Peptide Fragmentation Regimes Studied By Harmonic Field Reflectron MALDI ToF-ToF

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Overview

Aim

To improve the reliability of identification of proteins from a 2D gel spot after digestion with an enzyme by:

- Establishing the amino acid sequence of the peptides in the digest.
- Confirming the identity of the protein from a database using the peptide mass and sequence data.

Method

A MALDI ToF-ToF with Harmonic Field Reflectron was used to study the fragmentation of tryptic peptides by:

- Unimolecular dissociation.
- Collisional dissociation.
- A chemical modification of the peptide 'N' terminus which is designed to give only 'Y' fragmentation via unimolecular dissociation.

Results

- For tryptic peptides unimolecular dissociation by post source decay is suppressed because the proton is preferentially attached to the arginine residue.
- Surprisingly, collisional excitation does not enhance the efficiency of fragmentation. However, it does promote the formation of immonium ions.
- Chemical modification of the 'N' terminus yields a virtually complete spectrum of 'Y' ions with increased sensitivity over the other two methods. This 'Y' ion spectrum establishes the amino acid sequence of the peptide.

Introduction

Use of MALDI ToF - ToF to study peptide fragmentation by the following methods:

- Seamless Post Source Decay (sPSD).
- Seamless Collision Induced Dissociation (sCID).
- Chemical derivitization of the 'N' terminal followed by Seamless PSD.

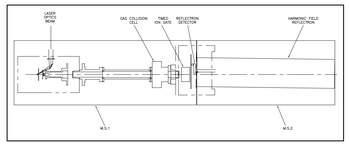
Methods

Instrumentation

MALDI ToF - ToF manufactured by Amersham Pharmacia Biotech AB, Uppsala, Sweden and SAI Ltd., Manchester, England, where:

ToF 1 Pulsed extraction MALDI ion source and in-line time of flight with 66 cm flight path.

ToF 2 Harmonic field ($V \propto z^2$) reflectron ToF (See schematic).

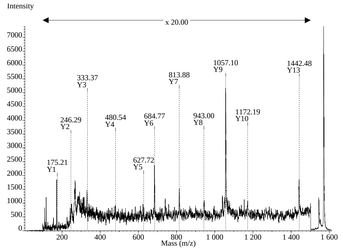


Instrument Schematic

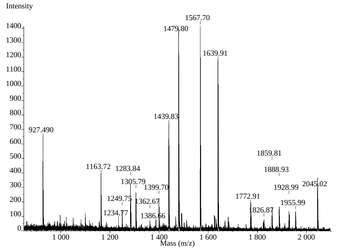
Fragmentation Regimes

- Seamless PSD. Unimolecular fragmentation in field free drift space.
- Seamless CID. He - (M + H)+ pulsed gas collision cell with ~ 18 eV/Dalton centre of mass collision energy.
- Derivitized - Seamless PSD. Derivitization chemistry *in aqua* followed by unimolecular dissociation in field free drift space.

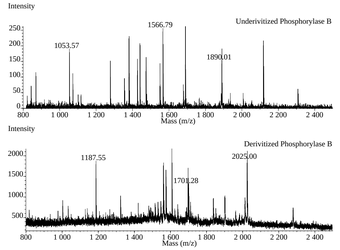
Results



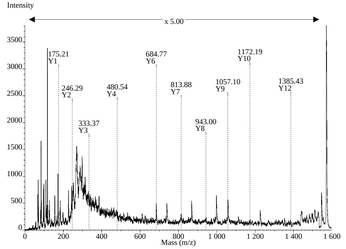
Model tryptic peptide 'EGVNDNEEGFFSAR' showing native sPSD spectrum.



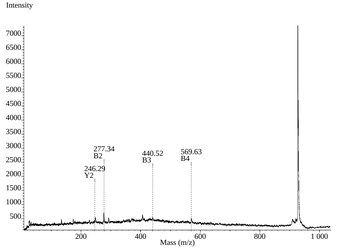
BSA -4VP tryptic digest.



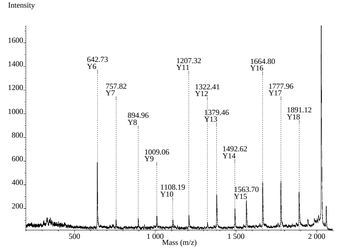
Digested protein Phosphorylase B before and after derivitization. Note: Mass shift due to the derivitization.



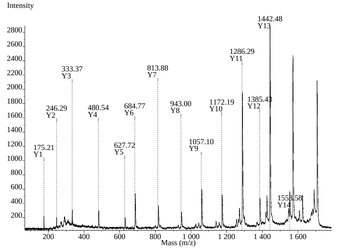
Model tryptic peptide 'EGVNDNEEGFFSAR' showing the sCID spectrum. Note the appearance of the immonium ions.



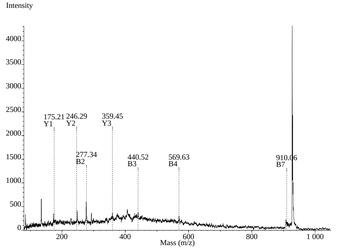
Digested protein of BSA showing sPSD of the tryptic peptide 'YLYEIAR' at m/z 927.5.



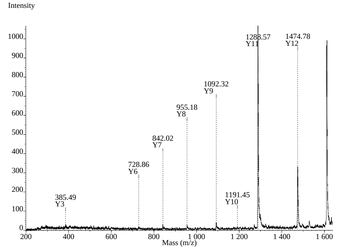
sPSD spectrum of the derivitized tryptic peptide 'LITAIGDVVNHDPVVGDR' at m/z 2027 from the sample shown above.



Derivitized model peptide 'EGVNDNEEGFFSAR' showing the sPSD spectrum.



Digested protein of BSA showing sCID of tryptic peptide 'YLYEIAR' at m/z 927.5.



sPSD spectrum of the derivitized tryptic peptide 'WPVHLLETLLPR' at m/z 1611 from the sample shown above.

Conclusion

For tryptic peptides with arginine at the 'C' terminus:

- Seamless Underivitized PSD gives very incomplete fragmentation and poor sensitivity.
- Seamless Underivitized CID produces immonium ions and increases the yield of fragment ions slightly.
- Derivitized Seamless PSD gives an almost complete 'Y' ion fragmentation, with increased sensitivity, which is easy to interpret.