Mass Spectrometry of Biological Molecules

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Pharmacology and Molecular Sciences

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Mass Spectrometry at the JHU/SOM

References.


Specific references in the text.
General Principles: What is a mass spectrometer?

- **Chemical**
  - EI: electron impact
  - GD: glow discharge
  - CI: chemical ionization

- **Biological**
  - MALDI: matrix-assisted laser desorption/ionization
  - ESI: electrospray ionization
  - AP/MALDI: atmospheric pressure MALDI

**Vacuum System**
- ion source
- mass analyzer
- detector

- TOF: time-of-flight
- ITMS: ion trap mass spectrometer

- multiplier
- channelplate

- a balance for weighing molecules

Topics in Biophysics 2001: Mass Spectrometry
**Basic Components of Mass Spectrometers**

<table>
<thead>
<tr>
<th>Ionization Sources</th>
<th>Mass Analyzers</th>
<th>Detectors</th>
</tr>
</thead>
<tbody>
<tr>
<td>thermal ionization</td>
<td>magnetic (B)</td>
<td>photographic plate</td>
</tr>
<tr>
<td>spark source</td>
<td>double-focusing (EB)</td>
<td>faraday cup</td>
</tr>
<tr>
<td>electron impact (EI)</td>
<td>reversed geometry (BE)</td>
<td>electron multiplier</td>
</tr>
<tr>
<td>photoionization (PI)</td>
<td>on cyclotron resonance (ICR)</td>
<td>magnetic electron multiplier</td>
</tr>
<tr>
<td>chemical ionization (CI)</td>
<td>quadrupole (Q)</td>
<td>continuous dynode multiplier</td>
</tr>
<tr>
<td>field ionization (FI)</td>
<td>quadrupole ion trap (ITMS)</td>
<td>dual channelplate</td>
</tr>
<tr>
<td>field desorption (FD)</td>
<td>radio frequency (RF)</td>
<td>Daly detector</td>
</tr>
<tr>
<td>multiphoton ionization (MPI)</td>
<td>time-of-flight (TOF)</td>
<td>diode array detector</td>
</tr>
<tr>
<td>fast atom bombardment (FAB)</td>
<td>Fourier transform (FTMS)</td>
<td>image currents</td>
</tr>
<tr>
<td>plasma desorption mass spectrometry (PDMS)</td>
<td>triple quadrupole (QQQ)</td>
<td>inductive detector</td>
</tr>
<tr>
<td>secondary ion mass spectrometry (SIMS)</td>
<td>four sector (EBEB)</td>
<td></td>
</tr>
<tr>
<td>thermospray (TS)</td>
<td>hybrid (EBQQ)</td>
<td></td>
</tr>
<tr>
<td>infrared laser desorption (IRLD)</td>
<td>hybrid (EB/TOF)</td>
<td></td>
</tr>
<tr>
<td>matrix-assisted laser desorption (MALDI)</td>
<td>tandem TOF/TOF</td>
<td></td>
</tr>
<tr>
<td>electrospray ionization (ESI)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mass spectrometers can be characterized by the ionization, mass analysis and detection methods that are used. Photographic plates were used on early mass spectrometers that employed static magnetic fields to achieve mass separation by spatial dispersion. These include the magnetic sector, double focusing (both Mattauch-Herzog and Nier-Johnson), and reversed geometry instruments. All others are dynamic instruments that disperse ions according to frequency or time. A particularly interesting case is that of Fourier transform mass spectrometers (FTMS). In this case, excitation of ions in an ion cyclotron resonance mass spectrometer is recorded from the image currents induced on plates positioned orthogonal to the excitation signal. Not all ionization techniques have been utilized with each mass analyzer. In general, magnetic field and quadrupole mass spectrometers are most appropriately used with continuous ionization techniques, while time-of-flight mass spectrometers are most suitable for pulsed methods. The triple quadrupole, four sector, hybrid and TOF/TOF analyzers are tandem instruments. Additionally, both FTMS (ICR) and quadrupole ion trap mass spectrometers can be utilized as tandem instruments.
General Principles

Mass Spectrometers and mass spectra

\[ \text{H}_3\text{C} - \text{C} - \text{O} - \text{CH}_2\text{CH}_3 \]

\[ m/z = 88 \]

\[ \text{H}_3\text{C} - \text{C} = \text{O} : \]

\[ m/z = 43 \]

\[ \text{CH}_3^+ \]

\[ m/z = 15 \]

Topics in Biophysics 2001: Mass Spectrometry

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# Isotopic Abundances and Masses of Elements Encountered in Peptides and Other Biological Molecules

<table>
<thead>
<tr>
<th>element</th>
<th>nominal mass</th>
<th>exact mass</th>
<th>percent abundance</th>
<th>average mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>12</td>
<td>12.00000</td>
<td>98.9%</td>
<td>12.01115</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>13.00335</td>
<td>1.1%</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>1</td>
<td>1.00783</td>
<td>99.98%</td>
<td>1.008665</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.0140</td>
<td>0.02%</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>16</td>
<td>15.99491</td>
<td>99.8%</td>
<td>15.994</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>17.9992</td>
<td>0.20%</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>14</td>
<td>14.00307</td>
<td>99.63%</td>
<td>14.0067</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>15.00011</td>
<td>0.37%</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>32</td>
<td>31.97207</td>
<td>95.0%</td>
<td>32.064</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>32.97146</td>
<td>0.76%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>33.96786</td>
<td>4.22%</td>
<td></td>
</tr>
</tbody>
</table>
## General Principles

### Monoisotopic and average mass

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>Monoisotopic Mass</th>
<th>Average Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate</td>
<td>$C_4H_8O_2$</td>
<td>88.0522</td>
<td>89.0555</td>
</tr>
</tbody>
</table>

**First peak (100%)**
- $4 \text{ C}^{12}$: $4 \times 12.0000 = 48.0000$
- $8 \text{ H}^1$: $8 \times 1.0078 = 8.0624$
- $2 \text{ O}^{16}$: $2 \times 15.9949 = 31.9898$
- **Monoisotopic mass**: 88.0522

