

Development of a Mass Spectrometry Approach for Studying Mitochondrial Thiol Proteins

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OVERVIEW

Purpose

- Detection and identification of free, exposed mitochondrial thiol proteins that are potential targets of oxidative damage by using a thiol-specific probe

Methods

- HPLC separation
- LCQ, ESI-q-TOF, and MALDI-TOF/TOF
- MASCOT search

Results

- Identification and characterization of butyltriphenylphosphonium (BTP)-labeled model peptide derivatives
- Higher ionization efficiency found in BTP-labeled peptides
- Several mitochondrial thiol proteins identified

Methods

4-iodobutyl-triphenyl-phosphonium (IBTP)- d_7/d_{15} were synthesized by reacting 1,4-diiodobutane with triphenylphosphine (d_7/d_{15}). Model peptide, vasopressin, was reduced with TECP, and then reacted with IBTP for 2 hours at room temperature. BTP-labeled peptides were separated by C_4 HPLC and analyzed on a quadrupole ion trap mass spectrometer (LCQ).

Rat cardiac mitochondria were isolated by centrifugation, disrupted by several freeze-thaw cycles and incubated with IBTP(d_7/d_{15}) for 1 hour at 37°C. After reaction the mixture was centrifuged to pellet insoluble fractions. Tryptic digests of soluble BTP-labeled proteins were analyzed by nano-LC-ESI-q-TOF MS and LC-MALDI-TOF/TOF MS. Mascot was used for protein identification.

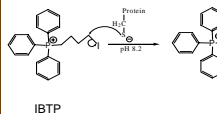
Introduction

Mitochondrial thiol proteins are putative targets of oxidation-related protein dysfunction in the aging heart. They are susceptible to numerous modifications but difficult to assess. In this study we attempted to detect and identify oxidized proteins in cardiac mitochondria from young and aged rat.

IBTP, a thiol-specific probe, was chosen as an alkylation reagent to detect the surface-exposed protein thiols. The alkylation reaction results in addition of BTP to the sulfhydryl moieties of cysteine side chains (Scheme 1). Deuterium substitution on the phenyl groups of IBTP produces a "heavy" stable isotope with an increased mass of 15 Da. This potentially enables MS-based relative quantitation of two independent samples by reacting each with either IBTP- d_7 or IBTP- d_{15} in MS analysis.

First, vasopressin was used to initially assess the influence of the BTP binding on mass spectrometric analysis. Additionally, subarcolemmal mitochondria (SSM) from young (3-months-old) and old (25-months-old) rat hearts were reacted with BTP- d_7/d_{15} , digested with trypsin and analyzed on ESI-q-TOF or MALDI-TOF/TOF MS.

Scheme 1. Modification reaction of protein thiols with IBTP



Results-Model peptide vasopressin

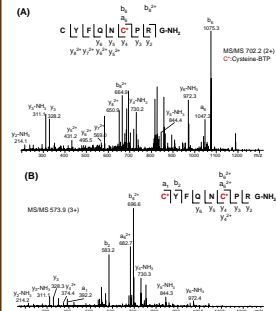


Figure 1. Tandem mass spectra of vasopressin containing one (A) and two (B) BTP-labeled cysteine residues.

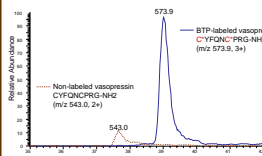


Figure 2. Increase in ionization efficiency of vasopressin after BTP-derivatization.

Results-Mitochondria sample

Table 1. List of IBTP-labeled peptides identified by MS

No.	sample	Identified protein	Sequence of BTP-labeled peptide	Ion score	MS instrument
1	d_7 YSSM ^b	Aconitate hydratase	VGLIGC [*] TFNSSYEDMGR	47	ESI-Q-TOF
2	d_7 YSSM	NADH-ubiquinone oxidoreductase 51kDa subunit	GAGAYIC [*] GEETALIESIEGK	90	ESI-Q-TOF
3	d_7 YSSM	Carnitine O-palmitoyltransferase II	C [*] SEAFVR	16	ESI-Q-TOF
4	d_7 YSSM	Creatine kinase	LGYLTC [*] PSNLGTGLR	69	ESI-Q-TOF
5	d_7 YSSM	Creatine kinase	LIDDFHFLDKPVSLLTC [*] AGMAR	29	ESI-Q-TOF
6	d_7 YSSM	ATP synthase O subunit	GEVPC [*] TVTTAFPLDEAVLSELK	32	ESI-Q-TOF
7	d_7 YSSM	Isocitrate dehydrogenase NADP	NILGGTVFREPIIC [*] K	47	ESI-Q-TOF
8	d_7 YSSM	ATP synthase D chain	NC [*] AQFVTSQAR	43	MALDI-TOF/TOF
9	d_7 YSSM	ATP synthase gamma chain	GLC [*] GAHSSVAK	36	MALDI-TOF/TOF
10	d_{15} YSSM	ADP,ATP carrier protein 1	EFNLGDC [*] LTK	19	MALDI-TOF/TOF
11	d_{15} OSSM ^c	ATP synthase O subunit	GEVPC [*] TVTTAFPLDEAVLSELK	34	ESI-Q-TOF

^aNo.1-10 experiments were performed under denaturing conditions (SM 13), and no.11 was under non-denaturing conditions. ^bYSSM is subarcolemmal mitochondria (SSM) from young rat. ^cOSSM is subarcolemmal mitochondria (SSM) from old rat. ^{*}The asterisk * marks the cysteine residue which is modified by BTP moiety.

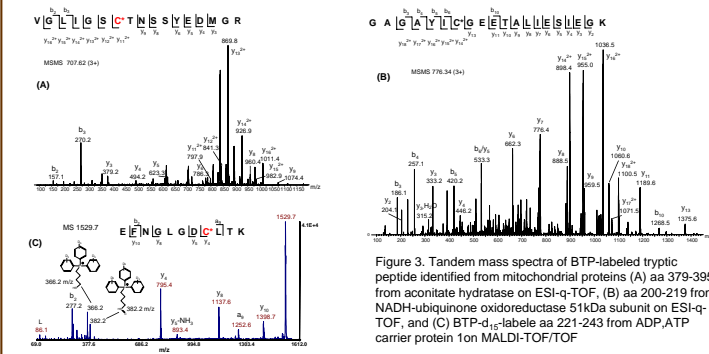
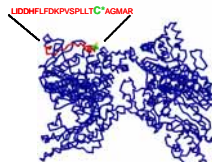


Figure 3. Tandem mass spectra of BTP-labeled tryptic peptide identified from mitochondrial proteins (A) aa 379-395 from aconitate hydratase on ESI-q-TOF, (B) aa 200-219 from NADH-ubiquinone oxidoreductase 51kDa subunit on ESI-q-TOF, and (C) BTP- d_{15} -labeled aa 221-243 from ADP,ATP carrier protein 1 on MALDI-TOF/TOF

Conclusion

* The introduction of BTP moieties enhances charge-directed fragmentation, and increases the ionization efficiency of labeled peptides, which make these labeled peptides easier to detect by mass spectrometry.

* IBTP, a thiol-specific probe can react with the free, exposed thiol groups in mitochondrial proteins, which help to detect and identify the potential targets of oxidative damage in mitochondria. For instance, the following picture shows the BTP-labeled aa 221-243 in Creatine kinase.



Acknowledgement

This project is supported by the National Institute on Aging and the Medical Research Foundation of Oregon. The authors wish to acknowledge the Molecular Structure and Interactions core facility and the Mass Spectrometry core facility of the Environmental Health Sciences Center at Oregon State University.

References

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