Lecture 1
Introduction- Protein Sequencing

Production of Ions for Mass Spectrometry

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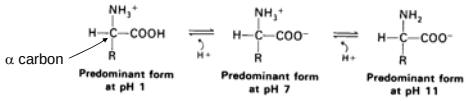
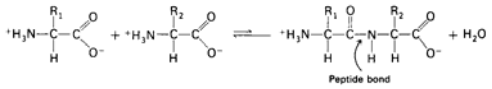
Introduction to Proteins

Amino Acid- structural unit of a protein



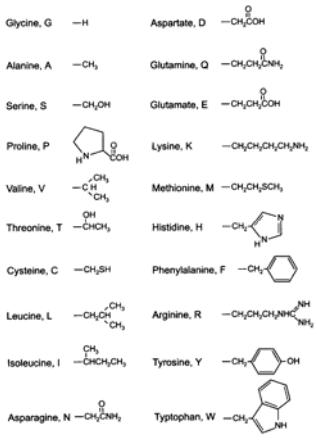
Amino acids- linked by peptide (amide) bond



Amino Acids

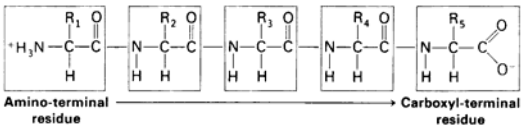
Proteins- 20 amino acids
(Recall DNA- 4 bases)

R groups-
Varying size, shape, charge,
H-bonding capacity,
& chemical reactivity



Introduction to Proteins

Polypeptide Chain (Protein) - Many amino acids linked by peptide bonds



By convention: Residue 1 starts at amino terminus.

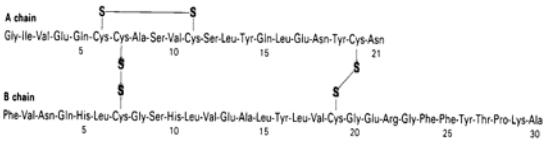
Polypeptides- a. Main chain i.e. regularly repeating portion
b. Side chains- variable portion

Introduction to Proteins

25,000 human genes ➡ ≥2X10^6 proteins

Natural Proteins - Typically 50-2000 amino acids
i.e. 550-220,000 molecular weight

Over 200 different types of post-translational modifications.
Ex: proteolysis, phosphorylation, acetylation, glycosylation



Ex: Insulin

Protein Complexity Is Very Large

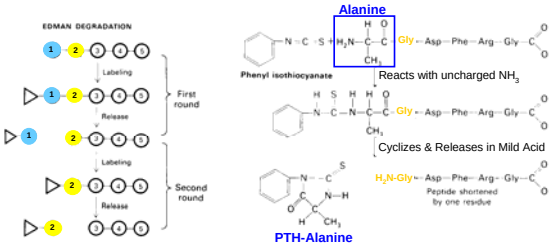
Over 200 different types of post-translational modifications.

Table 7.1 Post-translational modifications and corresponding mass variations

Post-translational modification	Mass difference (Da)
Methylation	14.03
Propylation	42.08
Sulfation	80.06
Phosphorylation	79.98
Glycosylations by:	
Deoxyhexoses (Fuc)	146.14
Hexosamines (GlcN, GalN)	161.16
Hexoses (Glc, Gal, Man)	162.14
N-Acetylhexosamines (GlcNAc, GalNAc)	203.19
Pentoses (Xyl, Ara)	132.12
Sialic acid (NeuNAc)	291.26
Reduction of a disulfide bridge	2.02
Carbamidomethylation	57.03
Carboxymethylation	58.04
Cysteinylation	119.14
Ethylpyridylation	105.12
Acetylation	42.04
Formylation	28.01
Biotinylation	226.29
Farnesylation	204.36
Myristoylation	210.36
Pyridoxal phosphate Schiff condensation	231.14
Stearoylation	266.47
Palmitoylation	238.41
Lipoylation	188.30
Carboxylation of Asp or Glu	44.01
Deamidation of Asn or Gln	0.98
Hydroxylation	16.00
Methionine oxidation	16.00
Proteolysis of a peptide bond	18.02
Deamination from Gln to pyroglutamic	−17.03

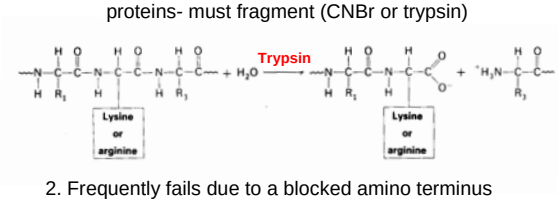
The Problem of Protein Sequencing.

