

# Relative quantification of oxylipid-modified peptides using a QTrap instrument

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## Overview

### Purpose

● To determine the relative abundance of oxidative damage by acrolein occurring in-vivo for several mitochondrial proteins

### Methods

● 4000 Q-Trap hybrid tandem mass spectrometer with a nano-ESI source

### Results

● The ion chromatographies for six pairs of native and acrolein-modified mitochondrial peptides in in-vivo were obtained.

## Methods

All experiments were carried out on a 4000 Q-Trap hybrid tandem mass spectrometer (AB/MDS SCIEX, Concord, Ontario, Canada) using a nano-ESI source. MRM mode was run with Q1 and Q3 set at unit resolution to increase the specificity.

Nano liquid chromatography was achieved with the LC Packings Integrated System: Famos autosampler with Switchos and Ultimate pumps (Dionex, San Francisco, CA, USA). The column used was a 75 µm id x 15 cm C18 PepMap coupled to the MS instrument using a 15 µm id nanospray tip (New Objectives, Cambridge, MA, USA) via a liquid junction. The flow rate was 280 nanoliter/min. The needle voltage was set to +2500 V for analysis in positive mode. 95% of 0.5 mg digested mitochondrial proteins were used to enrich the oxylipid-modified peptides by a monomeric avidin affinity column. The enriched sample was combined with the remaining 5% of the digested mitochondrial peptides for final analysis.

## Introduction

The adduction of proteins and other biomolecules by electrophilic lipid peroxidation products such as 4-hydroxynonenal or acrolein is thought to be an initiating and/or propagating factor in the pathophysiology of several diseases such as atherosclerosis, diabetes, Alzheimer's, Parkinson's and other age-related disorders. Determining the extent or relative amounts of this oxidative damage could provide valuable insights into the molecular mechanisms of these disorders. Relative quantification of oxylipid-modified proteins in biological samples is a challenging problem because of the complexity and extreme dynamic range that characterize these samples. Here we demonstrate a method for relative quantification of oxylipid modified peptides to their corresponding unmodified peptides. By using an affinity labeling and enrichment technique along with multiple reaction monitoring (MRM) mode on a hybrid linear ion trap mass spectrometer we are able to detect modified peptides of very low natural abundance along with the more abundant native peptide with high selectivity. Here we demonstrate the potential of this method to determine the relative abundance of oxidative damage by acrolein occurring in-vivo for several mitochondrial proteins.

### Sample Preparation:

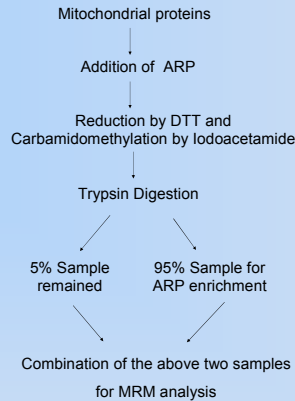


Fig. 1 Formation of ARP-labeled protein-oxylipid adduct

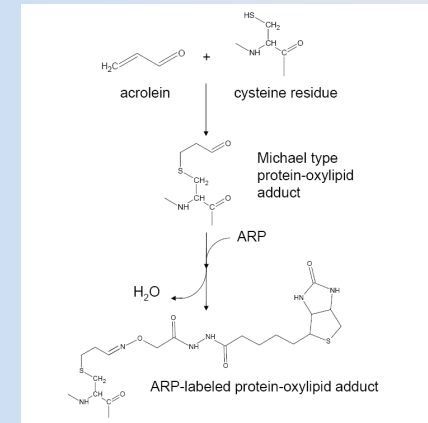


Fig. 2 MS/MS spectra for native and ACR-ARP modified peptides (EGVIECSFVQSK). The intense ions:  $y_8$ (984.4 Th) and  $y_8^*$ (1296.6 Th) were selected for further MRM analysis.

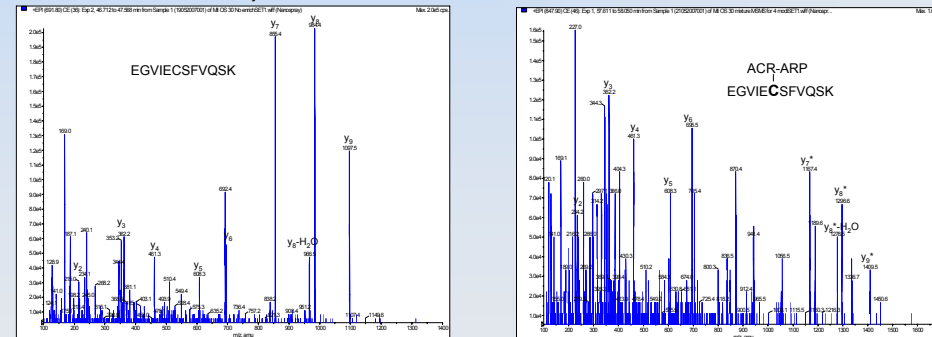
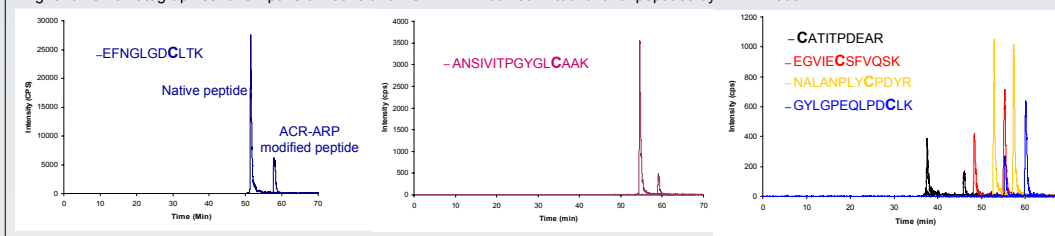


Fig. 3 Ion chromatographies for six pairs of native and ACR-ARP modified mitochondrial peptides by MRM mode



Peptides and the corresponding Mitochondrial Proteins:

Peptides	Mitochondrial Proteins
EFNGLGDCLTK	ADT1-MOUSE
CATITPDEAR	IDHP-BOVIN
EANSIVITPGYGLCAAK	NNTM-MOUSE
EGVIECSFVQSK	MDHM-RAT
NALANPLYCPDYR	UQCR2-HUMAN
GYLGPEQLPDCLK	MDHM-RAT

## Conclusions

The Information Dependent Acquisition (IDA) and Enhanced Product Ion (EPI) methods were used to identify the six acrolein modified peptides and their corresponding unmodified peptides and their retention times. For each peptide the most intense fragment ion was selected for the MRM analysis to obtain good sensitivity and minimize interference. The ion chromatographies for six pairs of native and acrolein-modified mitochondrial peptides in in-vivo were obtained. Future plans include synthesizing isotopic peptides for use as internal standards to calibrate the relative intensity.

## Acknowledgements

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## References

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