**Second peak (4.56%)**
- $3 \text{ C}^{12}$: $3 \times 12.0000 = 36.0000$
- $1 \text{ C}^{13}$: $1 \times 13.0033 = 13.0033$
- $8 \text{ H}^1$: $8 \times 1.0078 = 8.0624$
- $2 \text{ O}^{16}$: $2 \times 15.9949 = 31.9898$
- (1.1% x 4 = 4.4%)
- **Average mass**: 89.0555

**Second peak (0.25%)**
- $4 \text{ C}^{12}$: $4 \times 12.0000 = 48.0000$
- $7 \text{ H}^1$: $7 \times 1.0078 = 7.0546$
- $1 \text{ H}_2$: $1 \times 2.0140 = 2.0140$
- $2 \text{ O}^{16}$: $2 \times 15.9949 = 31.9898$
- (0.25 X 8 = 0.16%)
- **Average mass**: 89.0584
**General Principles**

**Monoisotopic and average mass**

<table>
<thead>
<tr>
<th>glucagon</th>
<th>C\textsubscript{153}H\textsubscript{225}N\textsubscript{42}O\textsubscript{50}S</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>first peak:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>second peak:</td>
<td>(^{12}\text{C} \rightarrow ^{13}\text{C}) 153 x 1.1%</td>
<td>170%</td>
</tr>
<tr>
<td></td>
<td>H \rightarrow \text{D}</td>
<td>225 x 0.02%</td>
</tr>
<tr>
<td></td>
<td>(^{14}\text{N} \rightarrow ^{15}\text{N}) 42 x 0.37%</td>
<td>15.5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>190%</td>
</tr>
<tr>
<td>third peak:</td>
<td>six possibilities (multiplicity)</td>
<td></td>
</tr>
</tbody>
</table>

Monoisotopic mass: 3,482.61

Average mass: 3,484.75
General Principles

Mass resolution and mass accuracy

Resolution = 1/3,000

Resolution = 1/500

Monoisotopic mass: 3,482.61 Average mass: 3,484.75
MALDI/TOF Mass Spectrometry

Matrix-assisted laser desorption/ionization

MALDI mechanism

1. The matrix absorbs UV or IR energy from the pulsed laser

2. The matrix ionizes and dissociates; it undergoes a phase change to a supercompressed gas; charge is passed to some of the analyte molecules

3. Matrix expands at supersonic velocity; additional analyte ions are formed in the gas phase; ions are entrained in the expanding plume
MALDI/TOF Mass Spectrometry

Matrix-assisted laser desorption/ionization

MALDI matrices

Nicotinic acid (NA)

Caffeic acid (CA)
3,4-Dihydroxycinnamic acid

Sinapinic acid (SA)
3,5-Dimethoxy-4-hydroxycinnamic acid

Gentisic acid (DHBA)
2,5-Dihydroxybenzoic acid

3-Hydroxypicolinic acid (HPA)
3-Hydroxy-2-pyridinecarboxylic acid

α-Cyano-4-hydroxycinnamic acid (CHCA)
MALDI/TOF Mass Spectrometry

Time-of-flight mass spectrometry

Short source region (s) with a high field for extracting the ions

Longer field-free drift region (D)

Flight times follow a simple square root dependence on mass

\[ t = \left( \frac{m}{2eV} \right)^{1/2} D \]
Mass Spectrometric Analysis of Peptides and Proteins

Protein molecular weight measurements

Molecular weight strategies

Topics in Biophysics 2001: Mass Spectrometry
Mass Spectrometric Analysis of Proteins
Protein molecular weight measurements

Improved mass resolution using a reflectron

SINGLE-STAGE REFLECTRON TOF MASS SPECTROMETER
FLIGHT TIMES IN THE SINGLE-STAGE RTOF MASS SPECTROMETER

\[ t = \left( \frac{m}{2eV} \right)^{1/2} \left[ L_1 + L_2 + 4d \right] \]

DUAL-STAGE REFLECTRON TOF MASS SPECTROMETER
Mass Spectrometric Analysis of Proteins
Protein molecular weight measurements

Improved mass resolution using delayed extraction

TIME-LAG FOCUSING
DELAYED EXTRACTION WITH MALDI


Mass Spectrometric Analysis of Proteins

Protein molecular weight measurements

Improved mass resolution using delayed extraction


FIGURE 7.25 Delayed-extraction MALDI mass spectra of the molecular ion regions of the peptides: (a) bradykinin (MH+, MW = 1060.6) obtained at an extraction pulse potential of 0.9 kV, (b) LYPVKLPVK (MH+, MW = 1219.7) at 1.0 kV, (c) Ac-KLEALEKLEALEA-NH₂ (MH+, MW = 1568.3) at 1.3 kV, (d) Ac-EAKAAKE-AEKGAKEAEK-NH₂ (MH+, MW = 1958.0) at 1.75 kV, (e) Ac-ELEKLKECEKLEKLEK-EK-NH₂ (MH+, MW = 2256.3) at 2.05 kV, and (f) Ac-KLEALEKLEALEKLEALEKLEALEA-NH₂ (MH+, MW = 3077.7) at 2.5 kV. (Reprinted with permission from reference 41).
**Mass Spectrometric Analysis of Proteins**

Improved mass resolution using *delayed extraction*

- **Glucagon-like peptide**
  - R = 13,370

- **Angiotensin II**
  - R = 1,990
# Mass Spectrometric Analysis of Peptides and Proteins

