

Assessing the effect of aging on proteins using an aldehyde-specific reagent, affinity-staining and MS

Duane T. Mooney and Claudia S. Maier

Department of Chemistry, Oregon State University, Corvallis, OR

OVERVIEW

Purpose

Identification and characterization of mitochondrial proteins that are putative targets of oxidative modification by lipid peroxidation products as a consequence of aging

Methods

- Mass spectrometry-based functional proteomics approach using N'-aminomethylcarbonylhydrazino D-biotin as aldehyde-specific reagent
- 1D and 2D gel electrophoresis, affinity staining using HRP-labeled streptavidin, LC-ESI-MS/MS
- Rat heart mitochondria

Results

Several putative protein targets of oxidative stress and aging-related protein damage identified, e.g. ATPase alpha and beta polypeptide chain, electron transfer flavoprotein alpha-subunit (ETF α)

Methods:

Mitochondria were isolated from rat heart according to Suh et al. 2003. Subsarcolemmal (SSM) and interfibrillary (IFM) mitochondria were isolated from the myocardium of young and old rats. Mitochondria were disrupted by several freeze-thaw cycles. Soluble and insoluble fractions were obtained by centrifugation. The fractions were incubated with ARP, and the proteins were subjected to one or two-dimensional SDS-PAGE. After blotting to nitrocellulose, ARP-labeled proteins were visualized using HRP-conjugated Neutravidin (Pierce). Bands of interest were cut out and subjected to trypsin digestion. Tryptic peptides were analyzed by nanoscale LC-ESI-MS/MS on a Micromass qToF2 Global Ultima mass spectrometer. Mascot was used for protein identification.

Introduction

The oxidatively modified proteins are suspected to be associated with cellular dysfunctions observed during the aging process. Mitochondria are considered to be a principal source, and target of reactive oxygen species (ROS). Aging-associated decline of mitochondrial function has been correlated with ROS. Post-translational oxidative modification to proteins has been shown to increase with age, and it is thought that oxidative protein modifications are at least partly responsible for loss of structural and functional integrity of some proteins and/or altered protein degradation.

In this study, we report an aldehyde/keto-specific labeling strategy for assessing aging-related oxidative damage to proteins. The probe, N'-aminomethylcarbonylhydrazino D-biotin or aldehyde-reactive probe(ARP), has been commonly used to detect abasic sites in DNA. Here we show that ARP reacts readily with the aldehyde functionalities introduced to proteins by conjugation reactions with lipid peroxidation products.

Further, soluble and membrane-associated protein extracts from SSM and IFM-type mitochondria (isolated from young (4 months) and old (24-28 months) rats) were analyzed for oxidatively modified proteins using ARP. Protein extracts were treated with ARP, resolved by SDS-PAGE, blotted to nitrocellulose, and biotinylated proteins were detected by affinity staining using peroxidase-labeled streptavidin. LC-ESI-MS/MS was used for protein identification.

Figure 1: Modification of aldehyde functionalities by N'-aminomethylcarbonylhydrazino D-biotin (ARP)

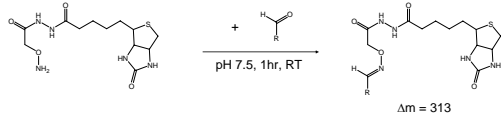
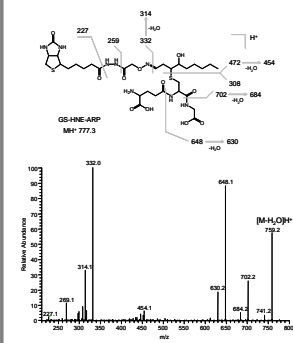
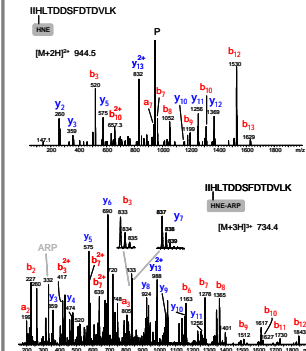


Figure 2: Mass spectrometric fragmentation study of ARP-tagged HNE-modified glutathione



The presence of HNE-ARP conjugate on GSH was assessed by tandem mass spectrometry using an ESI-ion trap mass spectrometer (Thermo Finnigan LCQ).

Figure 3: ARP-labeling and MS-identification of tryptic peptides of HNE-modified thioredoxin



HNE-modified thioredoxin was reacted with ARP, and the reaction product was examined using an ESI-ToF mass spectrometer. Analysis of tryptic peptides of the HNE-ARP adducted thioredoxin utilized nanoscale LC-ESI-MS/MS on a Waters Micromass qToF2 Global Ultima mass spectrometer.

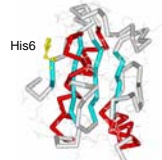
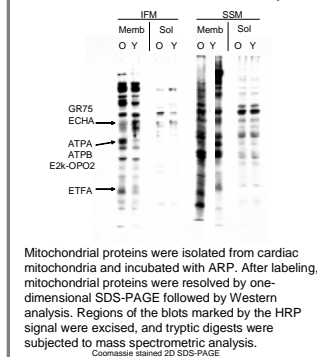
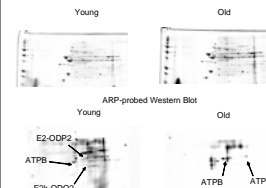


Figure 4: Affinity-staining and mass spectrometric identification of ARP-labeled mitochondrial proteins



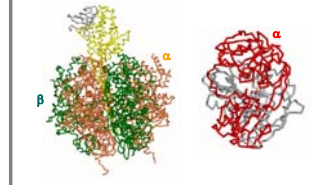
Mitochondrial proteins were isolated from cardiac mitochondria and incubated with ARP. After labeling, mitochondrial proteins were resolved by one-dimensional SDS-PAGE followed by Western analysis. Regions of the blots marked by the HRP signal were excised, and tryptic digests were subjected to mass spectrometric analysis.



To further refine our list of candidate proteins that may contain aldehyde-functionalities, ARP-labeled mitochondrial proteins were resolved by two-dimensional electrophoresis, and blotted. For protein identification tryptic peptides were analyzed by LC-ESI-MS/MS.

Results:

N'-aminomethylcarbonylhydrazino D-biotin (ARP) was used as aldehyde-specific reagent for the detection of oxidatively modified proteins in combination with affinity staining using peroxidase-labeled streptavidin. Using this new approach we were able to identify several putative protein targets of oxidative modification. For instance, we identified the alpha and beta polypeptide chains of the mitochondrial ATP synthase and the ETF-alpha chain as putative targets of oxidative modification in cardiac mitochondria.



Acknowledgements

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

- Fannin, S.W., Lesnfsky, E.J., Slabe, T.J. *Biochem. Biophys.* (1999) 372: 399-407
- Suh, J.H., Heath, S.-H., Hagen, T.M. *Free Radic. Biol. Med.* (2003) 35: 1064-1072
- Yanagisawa, H., Hirano, A., Sugawara, M. *Anal. Biochem.* (2004) 332: 358-367