

Differential Isotope Labeling of Interfibrillary Mitochondrial Thiol Proteins

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OVERVIEW

Purpose

- Identification and quantification of free, exposed interfibrillary mitochondrial (IFM) thiol proteins that are potential targets for oxidative modification using the cleavable isotope-coded affinity tags (cICAT) approach

Methods

- cICAT labeling
- SCX, C18, avidin affinity separation
- ESI-QTOF-MS/MS
- Mascot search engine

Results

- Quantification of thiol content ratios from 26 old/young ICAT-labeled IFM proteins
- A total of 11 proteins show the ICAT ratios different from 1

INTRODUCTION

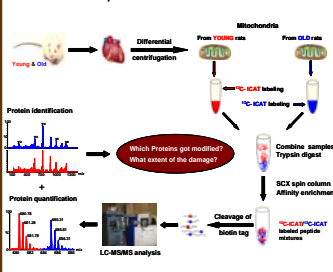
Mitochondrial thiol proteins play key roles in regulating mitochondrial functions. They are putative targets of oxidative stress-mediated modifications in the aging heart. During stages of increased oxidative stress, redox imbalance causes accumulation of oxidatively modified proteins leading potentially to mitochondrial dysfunction. In this study we attempt to identify and quantify the potential protein thiols which are linked to aging and age-related disorders in cardiac interfibrillary mitochondria (IFM) from young and old rats.

Commercial available sulfhydryl-specific reagents, cleavable isotope-coded affinity tags (cICAT),^{1,2} were chosen as the proteomic tools to identify the thiol proteins in complex mixtures for the relative quantification of peptides/proteins between young and old rats.

METHODS

Cardiac IFM from young (3 months) and old (24 months) rat hearts were isolated by differential centrifugation.³ Each sample was pooled from 3 different animals. There were a total of 4 samples in this study (12 old rats and 12 young rats). 1% Triton X-100 (v/v) was added to the mitochondria for 1 hour on ice with shaking. Same amount of young and old IFM samples were treated with light and heavy ICAT reagents individually for 2 hours at 37 °C in the dark followed by trypsin digestion. Proteolytic digests from ICAT-labeled protein mixtures were cleaned up by strong cation-exchange (SCX) spin columns and avidin affinity chromatography. The elutes were desalted and analyzed by nanoLC-ESI-QTOF. Mascot was used for protein identification, and R was used to for the statistical analysis.

Flow Chart of Experimental Procedure



RESULTS

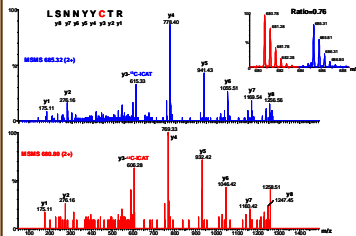


Figure 1. MS scan and tandem mass spectra of heavy/light ICAT-labeled peptides from NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 7 (NDUA7_MOUSE) aa 49-57.

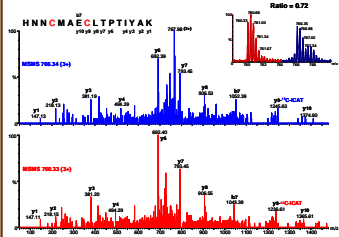


Figure 2. MS scan and tandem mass spectra of heavy/light ICAT-labeled peptides from Creatine Kinase (KCRS_RAT) aa 60-75.

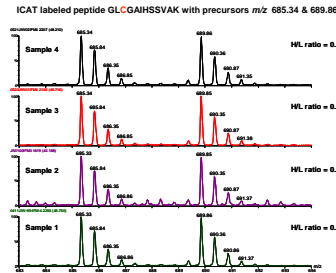


Figure 3. Illustration of reproducibility of the experiment. MS scan and intensity ratio of a ICAT-labeled peptide pair from ATP synthase subunit gamma (ATPG_RAT) aa 101-122 from all 4 samples. The average heavy/light ICAT-labeled peptide ratio is 0.876 (SE = 0.025, p-value = 0.002).

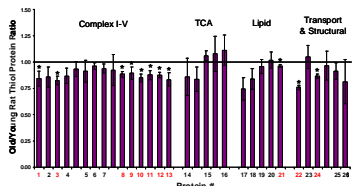


Figure 4. The average ICAT (heavy/light) ratios from proteins identified from more than 3 samples. Those proteins belong to different biological functional groups.

Table 1. ICAT-labeled peptide/protein ratios, SE, p-values and protein classification.