## Table I. Masses of the 20 Common Amino Acid "Residues"

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Nominal</th>
<th>Monoisotopic</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>alanine</td>
<td>Ala A</td>
<td>71</td>
<td>71.037</td>
</tr>
<tr>
<td>arginine</td>
<td>Arg R</td>
<td>156</td>
<td>156.101</td>
</tr>
<tr>
<td>asparagine</td>
<td>Asn N</td>
<td>114</td>
<td>114.043</td>
</tr>
<tr>
<td>aspartic acid</td>
<td>Asp D</td>
<td>115</td>
<td>115.027</td>
</tr>
<tr>
<td>cysteine</td>
<td>Cys C</td>
<td>103</td>
<td>103.009</td>
</tr>
<tr>
<td>glutamic acid</td>
<td>Glu E</td>
<td>129</td>
<td>129.043</td>
</tr>
<tr>
<td>glutamine</td>
<td>Gln Q</td>
<td>128</td>
<td>128.059</td>
</tr>
<tr>
<td>glycine</td>
<td>Gly G</td>
<td>57</td>
<td>57.021</td>
</tr>
<tr>
<td>histidine</td>
<td>His H</td>
<td>137</td>
<td>137.059</td>
</tr>
<tr>
<td>isoleucine</td>
<td>Ile I</td>
<td>113</td>
<td>113.084</td>
</tr>
<tr>
<td>leucine</td>
<td>Leu L</td>
<td>113</td>
<td>113.084</td>
</tr>
<tr>
<td>lysine</td>
<td>Lys K</td>
<td>128</td>
<td>128.095</td>
</tr>
<tr>
<td>methionine</td>
<td>Met M</td>
<td>131</td>
<td>131.040</td>
</tr>
<tr>
<td>phenylalanine</td>
<td>Phe F</td>
<td>147</td>
<td>147.068</td>
</tr>
<tr>
<td>proline</td>
<td>Pro P</td>
<td>97</td>
<td>97.053</td>
</tr>
<tr>
<td>serine</td>
<td>Ser S</td>
<td>87</td>
<td>87.032</td>
</tr>
<tr>
<td>threonine</td>
<td>Thr T</td>
<td>101</td>
<td>101.048</td>
</tr>
<tr>
<td>tryptophan</td>
<td>Trp W</td>
<td>186</td>
<td>186.079</td>
</tr>
<tr>
<td>tyrosine</td>
<td>Tyr Y</td>
<td>163</td>
<td>163.063</td>
</tr>
<tr>
<td>valine</td>
<td>Val V</td>
<td>99</td>
<td>99.068</td>
</tr>
</tbody>
</table>
Mass Spectrometric Analysis of Peptides and Proteins

Peptide Mapping

<table>
<thead>
<tr>
<th>TABLE II. CHEMICAL REAGENTS AND PROTEOLYTIC ENZYMES</th>
</tr>
</thead>
<tbody>
<tr>
<td>chemical reagents</td>
</tr>
<tr>
<td>cyanogen bromide (CNBr)</td>
</tr>
<tr>
<td>homoserine -30.1 Da</td>
</tr>
<tr>
<td>BNPS-skatole or DMSO+HCl acid hydrolysis</td>
</tr>
<tr>
<td>D/P then random</td>
</tr>
<tr>
<td>endopeptidases</td>
</tr>
<tr>
<td>trypsin</td>
</tr>
<tr>
<td>endoproteinase Lys-C</td>
</tr>
<tr>
<td>endoproteinase Glu-C</td>
</tr>
<tr>
<td>endoproteinase Asp-N</td>
</tr>
<tr>
<td>endoproteinase Arg C</td>
</tr>
<tr>
<td>chymotrypsin</td>
</tr>
<tr>
<td>pepsin</td>
</tr>
<tr>
<td>thermolysin</td>
</tr>
</tbody>
</table>
Mass Spectrometric Analysis of Peptides and Proteins

Peptide Sequencing

TABLE III. CHEMICAL REAGENTS AND PROTEOLYTIC ENZYMES (cont.)

<table>
<thead>
<tr>
<th>exopeptidases</th>
<th>selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>carboxypeptidase A</td>
<td>stops at R/PX, sometimes at G/S/D/E</td>
</tr>
<tr>
<td>carboxypeptidase B</td>
<td>cleaves at R/K</td>
</tr>
<tr>
<td>carboxypeptidase P</td>
<td>cleaves PX/D/E, sometimes stops at S/G</td>
</tr>
<tr>
<td>carboxypeptidase Y</td>
<td>cleaves at PX/E, sometimes stops at K/R/S/G</td>
</tr>
<tr>
<td>aminopeptidase M</td>
<td>non-specific</td>
</tr>
<tr>
<td>leucine aminopeptidase</td>
<td>stops at or near K/R/P</td>
</tr>
</tbody>
</table>

Mass balancing: the sum of the molecular weights of $n$ enzymatic fragments, minus $n-1$ water molecules, add up to the molecular weight of the intact protein.
**Example.** β-amyloid peptide (βA\textsubscript{1-40}):

\[
DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIQLMGGVV
\]

**tryptic digest:**

\[
\begin{array}{lll}
DAEFR & \text{MW} = 636.7 & \beta A_{1-5} & \text{MH}^+ \text{ observed} = 637.8 \\
HDSGYEVHHQK & \text{MW} = 1336.5 & \beta A_{6-16} & \text{MH}^+ \text{ observed} = 1337.1 \\
LVFFAEDVGSNK & \text{MW} = 1325.7 & \beta A_{17-28} & \text{MH}^+ \text{ observed} = 1326.7 \\
GAIIQLMGGVV & \text{MW} = 1085.5 & \beta A_{29-40} & \text{MH}^+ \text{ observed} = 1086.1 \\
\end{array}
\]

**cyanogen bromide:**

\[
\begin{array}{lll}
VGGVV & \text{MW} = 429.6 & \beta A_{36-40} & \text{MH}^+ \text{ observed} = 431.1 \\
VGGVVIA & \text{MW} = 613.8 & \beta A_{36-42} & \text{MH}^+ \text{ observed} = 614.2 \\
GAIIQL & \text{MW} = 673.9 & \beta A_{29-35} & \text{MH}^+ \text{ observed} = 626.0 \\
\text{homoserine} & \text{MW} = 643.8 & \\
\text{homoserine lactone} & \text{MW} = 625.8 & \\
\end{array}
\]

\[
636.7 + 1336.5 + 1325.7 + 1085.5 - 3(18) = 4,329.9
\]

*Note that cyanogen bromide digestion revealed a longer amyloid peptide!*
NOTE: cyanogen bromide (CNBr) cleaves the amide bond on the C-terminal side of a methionine residue:

\[
\text{HN-CH-} = \text{NH-CH-} = \text{CO} \\
\text{CH}_2 \\
\text{CH}_2 \\
\text{S} \\
\text{CH}_2
\]

forming a terminal homoserine

\[
\text{HN-CH-} = \text{NH-CH-} = \text{CO} \\
\text{CH}_2 - \text{CH}_2 - \text{OH}
\]

or homoserine lactone residue

\[
\text{HN-CH-} = \text{CH-} = \text{CO} \\
\text{CH}_2 - \text{CH}_2 - \text{O}
\]
Mass Spectrometric Analysis of Peptides and Proteins

Peptide Mapping

SDS-PAGE depends upon shape and size as well as mass.

