot Score	Old
Oxidative Phosphorylation				
Complex IV				
P192146NDH2-ubiquinol-cytochrome b240 subunit	174	100		
Complex III				
ORF1713Ubiquinol-cytochrome b240 subunit COOX	158	246		
P192146NDH2-ubiquinol-cytochrome b240 subunit	157	220		
Complex III				
P192146NDH2-ubiquinol-cytochrome b240 subunit	67	47		
P192146NDH2-ubiquinol-cytochrome b240 subunit	67	47		
ORF1713Ubiquinol-cytochrome b240 subunit (Site 677)*	119	122		
Complex IV				
ORF1713Ubiquinol-cytochrome b240 subunit	216	280		
Complex IV				
P192146NDH2-ubiquinol-cytochrome b240 subunit	40	134		
P192146NDH2-ubiquinol-cytochrome b240 subunit	60	72		
P192146NDH2-ubiquinol-cytochrome b240 subunit	96	111		
Complex IV				
P192146NDH2-ubiquinol-cytochrome b240 subunit	87	43		
P192146NDH2-ubiquinol-cytochrome b240 subunit	221	241		
P192146NDH2-ubiquinol-cytochrome b240 subunit	159	151		
P192146NDH2-ubiquinol-cytochrome b240 subunit	462	361		
Complex IV				
P192146NDH2-ubiquinol-cytochrome b240 subunit	652	620		
P192146NDH2-ubiquinol-cytochrome b240 subunit	740	659		
P192146NDH2-ubiquinol-cytochrome b240 subunit	188	153		
Other Proteins				
P192146NDH2-ubiquinol-cytochrome b240 subunit	118	44		
ORF1713Ubiquinol-cytochrome b240 subunit	86	302		
P192146NDH2-ubiquinol-cytochrome b240 subunit	104	100		
ORF1713Ubiquinol-cytochrome b240 subunit	21	21		
P192146NDH2-ubiquinol-cytochrome b240 subunit	72	47		
ORF1713Ubiquinol-cytochrome b240 subunit	38	41		
P192146NDH2-ubiquinol-cytochrome b240 subunit	246	250		
P192146NDH2-ubiquinol-cytochrome b240 subunit	257	250		
P192146NDH2-ubiquinol-cytochrome b240 subunit	177	151		
P192146NDH2-ubiquinol-cytochrome b240 subunit	93	74		
P192146NDH2-ubiquinol-cytochrome b240 subunit	108	108		
P192146NDH2-ubiquinol-cytochrome b240 subunit	139	106		
P192146NDH2-ubiquinol-cytochrome b240 subunit	246	169		
* 64 Proteins				
P192146NDH2-ubiquinol-cytochrome b240 subunit	213	261		
P192146NDH2-ubiquinol-cytochrome b240 subunit	87	63		
P192146NDH2-ubiquinol-cytochrome b240 subunit	233	123		
P192146NDH2-ubiquinol-cytochrome b240 subunit	118	118		
P192146NDH2-ubiquinol-cytochrome b240 subunit	30	45		
P192146NDH2-ubiquinol-cytochrome b240 subunit	30	44		
P192146NDH2-ubiquinol-cytochrome b240 subunit	47	47		
P192146NDH2-ubiquinol-cytochrome b240 subunit	76	81		
ORF1713Ubiquinol-cytochrome b240 subunit	72	176		
ORF1713Ubiquinol-cytochrome b240 subunit	212	279		
ORF1713Ubiquinol-cytochrome b240 subunit	80	81		
ORF1713Ubiquinol-cytochrome b240 subunit	85	85		
ORF1713Ubiquinol-cytochrome b240 subunit	140	140		
* 66 Proteins				
ORF1713Ubiquinol-cytochrome b240 subunit	1636	869		
ORF1713Ubiquinol-cytochrome b240 subunit	103	86		
ORF1713Ubiquinol-cytochrome b240 subunit	44	44		
ORF1713Ubiquinol-cytochrome b240 subunit	86	86		
P192146NDH2-ubiquinol-cytochrome b240 subunit	63	149		

Results cont.

Table 1. Cont.

Protein	Young	Old
ORF1713Ubiquinol-cytochrome b240 subunit	46	36
ORF1713Ubiquinol-cytochrome b240 subunit	81	81
ORF1713Ubiquinol-cytochrome b240 subunit	81	46
ORF1713Ubiquinol-cytochrome b240 subunit	166	146
ORF1713Ubiquinol-cytochrome b240 subunit	143	36
ORF1713Ubiquinol-cytochrome b240 subunit	61	36
P192146NDH2-ubiquinol-cytochrome b240 subunit	101	230
ORF1713Ubiquinol-cytochrome b240 subunit	74	62
ORF1713Ubiquinol-cytochrome b240 subunit	74	62
ORF1713Ubiquinol-cytochrome b240 subunit	40	40
ORF1713Ubiquinol-cytochrome b240 subunit	109	109
ORF1713Ubiquinol-cytochrome b240 subunit	119	113
ORF1713Ubiquinol-cytochrome b240 subunit	22	38
ORF1713Ubiquinol-cytochrome b240 subunit	60	62

* Proteins that were detected in all samples from both groups

Conclusion

Between- and within-group comparisons of shotgun proteomics experiments from 3-year-old and 3-old rat hearts shows that considerable variability of results exists. Physiologically relevant differences between groups are likely to be evident among most if not all members of a given group. Thus, MS-based quantitative comparisons need to establish within-group consistency and this is likely to necessitate a minimum n of 3.

Acknowledgements

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References

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 - Suh, J.H., Heath, S.-H., Hagen, T.M. Free Radic. Biol. Med. 2003 (35): 1064-1072
- DBParser freeware website:
<http://www.proteomecommons.org/archiv/11091210.60785/DBParserMain.html>