MS/MS of Peptides
Manual Sequencing of Protonated Peptides

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Peptides – Product Ion Scan

• Product ion spectra contain many types of fragment ions
  • charge directed
  • charge remote
  • “a”, “b”, “y” type sequencing ions
  • internal fragments, immonium ions

• Important for sequencing
  • amino acid determined from Δ mass between peaks in spectrum
  • “y” ions series
  • “b” ions series
  • immonium ions (identify amino acids in the peptide)
  • “a” ions (confirm “b” ion after a loss of CO, 28 amu)

• Presented here:
  • peptide fragment ions
  • a mechanism for fragment ion formation
  • a peptide to sequence
A mechanism of peptide fragmentation

(1) Δ positive charge

(2) Nucleophilic attack

(3) cyclic intermediate

Ref: Wysocki, 2000

A mechanism of peptide fragmentation

(4) Rearrangement

b oxazolone ion neutral

Ref: Wysocki, 2000
A mechanism of peptide fragmentation

(4) Rearrangement

oxazolone neutral (or other structure)

y ion

Ref: Wysocki, 2000

Acidic group of Asp (D) can cause cleavage

Ref: Wysocki, 2000
Peptides fragment in a predictable way

If doubly charged parent:
possible b/y ion pair
or doubly charged b or y
fragment ions

Peptides fragment in a predictable way
resulting in a series of peptide fragment ions
- b/y ion series commonly used for sequencing common with CID
- **Alternative** activation methods (ETD, ECD) generate c/z ion series
  - Can also be used to sequence peptides

\[
[M + 3H]^3^+ + A^- \rightarrow [M + 3H]^{2^+} + A
\]

\[
[M + 3H]^{2^+} \rightarrow [C+2H]^{1^+} + [Z+H]^{1^+}
\]

1

**c/z Ion formation mechanism**

c/z Ion formation mechanism

\[ \Delta \text{ between ion series} = \text{Residue mass} \]

\[ \begin{align*}
\text{Peptide bond fragment ions} \\
\text{b}_1 & \quad y_3 \quad \text{m/z} 190 \\
\text{b}_2 & \quad y_2 \quad \text{m/z} 133 \\
\text{b}_3 & \quad y_1 \quad \text{m/z} 76
\end{align*} \]

Residue Mass
specific to amino acid
present in sequence
### Peptide Sequencing

<table>
<thead>
<tr>
<th>Residue</th>
<th>3-letter code</th>
<th>1-letter code</th>
<th>Immonium ion*</th>
<th>Related ion*</th>
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<tbody>
<tr>
<td>Alanine</td>
<td>Ala</td>
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<td>71 u.</td>
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<td>Arginine</td>
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<td>115 u.</td>
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<td>Aspartic Acid</td>
<td>Asp</td>
<td>D</td>
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<td>Cysteine</td>
<td>Cys</td>
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<tr>
<td>Glutamic Acid</td>
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<td>Histidine</td>
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</table>

**Immonium and related ion masses after Falk, 1992 #690 and Papayiannopoulo, 1995 #681.**Bold face indicates strong signals, italic indicates weak.

http://www.matrixscience.com/help/fragmentation_help.html
LEARNING CHECK

Tryptic Peptide Sequencing Exercise performed in a low res ion trap MS/MS

Ion Chromatogram
Peak chosen at 26.47 min

Mass at 571.36 chosen for MS/MS

Ion Current over 60 min

MS/MS
Peptide precursor ions observed by MS

MH\(^+\) m/z = 1141.3

\[ [M+H]^+ \times \text{m/z measured} \]
\[ 1142.4 \] [M+2H]
\[ 1141.4 \] [M+H]

HOW?

MS/MS of 571.2

This spectrum will tell us peptide sequence eventually HOW?
An MS/MS spectrum of the m/z = 571.4 peptide. We will sequence this together.
y series ions

87 = Serine
156 = Arginine
243
19 = mass of H + OH + H
262
Sequencing a Peptide

Peptide Mass MH+(monoisotopic): 1025.5374
Sequence: TELAAEVHR

Computer programs search databases that contain information and sequence of proteins

<table>
<thead>
<tr>
<th>Measured peptide</th>
<th>Mass</th>
<th>GI</th>
<th>Protein</th>
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<tbody>
<tr>
<td>278.39</td>
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<td>2343.72</td>
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<td>322.38</td>
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This works because the fragments are predictable

### MS-Product Search Results

**Parameters**

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**Protein Prospector: [http://prospector.ucsf.edu/](http://prospector.ucsf.edu/)**

More peptides identified increases confidence in ID

If all of these peptides belonged to an unknown protein, MS/MS could potentially reveal protein identity
Sequence more peptides

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Spectra</th>
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<tbody>
<tr>
<td>VFGTDMDNSR</td>
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<td>IFDDSDQTK</td>
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<td>IFDDSDQTK</td>
<td>Spectra 4</td>
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<td>GLYDKDFR</td>
<td>Spectra 5</td>
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Note that Peptide 1 & 4 are the same

1 = doubly charged precursor
4 = singly charged precursor