Protein #	Protein ID	Peptide Sequence	Average HL/ICAT Ratio	SE	p-value	
1	Complex I	NDUA7_MOUSE	LSNYYICTR	0.84	0.07	0.02
2		NDUA8_RAT	LWVLCALNFR	0.96	0.10	0.06
3		NDUA8_RAT	VIVDNGSGK	0.83	0.04	0.02
4		NDU1F_MOUSE	LVSDVGR	0.87	0.03	0.00
5		NDU5F_MOUSE	MCLVVEKAPF	0.84	0.07	0.18
6		NDU5F_MOUSE	MCSDGGSLGPPK	0.92	0.10	0.20
7		NDU1V_MOUSE	LVECLLVIGOR	0.96	0.03	0.00
8	Complex II	DHSA_RAT	AFQGLSEAGNTACTLK	0.94	0.04	0.13
9		DHSA_RAT	VQGLDGLGK	0.92	0.15	0.47
10		DHSE_RAT	CGPWVLDALM	0.89	0.03	0.00
11	Complex III	OC1F_MOUSE	NALSLKDTPTFCEDNR	0.90	0.06	0.04
12		OC2F_MOUSE	NALANPLYCPDPR	0.85	0.04	0.00
13	Complex V	ATPG_MOUSE	NCASVYDGGK	0.88	0.04	0.01
14		ATPG_MOUSE	GLCGAIHSSVAK	0.88	0.03	0.00
15		OPT1B_MOUSE	ICTSEISTSPFR	0.83	0.06	0.01
16	TCA	KCRS_MOUSE	LVYLTLDPRNLTGLR	0.88	0.18	0.21
17		KCRS_MOUSE	RFTSYVFLDGLDTGVDPNHPFK	0.84	0.12	0.14
18		MDH8_MOUSE	GDVIVVPPAGVPR	1.06	0.05	0.16
19		MDH8_MOUSE	TFLSISGCTPK	1.08	0.17	0.40
20		ODPB_MOUSE	EGIEEVLNLR	1.12	0.14	0.30
21	Lipid	AC1L_MOUSE	GIQVINDGFLQSSR	0.75	0.11	0.05
22		AC1L_MOUSE	IVYEAATGTFPFCALLR	0.84	0.10	0.11
23		ETPD_MOUSE	YIFAEGLHGKALR	0.96	0.07	0.30
24		HCSR_MOUSE	TFEELVQIK	1.02	0.08	0.71
25		HCSR_MOUSE	RMKQKAK	0.96	0.09	0.04
26	Transport	ADT1_MOUSE	EFNGLDGLTK	1.70	0.02	0.00
27		KCAT_MOUSE	CLLDIASSGK	1.95	0.11	0.43
28		ND1A_MOUSE	GANSVYDGYLCAAK	0.87	0.02	0.01
29		ND1A_MOUSE	KTCALDAK	0.97	0.14	0.70
30		ND1A_MOUSE	TVQGGDGLR	0.82	0.12	0.18
31	Structural	MYL1_MOUSE	ITVQGGDGLR	0.82	0.11	0.18

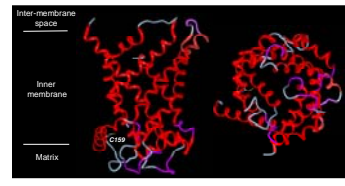


Figure 5. 3D structure of ADP/ATP translocase 1 (ADT1_RAT). C-159 is one of the ATP binding sites in the matrix.

CONCLUSIONS

- There are total 26 light/heavy ICAT-labeled proteins reproducibly identified from young/old rat heart interfibrillary mitochondria.
- These 26 proteins belong to different biological functional groups, for instance, the mitochondrial respiratory chain, lipid metabolism, TCA cycle, and transportation.
- Most heavy/light ICAT ratios are close to 1. 11 of them are different from 1 (p-value < 0.05). The ratio from ADP/ATP translocase 1 shows the greatest difference (average ratio 0.76) among all the proteins.
- ADP/ATP translocase 1 belongs to mitochondrial carrier family, is an integral membrane protein. It catalyzes the exchange of ADP and ATP across the mitochondrial inner membrane. The ICAT-labeled site C-159 is one of the ATP binding sites in the matrix.
- There are 10 proteins previously observed modification by acrolein, for instance ATP synthase subunit d (ATP5H_MOUSE) aa 100-111 in complex V.

ACKNOWLEDGEMENT

This project was supported by the National Institute on Aging (AG025372) and the Medical Research Foundation of Oregon. The authors wish to acknowledge the Mass Spectrometry core facility of the Environmental Health Sciences Center at Oregon State University (ES00210).

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