Edman Degradation: Step-wise cleavage of an amino acid from the amino terminus of a peptide.



Edman Degradation

1. Must be short peptide (≤50 a.a.)
   amino acid release- 98% efficiency
   proteins- must fragment (CNBr or trypsin)



2. Frequently fails due to a blocked amino terminus
3. Intolerant of impurities
4. Tedious & time consuming (hours-days)
   1 amino acid cycle- 2 hours

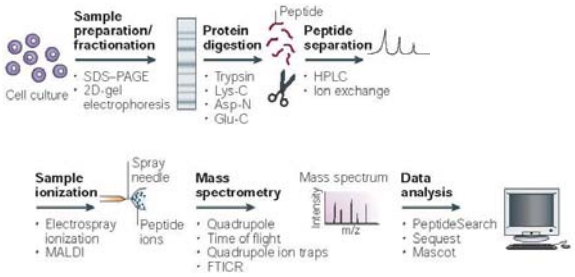
Solution: Mass Spectrometry

1. Impurities are tolerated.
2. Fast (sec - min).
3. Amino terminus can be blocked.
4. Always get some information.

Steps in Mass Spectrometry

1. Production of Ions
2. Ion Separation
3. Ion Detection
4. Data Acquisition & Reduction

Protein Sequencing By MS



Steen & Mann Nat, Rev, Molec. Cell Bio. 2004, 5:699-711.

Units and Numbers

m/z = mass/(# of e charges on the ion)
m = Daltons (Da) or atomic mass units (U)
m/z units = Thompson (Th)

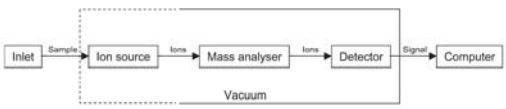
Ex: methanol (CH_3Cl)
$m_H = 1$; $m_{Cl} = 35$ (76%); $m_{Cl} = 37$ (24%)
$m_C = 12$ (99%) & $m_C = 13$ (1%)

Atomic Weight = average mass using isotope prevalence
50.5 Da

MS gives peaks for monoisotopic masses i.e.
Two major peaks- 50 & 52 Da
Very minor peaks- 51 & 53 Da

Elements of a Mass Spectrometer

1. Device to insert sample into a mass spec.
   sample probe, chromatograph, capillary
2. Source to produce ions from the sample.
3. Analyzer ($\geq$1) to separate ions by m/z.
4. Detector to count ions.
5. Computer to control instrument and collect & analyze data.



Mean Free Path of an Ion

L = mean free path traveled by a molecule before colliding with another

$L = kT/[p\sigma(2)^{1/2}]$; k = Boltzman Constant
T = temperature
p = pressure
σ = collision X-section = πd^2
d = sum of radii of colliding ions

L (cm) = 4.95/p for p in milliTorr

For MS analyzers, $L \geq 1$ meter then $p \leq 10^{-5}$ Torr
In practice- $p \leq 10^{-5}$ Torr
Need very efficient vacuum pumps for MS!

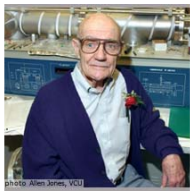
Ionization Methods

Characteristics:

1. Energy Imparted:
Soft Ionization (less fragmentation)-
MALDI- matrix-assisted laser desorption/ionization
ESI- electrospray ionization
Hard- EI (electron Impact Ionization),
FAB (fast atom bombardment),
SIMS (secondary ion mass spectrometry)

2. Sample State:
Gas- EI, CI
Liquid- nebulization to introduce droplets, **ESI**, thermospray
Solid- uses an absorbing matrix &
irradiate with particles or photons
MALDI, FAB, field & plasma desorption

Ionization Methods: ESI & Laser Desorption 2002 Nobel Prize in Chemistry



John B. Fenn is the chemist who invented the electrospray method. Today it is used in laboratories all over the world.

