Waters

Identification and Characterization of Oxidation and Deamidation of Proteins and Peptides by LC/MS(/MS)

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Introduction

Characterization of proteins is an important analytical challenge that must be met in pharmaceutical and biotechnology companies today. Reversed-phase chromatography coupled with mass spectrometry has rapidly become the technique of choice for the determination of post-translational modifications. We have developed a simple and rapid LC/MS method for identifying the presence and degree of oxidation and deamidation of intact proteins or peptides in a mixed sample using either single quadrupole or Q-TOF type mass spectral instrumentation.

Experimental

Intact Proteins

Waters Alliance™ LC and 996 PDA Detector

Waters ZQ™ Mass Detector or Micromass Q-TOF2™

–Source = ESI (+): Capillary (kV) = 3.3, Cone (V) = 25
–Temperature (°C): Source = 150, Desolvation = 350
–Gas flow (L/Hr): Cone = 50, Desolvation = 500
–Scan Mode

Separation Method:

–Symmetry 300® C_{18}, 4.6 X 50 mm, 5.0 µm
–A: 0.1% Formic acid in water
–B: 0.1% Formic acid in ACN
–Gradient from 15% B to 80% B in 15 min
–0.5 mL/min, Split Flow to ~0.2mL/min diverted to ZQ

Peptides

Waters ZQ™ Mass Detector

–Source = ESI (+): Capillary (kV) = 3.3, Cone (V) = 30
–Temperature (°C): Source = 150, Desolvation = 350
–Gas flow (L/Hr): Cone = 50, Desolvation = 500
–Scan Mode

Micromass Q-TOF2™

–Source = ESI (+), Capillary (kV) = 3.3, Cone (V) = 30
Collision Energy=30
–Survey Scan MS 200-1600 m/z, MS/MS 50-1600 m/z
–MaxEnt3 Processed Spectrum
–BioLynx™ Peptide Sequencing

Separation Method:

–Atlantis™ dC_{18}, 4.6 X 50 mm, 3 µm
–A: 0.1% Formic acid in water
–B: 0.1% Formic acid in ACN
–Gradient from 0% B to 40% B in 45 min
–0.75 mL/min, split flow to ~0.2mL/min diverted to ZQ

Samples

Oxidation:

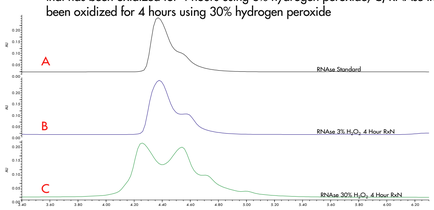
–Proteins and Peptides reacted for 4 hours using 3-30% H_2O_2 (v/v)

Deamination:

–Heating sample at 65°C for 1 day to1 month

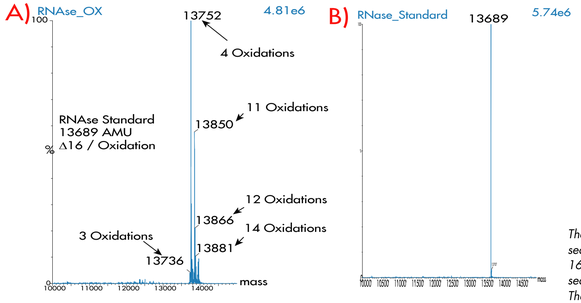
Protein Oxidation LC/UV

The effects of varying degrees of oxidation on the retention time for RNAse. The chromatogram is based on UV at 280 nm for A) RNAse Standard Protein; B) RNAse that has been oxidized for 4 hours using 3% hydrogen peroxide; C) RNAse that has been oxidized for 4 hours using 30% hydrogen peroxide



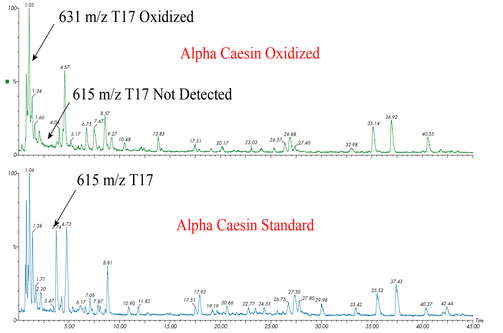
Protein Oxidation Ladder: LC/MS

The effects of oxidation on the mass spectra for RNAse. A) RNAse protein sample showing a heterogenous mixture containing varying amounts of oxidation; B) Unmodified RNAse Deconvoluted Spectrum. Note: That even within the standard, there is one major oxidized protein species.



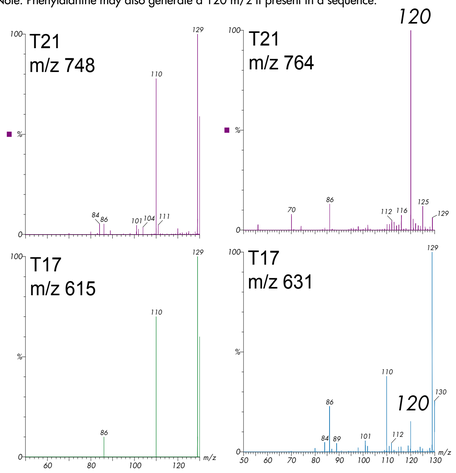
Peptide Oxidation: LC/MS

The LC/MS total ion chromatogram of peptides separated on an Atlantis™ dC_{18} column. Highlighted is the T17 peptide fragment. There is a decrease in retention time due to oxidation.



