

A MASS SPECTROMETRY-BASED PROTEOMICS APPROACH FOR MITOCHONDRIAL THIOL PROTEINS

Gretchen L. Clark-Scannell¹, Duane T. Mooney¹, Jan F. Stevens² and Claudia S. Maier¹

¹ Department of Chemistry, Oregon State University, Corvallis, OR

² Department of Chemistry and Linus Pauling Institute, Oregon State University, Corvallis, OR

OVERVIEW

Purpose

- Identification and characterization of mitochondrial thiol proteins that are potential targets of oxidative damage
- Comparative quantification of thiol proteins

Methods

- Mass spectrometry-based functional and quantitative proteomics approach using a stable isotope-coded thiol specific probe, namely (4-iodobutyl)triphenylphosphonium IBTP- d_0 and IBTP- d_{15}
- 2D gel electrophoresis, LC-ESI-MS/MS, LC-MALDI-MS/MS
- Rat heart mitochondria

Results

- Several potential protein targets of oxidative stress and aging-related protein damage identified
- Mass-coded IBTP reagents allows relative quantification of thiol proteins
- IBTP tag has properties that allows the use of database search engines for protein identification

Methods

IBTP reagents were synthesized according to the procedure described by Lin et al. 2002.

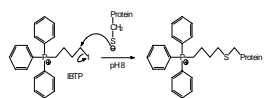
Mitochondria were isolated from rat heart according to Suh et al. 2003. Mitochondria were incubated with IBTP in phosphate buffer (pH 8) for 15 min at 37°C. Mitochondria were disrupted by several freeze-thaw cycles. Soluble and insoluble protein fractions were obtained by centrifugation. Proteolytic digests of IBTP-labeled proteins were analyzed by nanoscale LC-ESI-MS/MS using a Micromass q-ToF2 Global Ultima mass spectrometer. In addition, peptide mixtures were separated on a capillary LC and the eluting peptide were directly spotted on MALDI targets and analyzed using an AB 4700 Proteomics Analyzer. For protein identification the search engines Mascot and GPS explorer were used.

Introduction

Mitochondria are considered as a principal source and target of reactive oxygen species (ROS) with mitochondrial damage and dysfunction observed in aging and aging-related pathologies. Potential modification of protein thiols that can alter function include disulfide formation, S-glutathionylation, formation of higher oxidation states, and S-nitrosylation. Although increased levels of post-translationally modified protein thiols are recognized as biomarkers of oxidative stress and aging-related damage in mitochondria, the identity of distinct protein targets and the specific sites of modification are largely unknown.

In this study, we report an experimental strategy for comparative quantification of thiol proteins in two different mitochondria samples. Because of the nucleophilicity of the sulfhydryl functionality, thiol proteins are potential targets of oxidative stress-related modification reactions. In order to identify proteins with surface-exposed thiol groups, we use as a thiol-specific probe (4-iodobutyl)triphenylphosphonium (IBTP). IBTP forms stable thio-ether bonds with exposed sulfhydryl groups (Figure 1) and accumulates in the mitochondrial matrix due to the mitochondrial membrane potential (Lin et al. 2002). In combination with a quantitative mass spectrometry-based proteomics approach, stable isotope-coded light (d_0) and heavy (d_{15}) IBTP reagents were utilized. The mass spectrometric properties of IBTP-tagged peptides were studied by ESI-MS/MS and MALDI-MS/MS. The mass-coded IBTP reagent was successfully applied toward the identification and quantification of thiol proteins of rat heart mitochondria.

Figure 1: Modification reaction of protein thiolates with (4-iodobutyl)triphenylphosphonium (IBTP)



Lin et al. 2002

Figure 2: Mass spectrometric properties of IBTP-labeled peptides

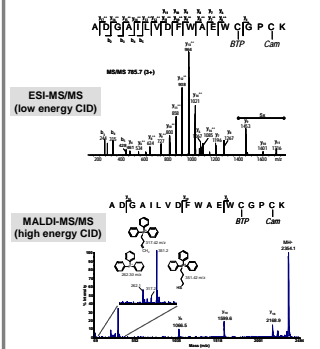


Figure 5: 2D gel electrophoretic separation of rat heart mitochondrial proteins and MALDI-MS/MS for protein identification

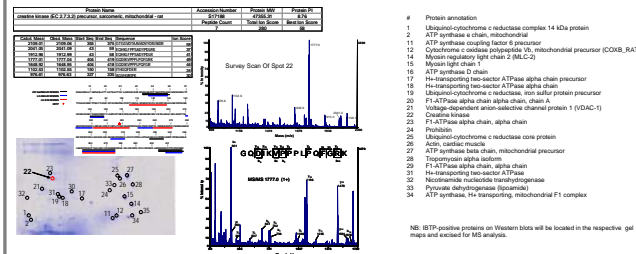


Figure 3: Functional and quantitative proteomics strategy for the characterization of mitochondrial thiol proteins

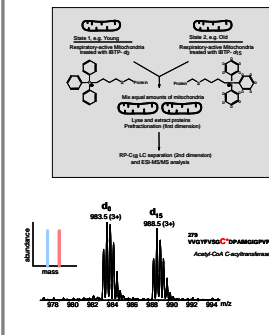
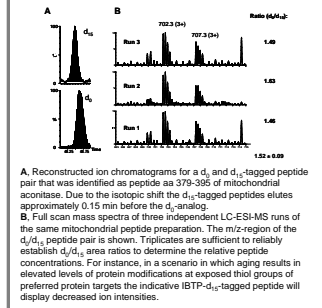
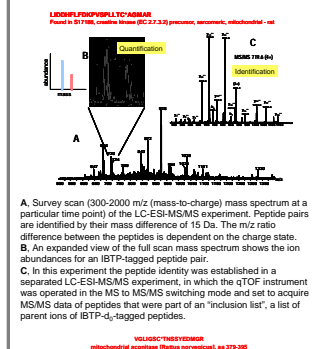


Figure 4: Quantitative mitochondrial redox proteomics with stable-isotope coded IBTP probes



Results

Using a combination of mass spectrometry-based functional and quantitative proteomics approaches we were able in this proof-of-concept study to uncover a number of mitochondrial proteins with reactive thiols which are likely targets of oxidative modification reactions. The mass coded-IBTP labeling strategy allows not only the identification of mitochondrial thiol proteins but also provides (i) site-specific information regarding particularly susceptible thiols and (ii) the relative extent of oxidative damage to distinct protein thiol sites.

Prominent examples of positive thiol protein identifications include creatine kinase (mtCK), aconitase, acetyl CoA C-acyl transferase, isocitrate dehydrogenase, and dihydroliipoamide succinyl-transferase.

We are currently using the comparative IBTP mass tagging approach to identify and quantify thiol proteins in heart mitochondria that are particularly susceptible to aging-related oxidative damage in rats.

Acknowledgements

This project is supported by the Environmental Health Sciences Center (to C.S.M.), the Medical Research Foundation of Oregon (to C.S.M.) and the American Heart Association (to J.F.S.). MS experiments were conducted in the Mass Spectrometry Core of the Environmental Health Sciences Center at OSU (P30 ES00210 NIEHS).

References

Suh, J.H., Heath, S.-H., and Hagen, T.M. Free Radic. Biol. Med. 35: 1064-1072 (2003)
 Lin, T.K., Hughes, G., Muratovska A., Blaikie, F.H., Brookes, P.S., Darley-Usmar, V., Smith, R.A. and Murphy, M.P. J. Biol. Chem. 277: 17048-17056 (2002)