MALDI mass spectrometry measures mass with an accuracy of 0.1% to 0.01%.

With this accuracy, it is difficult to identify a protein based only on its measured molecular weight.

However, it provides an important method to limit database identification using tryptic maps.


Mass Spectrometric Analysis of Peptides and Proteins

Identifying proteins from databases

Table 2. Nine Proteins Identified from HEL Cell CBB 2.D Gel

<table>
<thead>
<tr>
<th>gel</th>
<th>enzyme</th>
<th>MW /pI</th>
<th>SwissProt access. No.</th>
<th>protein name</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>trypsin</td>
<td>18012.6/7.68</td>
<td>PO5092</td>
<td>PPIase</td>
</tr>
<tr>
<td>G2</td>
<td>trypsin</td>
<td>26669.6/6.45</td>
<td>PO0938</td>
<td>triosephosphate isomerase</td>
</tr>
<tr>
<td>G3</td>
<td>trypsin</td>
<td>26669.6/6.45</td>
<td>PO0938</td>
<td>TIM</td>
</tr>
<tr>
<td>G8</td>
<td>trypsin</td>
<td>29032.8/4.75</td>
<td>P12324</td>
<td>tropomyosin, cytoskeletal type</td>
</tr>
<tr>
<td>G10</td>
<td>trypsin</td>
<td>32575.2/4.64</td>
<td>PO6748</td>
<td>NPM</td>
</tr>
<tr>
<td>G11</td>
<td>trypsin</td>
<td>41737.0/5.29</td>
<td>PO2570</td>
<td>β-actin</td>
</tr>
<tr>
<td>G12</td>
<td>trypsin</td>
<td>61055.0/5.70</td>
<td>P10809</td>
<td>HSP-60</td>
</tr>
<tr>
<td>G13</td>
<td>trypsin</td>
<td>56782.7/5.99</td>
<td>P30101</td>
<td>ERP60</td>
</tr>
<tr>
<td>G14</td>
<td>trypsin</td>
<td>47169.2/7.01</td>
<td>PO6733</td>
<td>α-enolase</td>
</tr>
</tbody>
</table>

Larger proteins will give more false hits since they have more peptide fragments; restrict search by MW of protein.

# Mass Spectrometric Analysis of Peptides and Proteins

## Peptide Mapping and Sequencing

<table>
<thead>
<tr>
<th>name</th>
<th>site</th>
<th>modification</th>
<th>$\Delta m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-terminal acetylation</td>
<td>terminal NH$_2$-</td>
<td>replaced by CH$_3$CONH-</td>
<td>+42</td>
</tr>
<tr>
<td>N-terminal formylation</td>
<td>terminal NH$_2$-</td>
<td>replaced by HCONH-</td>
<td>+28</td>
</tr>
<tr>
<td>N-terminal myristylation</td>
<td>terminal NH$_2$-</td>
<td>rep’d by CH$_3$(CH$<em>2$)$</em>{12}$CONH-</td>
<td>+210</td>
</tr>
<tr>
<td>N-terminal palmitoylation</td>
<td>terminal NH$_2$-</td>
<td>rep’d by CH$_3$(CH$<em>2$)$</em>{14}$CONH-</td>
<td>+238</td>
</tr>
<tr>
<td>C-terminal amidation</td>
<td>terminal -COOH</td>
<td>replaced by -CONH$_2$</td>
<td>-1</td>
</tr>
<tr>
<td>disulfide bonds</td>
<td>2 Cys -SH</td>
<td>replaced by -S-S-</td>
<td>-2</td>
</tr>
<tr>
<td>glycosylation (N-linked)</td>
<td>$N$-$X$-$S/T$</td>
<td>see TABLE</td>
<td></td>
</tr>
<tr>
<td>glycosylation (O-linked)</td>
<td>$S/T$</td>
<td>see TABLE</td>
<td></td>
</tr>
<tr>
<td>sulfation</td>
<td>-OH of $Y$</td>
<td>replaced by -OSO$_3$H</td>
<td>+80</td>
</tr>
<tr>
<td>phosphorylation</td>
<td>-OH of $Y$/S/T</td>
<td>replaced by -OPO$_3$H$_2$</td>
<td>+80</td>
</tr>
<tr>
<td>N-methylation</td>
<td>-NH$_2$ of $K/R/H/Q$</td>
<td>replaced by -NHCH$_3$</td>
<td>+14</td>
</tr>
<tr>
<td>O-methylesterification</td>
<td>-COOH of $E/D$</td>
<td>replaced by -COOCH$_3$</td>
<td>+14</td>
</tr>
<tr>
<td>carboxylation</td>
<td>-NH$_2$ of $E/D$</td>
<td>replaced by -NHOCH$_3$</td>
<td>+30</td>
</tr>
<tr>
<td>hydroxylation</td>
<td>-NH$_2$ of $P/K/D$</td>
<td>replaced by -NHOH</td>
<td>+16</td>
</tr>
</tbody>
</table>
TABLE V. CONSENSUS SITES FOR PROTEIN PHOSPHORYLATION BY KINASES

<table>
<thead>
<tr>
<th>enzyme</th>
<th>consensus sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>protein kinase C</td>
<td>(R/K_{1-3},X_{2-0})-S/T-(X_{2-0},R/K_{1-3})</td>
</tr>
<tr>
<td>cAMP-dependent PK</td>
<td>R-R-X-S/T</td>
</tr>
<tr>
<td>cGMP-dependent PK</td>
<td>R/K_{2-3}-X-S/T</td>
</tr>
<tr>
<td>casein kinase I</td>
<td>S[P]-X_{1-3}-S/T</td>
</tr>
<tr>
<td>casein kinase II</td>
<td>S/T-(D/E/S[P])<em>{1-3},X</em>{2-0}</td>
</tr>
</tbody>
</table>