Koichi Tanaka's idea was to use the energy from laser light, ingeniously transferred to the proteins, to get them to let go of one another and hover freely.

http://nobelprize.org/chemistry/laureates/2002/illpres/mass.html

Electrospray Ionization

Recall: Mean Free Path = L (cm) = 4.95/p for p in milliTorr

Problem #1:
Most ionizers Need at P = ~0.5 Torr so that L = ~0.1 mm
Recall the analyzer is at $\leq 10^{-5}$ Torr

Solution #1:
Increase collisions ⟶ Better ionization
Higher pressures ⟶ Increase collisions

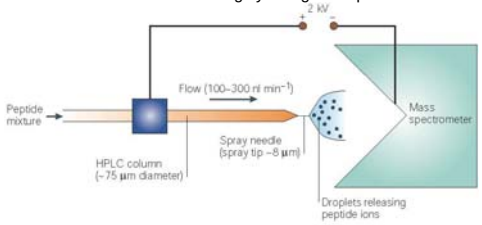
ESI- Ionization at atmospheric pressure (760 Torr)
(10^3-10^4 more efficient ionization)

Enabled by: 1. focusing lenses with small openings
2. focusing multipole lenses
3. very high capacity pumps

Electrospray Ionization

1. Capillary with a small orifice or tip.
2. Slowly, flowing liquid at atmospheric pressure.
3. High E field (~10^6 V/m) across the tip.

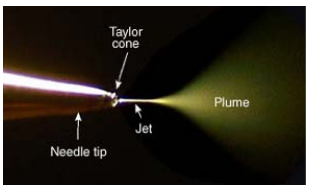
Charge accumulation at liquid surface at capillary tip.
Leads to formation of highly charged droplet.



Steen & Mann Nat. Rev. Molec. Cell Bio. 2004, 5:699-711.

Taylor Cone & Droplet Formation

Onset Voltage- Minimum voltage at which droplets form.



http://www.newobjective.com/electrospray/

Supplemental Info for Marginean et al, Anal. Chem. 2004, v76, p. 4202-7

Taylor Cone & Droplet Formation

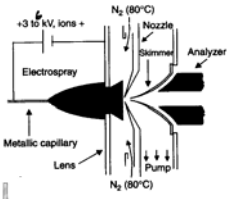
900 V - no spray
1000 V - Taylor-cone/droplet oscillation, more "drops" than spray
1100 V - cone/droplet oscillation. approx 50% spray
1200 V - cone/droplet oscillation, on the verge of a stable Taylor cone
1300 V - stable cone-jet
1400 V - cone-jet on the verge of "jumping", slight instability
1550 V - multiple cone-jets

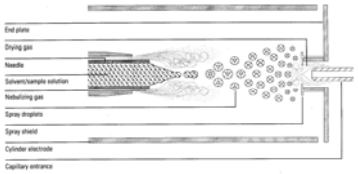
http://www.newobjective.com/electrospray/

Electrospray Ionization

Problem #2:
Sample cooling due to adiabatic expansion can yield ion clusters of sample.

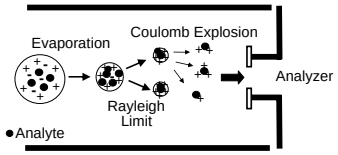
Solution #2: Evaporate residual solvent
Employ: 1. Hi temp. transfer tube or,
2. Heated N_2 counter flow or,
3. Heated gas curtain





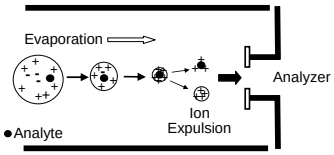
http://www.colorado.edu/chemistry/chem5181/MS3_Ionization_II.pdf

Mechanism of Ionization



Charged Residue Model
1. Solvent evaporates shrinking droplets
2. Coulombic forces exceed cohesive forces
3. Droplet break up
   Rayleigh Eqn: $q^2 = 8\pi^2\varepsilon_0\gamma d^3$
   q = charge; ε_0 = environment permittivity
   γ = surface tension; d = droplet diameter
4. Repeats eventually leading to single ions

Mechanism of Ionization



Ion Evaporation Model

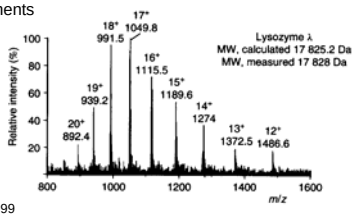
1. Solvent evaporates shrinking droplets
2. Coulombic forces exceed surface tension
3. Sufficiently large E field at droplet surface results in release of single ions.