Immonium Ions Indicative of Oxidation

The ion spectra of four peptide (standards 748, 615; Oxidized 764, 631 respectively) shows oxidized methionine produces a signature 120 m/z ion.
Note: Phenylalanine may also generate a 120 m/z if present in a sequence.



Peptide Oxidation: MS/MS Sequences

The tables illustrate the b and y ions obtained from the MSMS spectra. A) The amino acid sequences are compared between alpha casein T21 peptide and oxidized T21 peptide. The 16 m/z shift corresponds to oxidation of the methionine residue. B) The amino acid sequences are compared between the alpha casein T17 peptide and oxidized T17 peptide. The 16 m/z shift corresponds to oxidation of the methionine residue.

A) T T M P L W

Alpha Casein T21 Fragment

b ion Series				y ion Series			
Ion	m/z standard	Δ m/z	m/z Ox.	Ion	m/z standard	Δ m/z	m/z Ox.
b1	102	Δ0	102	y1	205	Δ0	205
b2	203	Δ0	203	y2	318	Δ0	318
b3	334	Δ16	350	y3	415	Δ0	415
b4	431	Δ16	447	y4	546	Δ16	562
b5	544	Δ16	560	y5	-	-	-
bmax	730	Δ16	746	ymax	748.5	Δ16	764.5

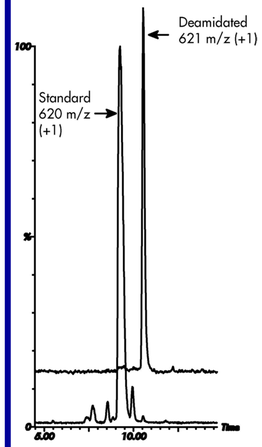
B) L H S M K

Alpha Casein T17 Fragment

b ion Series				y ion Series			
Ion	m/z standard	Δ m/z	m/z Ox.	Ion	m/z standard	Δ m/z	m/z Ox.
b1	-	-	-	y1	147	Δ0	147
b2	251	Δ0	251	y2	278	Δ16	294
b3	338	Δ0	338	y3	365	Δ16	381
b4	467	-	-	y4	502	-	-
bmax	597	Δ16	613	ymax	615	Δ16	631

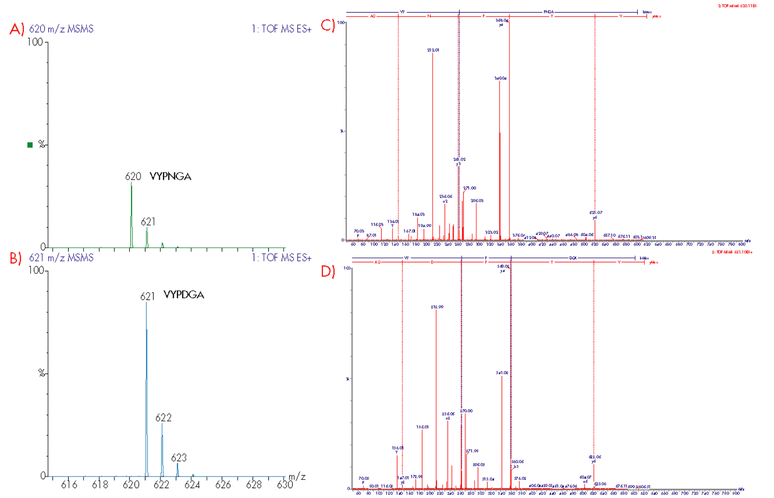
Peptide Deamidation LC/MS

Differences occur during deamidation where most peptides generally increase their retention time as illustrated here using ACTH hormone peptide fragments.



Peptide Deamidation LC/MS(/MS)

Identification of peptides which have been deamidated by LC/MS with confirmation by LC/MS/MS. Note: The 1 AMU difference between isotopes of the unmodified peptide in the +1 charge state and the deamidated peptide in the +1 charge state. A) The isotope pattern of the unmodified peptide; B) The isotope pattern of the deamidated peptide; C) The analysis and identification of the peptide sequence for the unmodified peptide; D) The analysis and identification of the peptide sequence for the deamidated peptide.



Conclusions

- **The LC/UV/MS/MS described here is a robust and universal method to identify oxidations and deamidations occurring in both intact proteins and peptides**
- **Identification of oxidation in intact proteins is characterized without having to perform peptide mapping.**
- **Isotope patterns and MSMS sequencing of peptides are successfully used for identification and characterization of deamidation.**
- **Oxidized Methionine produces a signature 120 m/z ion in all cases we observed.**

References

1)Paul Rainville, Claude Mallet, and Reb Russell II, Detecting and Mapping Carbamylation of Intact Proteins and Peptides by LC/UV/MS/MS. BioApps, SanDiego, June 25-28th

2)Robinson NE, Robinson AB, Merrifield RB. 2001. Mass spectrometric evaluation of synthetic peptides as primary structure models for peptide and protein deamidation. J Pept Res;57(6):483-93

3)Zhu H, Hunter TC, Pan S, Yau PM, Bradbury EM, Chen X. 2002. Residue-specific mass signatures for the efficient detection of protein modifications by mass spectrometry. Anal Chem; 74(7):1687-94