X = unspecified amino acid; S[P] = phosphorylated serine

Figure 5. S-linked Phosphopeptide: HPLC and Spectrum - 5 picomoles of the phosphopeptide KRPS(p)QRHGSKY-Am in 3 μl 0.1% TFA was mixed with 1 μl ACHC concentrated supernatant (in 50% EtOH : 50% 0.1% TFA) and air dried at 25°C.
Mass
Spectrometric
Analysis of
Peptides and
Proteins

Ladder
sequencing

Carboxypeptidase ladder of
DAEFR:

<table>
<thead>
<tr>
<th>Mass (Da)</th>
<th>[a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>134.1</td>
<td>Asp + 19</td>
</tr>
<tr>
<td>205.2</td>
<td>Ala</td>
</tr>
<tr>
<td>334.3</td>
<td>Glu</td>
</tr>
<tr>
<td>481.5</td>
<td>Phe</td>
</tr>
<tr>
<td>637.5</td>
<td>Arg</td>
</tr>
</tbody>
</table>

[1 115.1 71.1 129.1 147.2 156.2 17 1]
H - Asp - Ala - Glu - Phe - Arg - OH - H^+ 637.7

[1 115.1 71.1 129.1 147.2 17 1]
H - Asp - Ala - Glu - Phe - OH - H^+ 481.5

[1 115.1 71.1 129.1 17 1]
H - Asp - Ala - OH - H^+ 334.3

[1 115.1 17 1]
H - Asp - OH - H^+ 205.2

[1 115.1 71.1 17 1]
H - Asp - OH - H^+ 134.1
In situ digestion: several aliquots of the intact peptide are placed on different locations on the sample probe or slide. Enzyme (in excess) is added to each spot and quenched after a predetermined time by addition of the matrix solution. 

Timed-course digests provide a means to maximize amino acid sequence information.

Mass Spectrometric Analysis of Peptides and Proteins

Ladder sequencing

Aminopeptidase ladder of DAEFR:

<table>
<thead>
<tr>
<th>Mass (Da)</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>637.7</td>
<td>H-Asp-Ala-Glu-Phe-Arg-OH-H⁺</td>
</tr>
<tr>
<td>522.6</td>
<td>H-Ala-Glu-Phe-Arg-OH-H⁺</td>
</tr>
<tr>
<td>451.5</td>
<td>H-Glu-Phe-Arg-OH-H⁺</td>
</tr>
<tr>
<td>322.4</td>
<td>H-Phe-Arg-OH-H⁺</td>
</tr>
<tr>
<td>175.2</td>
<td>H-Arg-OH-H⁺</td>
</tr>
</tbody>
</table>

[b] Mass spectrum of DAEFR showing the mass shift upon aminopeptidase digestion.
Example of aminopeptidase ladder sequencing:

FIGURE 10.7 MALDI-TOF mass spectrum of the synthetic peptide AWCSDEAVPGSPRCDG digested with aminopeptidase M for 180 s at room temperature.
Ladder sequencing of a phosphopeptide:

For phosphopeptides, amino and carboxypeptidase ladder sequencing is carried out in the same manner, but the sequence includes a phosphoserine which has a residue mass of:

$$87 + 80 = 167$$
MHC Class I peptide from $5 \times 10^9$ *Salmonella*-infected murine L cells.

A. RP HPLC and MALDI mass spectrum of bioactive fraction.

B/C. Carboxy and amino peptidase digests consistent with Salmonella GroEL protein GMQFDRGYL or murine heat shock protein GMKFDRGYI.

C. Tryptic digest suggests bacterial GroEL protein.

Amino acid sequencing by fragmentation.

Fragmentation of protonated peptides

Because the protonated peptide is an even-electron ion, fragment ions will also generally be even-electron ions. Thus, if cleavage of the amide bond retains the two bonding electrons on nitrogen, b ions will be formed:

These ions can lose a neutral CO molecule to form a ions:

Both a and b ions involve retention of the positive charge on the amino-terminus and provide a series of peaks that reveal the amino acid sequence from the carboxy terminus. Alternatively, cleavage of the amide bond accompanied by H-transfer to the nitrogen atom results in formation of y ions:

which can be used to determine the amino acid sequence from the amino-terminus.
**Amino acid sequencing by fragmentation.**

**Fragmentation nomenclature**

\[
\text{H}_2\text{N} - \text{CH} - \text{C} \equiv \text{O} - \text{NH} - \text{CH} - \text{C} \equiv \text{O} - \text{NH} - \text{CH} - \text{C} \equiv \text{O} - \text{OH} \\
\text{a}_1 \quad \text{b}_1 \quad \text{c}_1 \quad \text{a}_2 \quad \text{b}_2 \quad \text{c}_2 \quad \text{a}_3 \quad \text{b}_3 \quad \text{c}
\]

\[y\text{-ion masses can be determined for a known sequence:} \]

\[
\begin{array}{cccccccc}
1 & 115.1 & 71.1 & 129.1 & 147.2 & 156.2 & 17 & 1 \\
\text{H} & \text{Asp} & \text{Ala} & \text{Glu} & \text{Phe} & \text{Arg} - \text{OH} + \text{H}^+ \\
\end{array}
\]

\[b\text{-ion masses can also be determined:} \]

\[
\begin{array}{cccccccc}
1 & 115.1 & 71.1 & 129.1 & 147.2 & 156.2 & 17 & 1 \\
\text{H} & \text{Asp} & \text{Ala} & \text{Glu} & \text{Phe} & \text{Arg} & \text{OH} + \text{H}^+ \\
\end{array}
\]

\[a\text{-ion masses are determined by subtracting 28 from the masses of the } b\text{-ions} \]
Amino acid sequencing by fragmentation.

Fragmentation nomenclature

De novo sequencing of an unknown is complicated by the fact that one does not know a priori which type of fragmentation will occur.

Approaches include the location of peaks 28 mass units apart (a and b series) and noting that the molecular ion is a y ion.)
**Mass Spectrometric Analysis of Peptides and Proteins**

**Amino acid sequencing by fragmentation**

**Post-source decay (PSD) using a reflectron**

As described above, the single-stage reflectron focuses molecular ions at flight times that follow a square root law:

\[
t = \left( \frac{m}{2eV} \right)^{1/2} \left[ L_1 + L_2 + 4d \right]
\]

All of the ions formed in the ion source are in focus at the detector.

