Ion Activation in MS/MS

Briefly introduce different activation methods
Provide framework for understanding differences

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If different activation parameters
  e.g., different energy distribution,
  different time frame
predict expected change in spectra
SORI-CID
ADSGEGDFLAEGGGVR + H⁺

msec to sec, collision gas in FTICR

SID (Surface-Induced Dissociation)
µsec Time Frame
Ion Activation in MS/MS
Traditionally Collision with Gas
but Other Types of Activation Exist

Q-SID-TOF
ADSGEGDFLAEGGGVR + H+

y₁

b₂

b₃

b₆

y₅

y₉

y₁₁

y₁₄

200 400 600 800 1000 1200 1400 1600

80 eV SID

[M+H]⁺

CID
Ion Activation in MS/MS

WHY DO WE NEED ADDITIONAL ACTIVATION METHODS?

More complete fragmentation
large biomolecules
molecules that fragment too little or too much

Further develop understanding of activation and
unimolecular dissociation

Improve ease of use small instruments, remote applications

Activation Methods

Post Source Decay (PSD) - MALDI instruments
*Collision Induced Dissociation (CID)
  low energy (eV)
  high energy (keV)
  single collision
  multiple collisions
  Sustained Off-Resonance Irradiation (SORI)
  Resonance Excitation (RE)
  in traps or transmitted through

*Infrared Multiphoton Dissociation (IRMPD)
Electron Capture Dissociation (ECD)
*Electron Transfer Dissociation (ETD)
Surface-Induced Dissociation (SID)
Two step Activation Process:
  activation + unimolecular dissociation

  Internal energy distribution

Molecule:
  Different unimolecular reaction pathways
  Energy dependent rate constants

Instrument Configuration:
  Time for reaction
Rate Theory: RRKM, QET (statistical)

Theory of Unimolecular Reactions

\[ [M] = [M]_0 e^{-kt} \]

- \([M]\) = ion abundance at time \(t\)
- \([M]_0\) = ion abundance at \(t=0\)
- \(k\) = unimolecular rate constant
- \(k\) depends on internal energy (E)

Unimolecular Decay
Assumptions
Separation of translational, rotational, vibrational, and electronic motion
Motion of the nuclei – classical mechanics
(quantum corrections, if necessary)

Postulates
Transition state (TS) on potential energy surface (PES) – boundary between reactants, R, and products, P
Each microstate has equal *a priori* probability

Other Assumptions
The time required for dissociation is long compared with the time of excitation
The rate of dissociation is slow relative to the rate of internal energy randomization over all degrees of freedom
The ion is an isolated system in a state of internal equilibrium
Fragmentation products are formed by a series of competing and consecutive unimolecular reactions
CID: For Whole Population, Range of Impact Parameters

Internal Energy Distribution, P(E)


Internal Energy Distribution P(E) and k(E) Curves

Direct bond cleavage
Rearrangement
RRK (simplified)

k(E) = (1 – E_0/E)^s-1
**Kinetic Shift:**

excess energy required to observe detectable dissociation within a certain experimental time frame

**Competitive Shift:**

excess energy required to cause dissociation to be competitive with pathway of lower appearance energy

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**Potential Energy Surface (PES)**

- A = local (global) minimum
- B = local (global) maximum
- S = saddle point

$\frac{\partial^{2}P(x,y)}{\partial x^2} > 0$

$\frac{\partial^{2}P(x,y)}{\partial y^2} < 0$

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Potential Energy Profile of a Reaction

$E_0$ (kinetic term) can be significantly different from $\Delta E$ ($\Delta H$) (thermodynamic term).

Kinetic shift is energy needed to drive a reaction with a given rate constant, $k$.


Potential Energy Profile of a Reaction

Internal Energy
Critical Energy
Reaction Path

What Determines $k$ at a given $E_{\text{int}}$?

- The ratio of microstates above the transition state ($n^\#$) and at the reactant ($m$)

\[
k(E) = \frac{\sigma \ G^*(E - E_0)}{\hbar \ \rho(E)}
\]


Loose and Tight TS

It is more difficult to build a road (or roads) in the Grand Canyon than in the Shenandoah Valley.