Analyte Charge

1. Ion formation due to:
   a. charge on droplets
   b. electrochemical processes
   c. adduct formation (Na^+, NH_4^+)
   d. gas-phase reactions
2. Produce multiply charged ions from large molecules
   Proteins- ~1 charge/1 kD on average
3. Multiple mass measurements due to multiple m/z

protonation- $(M + zH)^{z+}$
deprotonation- $(M - zH)^{z-}$

4. Very little fragmentation



DeHoffman & Stroobant,
Mass Spec. Wiley, 2nd Ed. 1999

Charge Distribution

For a peak located at m_1 i.e. peak 1:

$z_1m_1 = M + z_1m_p$ for M = mass of a molecular ion
m_1 = measured mass-to-charge
z_1 = # of added protons on m_1 peak
m_p = mass of proton

Consider a peak separated from peak 1 by j-1 peaks:

$(z_1\text{-}j)\ m_2 = M + (z_1\text{-}j)\ m_p$ for m_2 = measured mass-to-charge
$(z_1\text{-}j)$ = # of protons on the peak at m_2

Then for peak 1:

$z_1 = j\ (m_2 - m_p) / (m_2 - m_1)$ & $M = z_1\ (m1 - m_p)$

Multiply charged species permit calculation of z & M in ESI!

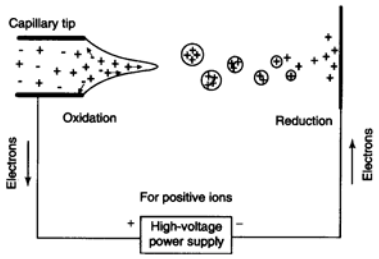
Electrochemistry in ESI

Recall: Ion charge is due to electrochemical processes at probe the tip

Total # of ions/time into the spectrometer is limited by the current at the probe tip.

Positive ion ESI - oxidation at tip

Negative ion ESI- reduction at tip



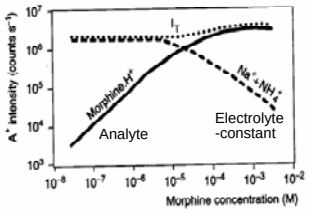
Electrochemistry in ESI

I_M = Limiting Ion Current (total current is limited by oxidation) (typically ~ 1 μA)

Consider 2 analytes, A and B:
$I_A = k_A [A]$ = current due to A & k_A = rate constant for A
$I_B = k_B [B]$ = current due to B & k_B = rate constant for B
$I_T = I_A + I_B$ = total current

At $I_T = I_M$
$I_A = I_M k_A [A] / (k_A [A] + k_B [B])$
$I_B = I_M k_B [B] / (k_A [A] + k_B [B])$

Consider $k_B [B] >> k_A [A]$
Then-
$I_A \sim I_M k_A [A] / k_B [B]$
$I_B \sim I_M$



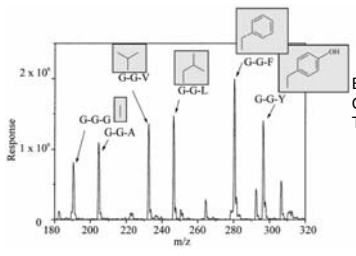
Kebarle & Tang Anal Chem., 1993, 65, 972A

Surface Activity in ESI

Equilibrium Partitioning Model: Molecules that prefer the surface of the droplets have a better ESI response.

Partitioning Coefficient (k) = $[Analyte]_{Surface}/[Analyte]_{Interior}$

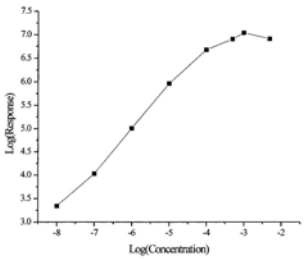
k depends on polarity, charge density, basicity.



Equimolar Conc. of Tripeptides

Cech & Enke, Anal. Chem. 2000, 72:2717.