The scheme shown might represent two peptides \((m_1, m_2)\) from a tryptic digest.
**Mass Spectrometric Analysis of Peptides and Proteins**

*Amino acid sequencing by fragmentation*

**Post-source decay (PSD) using a reflectron**

Fragmentation can be observed when it occurs (1) promptly, (2) in the flight tube of a reflectron TOF, or (3) as a result of collisions in a tandem mass spectrometer. When it occurs in the flight tube, it is referred to as *post-source decay*.

Fragment ions formed by *post-source decay* are dispersed but not all focused on a linear scale:

\[
    t_b = \left( \frac{m_a}{2eV} \right)^{1/2} \left[ L_1 + L_2 + 4 \frac{m_b}{m_a} d \right]
\]
When single-stage or dual-stage reflectrons are used to record post-source decay mass spectra, it is necessary to “step” the reflectron voltage in order to focus different mass regions.

The mass spectral segments are then “stitched” to form a finished mass spectrum.

CID/PSD mass spectrum of a wasp neurotoxin obtained by introducing argon gas. Mass spectra were acquired on a PE BioSystems Voyager Elite.

Mass Spectrometric Analysis of Peptides and Proteins
Amino acid sequencing by fragmentation

Post-source decay (PSD) using a curved-field reflectron

Mass Spectrometric Analysis of Peptides and Proteins

Amino acid sequencing by fragmentation

Post-source decay (PSD) using a curved-field reflectron
High resolution post-source decay mass spectrum of angiotensin II obtained on a Kratos AXIMA-CFR with a curved-field reflectron.
Nested PSD

PSD of intact peptide and one carboxypeptidase fragment

Peaks in both spectra with a common mass are a, b or c ions; peaks with shifted masses are x, y or z ions


Mass Spectrometric Analysis of Peptides and Proteins

Epitope mapping by mass spectrometry.


MALDI mass spectra of (a) peptide fragments produced by endoproteinase Lys-C digestion of melittin, (b) Endo Lys-C fragments isolated by immunoprecipitation with mab 83144, (c) chymotrypsin fragments of melittin, and (d) chymotrypsin fragments of melittin immunoprecipitated with antimelittin antibody mab 83144. Peaks labeled with an asterisk are dynorphan A1-13 added as internal mass calibrant, and peaks labeled 11, 12 and 1 are impurities.
Mass Spectrometric Analysis of Oligosaccharides and Glycoconjugates

N-linked and O-linked oligosaccharides, structures, glycosylation sites

N-linked: generally attached to asparagine at Asn-X-Ser/Thr

O-linked: single GlcNAc attached to a serine or threonine residue

Structural analysis of carbohydrates is very different from peptides:

- there are a limited number of residue masses for a number of isomers
- there is considerable branching (biantennary, triantennary, fucosylated sugars, etc.)
- glycopeptides are generally heterogeneous.
- glycosidases are specific for linkage
Mass Spectrometric Analysis of Oligosaccharides and Glycoconjugates

Mapping carbohydrate heterogeneity


![Image of mass spectrum]

**FIGURE 10.15** Positive ion plasma desorption TOF mass spectrum of the intact, nonradiolabeled gp432 glycopeptide from the 59-kDa VSG obtained from *T. brucei*. Reprinted with permission from reference 28.
## Mass Spectrometric Analysis of Oligosaccharides and Glycoconjugates

<table>
<thead>
<tr>
<th>monosaccharide</th>
<th>abbreviations</th>
<th>monoisotopic mass</th>
<th>average mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-fructose</td>
<td>Fru</td>
<td>162.052</td>
<td>162.143</td>
</tr>
<tr>
<td>L-fucose</td>
<td>Fuc</td>
<td>F</td>
<td>146.058</td>
</tr>
<tr>
<td>D-galactose</td>
<td>Gal</td>
<td>GL</td>
<td>162.052</td>
</tr>
<tr>
<td>N-acetyl-D-galactosamine</td>
<td>GalNAc</td>
<td>203.079</td>
<td>203.19</td>
</tr>
<tr>
<td>D-glucose</td>
<td>Glc</td>
<td>G</td>
<td>162.052</td>
</tr>
<tr>
<td>D-glucuronic acid</td>
<td>GlcA</td>
<td>176.032</td>
<td>176.126</td>
</tr>
<tr>
<td>N-acetyl-D-glucosamine</td>
<td>GlcNAc</td>
<td>203.079</td>
<td>203.19</td>
</tr>
<tr>
<td>hexose (non-specific)</td>
<td>Hex</td>
<td>162.052</td>
<td>162.143</td>
</tr>
<tr>
<td>D-mannose</td>
<td>Man</td>
<td>M</td>
<td>162.052</td>
</tr>
<tr>
<td>N-acetyl-D-mannosamine</td>
<td>ManNac</td>
<td>203.079</td>
<td>203.19</td>
</tr>
<tr>
<td>N-acetyleneuraminic acid</td>
<td>NANA NeuAc</td>
<td>291.095</td>
<td>291.26</td>
</tr>
<tr>
<td>Sialic acid</td>
<td>SA</td>
<td>291.095</td>
<td>291.26</td>
</tr>
</tbody>
</table>

### Topics in Biophysics 2001: Mass Spectrometry
Mass Spectrometric Analysis of Oligosaccharides and Glycoconjugates

Enzymatic methods for structural analysis

Endo- and exo-glycosidases used with mass spectrometry

<table>
<thead>
<tr>
<th>TABLE VII: GLYCOSIDASES AND THEIR CLEAVAGE SITES</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>endoglycosidases</strong></td>
</tr>
<tr>
<td>endo-β-N-acetylglucosaminidase F (Endo F)</td>
</tr>
<tr>
<td>endo-β-N-acetylglucosaminidase H (Endo H)</td>
</tr>
<tr>
<td>peptide-N-glycosidase F (PNGase F)</td>
</tr>
<tr>
<td><strong>exoglycosidases</strong></td>
</tr>
<tr>
<td>β-galactosidase (jack bean)</td>
</tr>
<tr>
<td>β-galactosidase (S. pneumoniae)</td>
</tr>
<tr>
<td>β-galactosidase (bovine testes)</td>
</tr>
<tr>
<td>b-N-acetylglucosaminidase (S. pneumoniae)</td>
</tr>
<tr>
<td>α-mannosidase (jack bean)</td>
</tr>
</tbody>
</table>
Figure 10.16 (a) is the MALDI mass spectrum of the intact glycopeptide, revealing five peaks at \( m/z \) 4820 (I), 5023 (II), 5185 (III), 5224 (IV) and (5388) (V). The glycopeptides were resistant to cleavage of the oligosaccharide from the peptide using PNGase F, suggesting the presence of a fucosyl residue on the GlcNAc proximal to asparagine. Proton-catalyzed defucosylation using 70% trifluoroacetic acid, resulted in shifting all five peaks by 146 Da, indicating that all species contained a fucosyl residue. Subsequent reaction with PNGase F resulted in removal of the oligosaccharides, giving the single peak at \( m/z \) 3651 shown in (b). This was consistent with the molecular weight calculated from the amino acid sequence (3649 Da), conversion of asparagine to aspartic acid, and protonation to form the MH\(^+\) ion.

Digestion of the intact glycopeptide mixture with \( \beta\)-galactosidase from jack bean (c) and bovine testes reduced the glycopeptides to I, II and IV (while that from \( S. pneumoniae \) did not), suggesting a Gal(\( \beta \)-1→3)HexNAc in III and V. Subsequent digestion of this mixture with \( \beta\)-N-acetylglucosaminidase removed a single GlcNAc residue from structure II and two GlcNAc residues from structure IV, reducing all of the glycopeptides to structure I (d). Digestion of this species with \( \alpha\)-mannosidase at low enzyme concentration removed a single mannose residue, corresponding to cleavage of Man(\( \alpha\)-1→3)Man(\( \beta\)-1→4), resulting in the peak at \( m/z \) 4659 in (e). Subsequent digestion with this same enzyme at high concentration resulted in loss of a second mannose, corresponding to cleavage of Man(\( \alpha\)-1→6)Man(\( \beta\)-1→4). The peak at \( m/z \) 4497 in (f) corresponds to a core glycan (consisting of 2 GlcNAc, 1 Man, 1 Fuc and 1 Xyl residues).
Determination of oligosaccharide structures


Topics in Biophysics 2001: Mass Spectrometry
Mass Spectrometric Analysis of Oligosaccharides and Glycoconjugates

Chemical degradation

Table 1. Components Generated from H⁺ Resin Hydrolysis of Oligomannose 6

<table>
<thead>
<tr>
<th>m/z</th>
<th>Na⁺ adduct composn</th>
<th>m/z</th>
<th>Na⁺ adduct composn</th>
</tr>
</thead>
<tbody>
<tr>
<td>1419.465</td>
<td>Man₅GlcNAc₂</td>
<td>851.260</td>
<td>Man₅</td>
</tr>
<tr>
<td>1257.412</td>
<td>Man₆GlcNAc₂</td>
<td>771.269</td>
<td>Man₆GlcNAc₂</td>
</tr>
<tr>
<td>1216.388</td>
<td>Man₇GlcNAc</td>
<td>730.233</td>
<td>Man₇GlcNAc</td>
</tr>
<tr>
<td>1095.361</td>
<td>Man₈GlcNAc₂</td>
<td>689.207</td>
<td>Man₈</td>
</tr>
<tr>
<td>1054.335</td>
<td>Man₉GlcNAc</td>
<td>568.182</td>
<td>Man₉GlcNAc</td>
</tr>
<tr>
<td>1013.321</td>
<td>Man₁₀</td>
<td>527.156</td>
<td>Man₁₀</td>
</tr>
<tr>
<td>933.309</td>
<td>Man₆GlcNAc₂</td>
<td>406.130</td>
<td>Man₆GlcNAc</td>
</tr>
<tr>
<td>892.285</td>
<td>Man₇GlcNAc</td>
<td>365.104</td>
<td>Man₇</td>
</tr>
</tbody>
</table>


Figure 2. ESI-FT-ICR mass spectrum of the oligomannose 6 hydrolysate products after a 24-h resin hydrolysis. Spectrum displays the ladder sequence of the N-linked oligosaccharide. The dashed lines in the oligosaccharide structure represent the glycosidic linkages that have been cleaved by acid hydrolysis.
Mass Spectrometric Analysis of Oligosaccharides and Glycoconjugates

Fragmentation methods: Nomenclature


FIGURE 10.20 (a) Delayed extraction IRLD mass spectrum of cellobiose. (b) Structure of cellobiose Glc(β1→4)Glc(β1→4)Glc (MW = 504 Da), illustrating the fragment ion nomenclature according to reference 39. Reprinted with permission from reference 38.
Mass Spectrometric Analysis of Oligonucleotides and DNA

Molecular weight measurements

IR-MALDI TOF mass spectra of (a) synthetic 21-nt DNA (MW = 6398), (b) plasmid DNA restriction enzyme digest (pBluescript-KS⁺; Bgl I and Rsa I; 87, 112, 285 and 433 kDa), (c) plasmid DNA restriction enzyme digest (pBluescript-KS⁻; Not I and Ssp I; 70, 170 and 673 kDa), and (d) 1206 nt RNA transcript (387 kDa). Wavelength 2.94 µm (Er:YAG laser) with glycerol matrix.

Mass Spectrometric Analysis of Oligonucleotides and DNA
Ladder methods

Demonstration of mock sequencing: (a) MALDI mass spectra of synthetic nucleotides corresponding to the first 24 DNA fragments generated in typical sequencing reactions using a standard M13mp19 template and the primer d(GTAAAACGACGGCCAGT) and (b) overlay of the spectra shown in (a). 0.5 pmol of each component was used and the matrix was 3-HPA.

Mass Spectrometric Analysis of Oligonucleotides and DNA
Hybridization methods

Electrospray ionization (ESI) mass spectrometry
Instrumentation and basic principles

Introduced by John Fenn:
Fenn, J.B.; Mann, M.; Meng, C.K.; Wong, S.F.; Whitehouse, C.M., Science 246 (1989) 64.

Topics in Biophysics 2001: Mass Spectrometry
Electrospray ionization (ESI) mass spectrometry

Multiply-charged ions

Multiply-charged ions are favored in ESI mass spectra, with ions having the formula:

\[(M + nH)^+\]

Average \(m/z\) are around 1000, which is compatible with quadrupole mass spectrometers.