Analyte Concentration for ESI



Cech & Enke,
Mass Spec. Rev.
2001, 20:362-87.

Typically: 10^{-8} M < [Analyte] < 10^{-5} M

Microspray and Nanospray

Table 3.2. Experimental conditions that distinguish electrospray, microspray, and nanospray ionization

	Electrospray[1]	Microspray[1]	Nanospray[2]
Typical flow rate	2 μL/min	0.2 μL/min	0.02 μL/min
Needle size (i.d.)	75 μL/min	75 μm	5 μm
Approximate limits of detection	10 femtomole	1 femtomole	50 attomole
Sheath liquid flow required	Yes	No	No

ESI

Points:

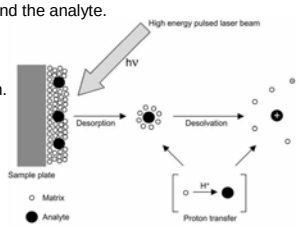
1. Simple
2. Good for large, nonvolatile biomolecules
   (up to 50 kD)
   a. 10 nM < Analyte < 10 μM
   b. Liquid solutions
   c. Must desalt sample
3. Easily coupled to HPLC, μ-HPLC, and CE
   a. Flow rates of pl/min-nl/min
   b. Also coupled to microfluidic chips
4. Little fragmentation
   a. Easy to determine M.W.

MALDI- Karas & Hillenkamp

1. Sample + Matrix
   Matrix- small organic molecule absorbing at laser λ.

2. Dried- Matrix crystals surround the analyte.

3. Ablate with pulsed laser
   a. Rapid matrix heating with localized sublimation.
   b. Gas phase matrix brings analyte along.
   c. Very little energy transferred to analyte.
   d. Ionization occurs by gas-phase proton transfer by photoionized matrix.

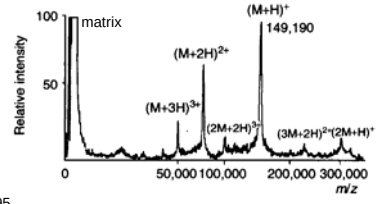


Lane,
Cell Molec. Life Sci.
2005, 62:848-869.

Role of the Matrix

1. Minimizes sample clustering
   matrix molecules >> analyte molecules
2. Absorbs laser energy minimizing sample damage
   Mainly the monocharged species i.e. $(M+H)^+$, $(M+Na)^+$, etc
3. Increases efficiency of energy transfer
   Enhances sensitivity- picomoles of peptides & proteins up to 300 kD
4. Improves analyte independence

MALDI MS of antibody



Hillenkamp & Karas Meth.
Enzym. 1990, 193: 280-295.

Fragmentation in MALDI

Threshold Irradiance- Minimum laser power resulting in matrix desorption.
At > threshold- increased fragmentation, decreased resolution

Lasers- UV (N_2, 337 nm; Nd:YAG, 260 or 355 nm)
IR (Er:YAG, 2.94 μm; CO_2, 10.6 μm)

Fragmentation:

1. Prompt- On sample surface during desorption
2. Fast- At source but after desorption & before acceleration
3. Post Source Decay- Occurs after onset of acceleration

Seen in MALDI Spectra

Peak broadening with decreased resolution & sensitivity.

MALDI Tolerates Impurities

Table 3.3. Tolerance limits of matrix-assisted laser desorption/ionization for various reagents used in protein and peptide isolation protocols.

Reagent	Approximate maximum tolerable concentration
Urea	0.5 M
Guanidine	0.5 M
Sodium dodecyl sulfate (SDS)	0.01 %
Detergents other than SDS	0.1 %
Dithiothreitol	0.5 M
Glycerol	1 %
Alkali metal cations such as Na^{2+}	0.5 M
Tris	50 mM
Phosphate	50 mM

MALDI Summary

Points:

1. Best mass accuracy- 0.01% (1 Da in 10 kDa)
2. Very fast and inexpensive (MALDI-TOF)
3. Can do large molecules ~300,000 kDa
4. Little fragmentation
   Good for determining molecular weights
5. Very sensitive
6. Nontrivial to couple to separation techniques
7. Frequently used with TOF analyzer
   for "Peptide Mass Fingerprinting"

References

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