---

**Fig. 3.** ESI mass spectrum of equine myoglobin. Peaks \(m/z\) 707.25 through \(m/z\) 1305 are centroid values for these multiply protonated (24+ through 13+) ions. The simultaneous relation of one pair, \(m/z\) 998.25 and \(m/z\) 1060.7, is illustrated where the integer value of \(n\) is 17. Calculation of \(M_r\) as discussed in the text.
Electrospray ionization (ESI) mass spectrometry
Mass analyzers used with electrospray

Triple Quadrupoles

DC and RF voltages set to pass a single mass
RF-only mode to pass all masses
Low energy, multiple collisions
DC and RF voltages scanned to record all masses in succession
Electrospray ionization (ESI) mass spectrometry
Mass analyzers used with electrospray

Ion trap mass spectrometers (ITMS)

- **Trapping cycle:** fixed amplitude of the fundamental rf voltage
- **Ion ejection cycle:** remove unwanted ions by symmetric or asymmetric pulses on endcaps; *stored waveform inverse Fourier transform* (SWIFT) or *filtered noise field* techniques
- **Mass analysis cycle:** mass selective instability mode: scan fundamental rf voltage; mass range approx. 650 resonance ejection mode: set supplemental rf voltage on endcaps and then scan fundamental rf voltage
- **MS/MS mode:** low amplitude supplemental rf voltage applied to endcaps

Fundamental rf applied to ring electrode (1.1 MHz)
dc and excitation voltages applied to the end caps
**Electrospray ionization (ESI) mass spectrometry**

Mass analyzers used with electrospray

ESI/ion traps: the LCQ

- RF-only quadrupole or octapole filters that collimate ions from high pressure ESI source.

Ion trap mass spectrometer can record MS, MS/MS and MS\(^n\) spectra

MALDI and AP/MALDI sources can be used in place of ESI
LCQ mass spectrometer with AP/MALDI source

Full Scan of Acetylated* Peptide Tryptic Digest Mixture

MS/MS of Tryptic Fragment 9-20*
Electrospray ionization (ESI) mass spectrometry
Mass analyzers used with electrospray

Fourier transform mass spectrometers

Figure 2. ESI-FT-ICR mass spectrum of the oligomannose 6 hydrolysate products after a 24-h resin hydrolysis. Spectrum displays the ladder sequence of the N-linked oligosaccharide. The dashed lines in the oligosaccharide structure represent the glycosidic linkages that have been cleaved by acid hydrolysis.

**Electrospray ionization (ESI) mass spectrometry**

Mass analyzers used with electrospray

**Time-of-flight (TOF) mass spectrometers with orthogonal acceleration**

Orthogonal acceleration from the ion storage region

Quadrupole ion guide operated in rf-only mode (passes all masses)

- quadrupole ion focusing
- orthogonal injection
- reflectron
Time-of-flight (TOF) mass spectrometers with orthogonal acceleration

Figure 2. ESI-TOF mass spectra of peptide LIXPIXDXIXNE (X = Q or K) showing the triply protonated molecule peak. The spectrum is internally calibrated with the triply charged molecule peaks of angiotensin I and neurotensin. The deconvoluted peaks show the mass for the uncharged molecule.

Table 2. Determination of the number of K and Q in peptide LIXPIXDXIXNE (X = Q or K) by comparison between the experimentally determined mass of 1437.8189 Da and the theoretically possible masses

<table>
<thead>
<tr>
<th>Number of</th>
<th></th>
<th>( M_{\text{exp}} ) (Da)</th>
<th>( \Delta M ) (mDa)</th>
<th>( M_{\text{exp}} - M_{\text{exp}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q 4</td>
<td>0</td>
<td>1437.7961</td>
<td>+22.8</td>
<td></td>
</tr>
<tr>
<td>Q 3</td>
<td>1</td>
<td>1437.7827</td>
<td>+36.2</td>
<td></td>
</tr>
<tr>
<td>Q 2</td>
<td>2</td>
<td>1437.8191</td>
<td>-0.2</td>
<td></td>
</tr>
<tr>
<td>Q 1</td>
<td>3</td>
<td>1437.8555</td>
<td>-36.6</td>
<td></td>
</tr>
<tr>
<td>Q 0</td>
<td>4</td>
<td>1437.8919</td>
<td>-73</td>
<td></td>
</tr>
</tbody>
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Combined quadrupole and time-of-flight mass spectrometers (QTOF)

$q_1$ is an RF-only quadrupole filter that collimates ions from high pressure source.

$Q_2$ is a quadrupole mass filter that selects the precursor mass.

$q_3$ is an RF-only quadrupole filter used as a collision chamber.

Collisions are low energy.

The TOF makes it possible to analyze product ions with higher $m/z$ than their precursors.
CID mass spectra of peptides: NFNRHLHFTLVKDR and LLSYDVDEAFIRDVAK


**Homework problem:** calculate the masses of b and y ions for the two peptides shown and compare with results obtained in their mass spectra.
Electrospray ionization mass spectrum of high mannose sugars derivatized with seven different amines.

Electrospray ionization CID mass spectrum of the $[\text{M+Na}]^+$ ion from the 3-AQ derivative of $(\text{GlcNAc})_2\text{Man}_3$.

Analysis of proteins and glycoproteins


Topics in Biophysics 2001: Mass Spectrometry
Mass Spectrometry at the JHU/SOM

Middle Atlantic Mass Spectrometry Laboratory (Biophysics B3)

- Kratos MALDI 4 time-of-flight mass spectrometer with time delayed extraction and curved-field reflectron
- Finnigan LCQ (electrospray ionization/quadrupole ion trap)
- Finnigan LCQ Duo (with atmospheric pressure MALDI)
- Kratos AXIMA-CFR high resolution MALDI time-of-flight mass spectrometer with curved-field reflectron

Department of Pharmacology (Biophysics B11)

- Sciex API150EX electrospray ionization/quadrupole mass spectrometer

Applied BioSystems Mass Spectrometry Facility at the Johns Hopkins Medical School (Physiology, Fifth Floor)

- Applied BioSystems (PE Sciex) QSTAR quad/TOF mass spectrometer