More microstates are available above the loose TS than above the tight TS.
Illustration of the subset of systems which includes all reacting species

$$k(E) = \left(\frac{\sigma}{h}\right) \frac{G^#(E - E_0)}{\rho(E)}$$

- $\sigma$ = reaction path degeneracy
- $h$ = Planck constant
- $G^#(E - E_0)$ = number of states above TS
- $\rho(E)$ = density of states in the reactant ion of internal energy $E$

Figure 7.1: The Wahrhaftig diagram: relationship of $P(E)$ and $k(E)$ for unimolecular ion decompositions of ABCD$^-$. See text for definitions. (Wahrhaftig 1962, 1986.)
Predict Shapes of $k(E)$ vs. $E$

which pathway highest $E_0$?
which pathway steepest rise?

$k$ vs. $E$ curve
breakdown curve
(abundance vs. $E$)
Need Internal Energy Distribution $P(E)$ to calculate spectrum

How can internal energy distribution be determined?

EI : PES

MS/MS: ?

Internal Energy Distributions for MS/MS

Thermometer molecule method:

Fragment molecule with known thermochemistry

Work backwards from spectrum to figure out $P(E)$ that must have been present to lead to measured spectrum
Energy Deposition from a Thermometer Molecule


Spectra Depend on Reaction Pathways, Energy Distribution Deposited, and Observation Time Window

**CH$_3$CO(Pro)OCH$_3$**

Predict fragments of Protonated molecule:
### IT 16%

- $[\text{MH} - \text{MeOH}]^+$
- $[\text{MH} - \text{HCOOME}]^+$
- $[\text{MH} - \text{H}_2\text{O-CO}]^+$

### QQQ 13 eV

- $[\text{MH} - \text{H}_2\text{O-CO}]^+$
- $[\text{MH} - \text{CH}_2=\text{C}=\text{O}]^+$
- $[\text{MH} - \text{H}_2\text{MeOH}]^+$
Q-TOF SID, 13eV

MH$^+$

MH$^-$CH$_3$OH$^+$

MH$^-$CH$_2$=C=O$^+$

MH$^-$CH$_3$OH$^-$CO$^+$

Proton Transfer to Ring (amide) N
Factors to Compare Qualitatively

Average amount of energy deposited

Distribution of energy deposited

Ease of variation of energy deposition
  (e.g., change a voltage, pressure)

Form in which energy is deposited
  (electronic vs. vibrational)

Time frame activation vs. dissociation

How readily activation can be driven

Typical P(E) Curves for keV and eV CID

Activation Can be “Energy-Sudden” or “Slow Heating”

Consider

\[ \text{CH}_3\text{CH}_2\text{CH}_2\text{OH}^{\ddagger\ddagger} \]

\[ \cdot\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}_2^+ \]

In ion trap CID, both lose water

What happens in triple quad?
Isomeric ion structures


IT shows loss of H$_2$O for both

Suggested Readings


Activation Methods

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Resonance Excitation (RE)
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Electron Capture Dissociation (ECD)
Electron Transfer Dissociation (ETD)
Surface-Induced Dissociation (SID)
Blackbody Infrared Radiative Dissociation (BIRD)

Post Source Decay (PSD)
not a "real" MS/MS
without gas (metastable) or with gas (keV CID)

$E_f = (m_f/m_p)E_p$

Linear reflectrons must be adjusted for $E_f$

Collision-Induced Dissociation (CID)

- High (keV) energy (electronic excitation possible)
  - Sector (μs time scale, single collision)
  - TOF/TOF (μs time scale, single collision)

- Low (eV) energy
  - QQQ, sector/Q (BEQQ), Q-TOF (μs, multiple collision)
  - IT (ms, multiple collision)
  - ICR (ms-min, multiple collision)
  - Orbitrap

Orbitrap: Collisional activation OUTSIDE orbitrap

Higher-energy C-trap dissociation (HCD), previously performed in the C-trap (a), is now performed in an octopole collision cell (b) (reference Olsen, 2007)
Maximum energy available

Depends on **target mass** and **kinetic energy**:

\[
E_{\text{cm}} = E_{\text{lab}} \left(\frac{M_t}{M_t + M_p}\right)
\]

**Example:** 
\([\text{YGGFL}]^+ = 556 \text{ u} = M_p\)

Internal energy, \(P(E \leq E_{\text{cm}})\)

- He (4 u) \(\leq 0.714\%\), \(E_{\text{lab}} (71.4 [10,000]; 0.36 [50])\)
- Ar (40 u) \(\leq 6.7\% \ E_{\text{lab}}\)
- Xe (131 u) \(\leq 19\% \ E_{\text{lab}}\)
Different Collision Gas TOF TOF

Collision Gas Kr  P=1x10^{-5}

Collision Gas He  P=2x10^{-6}

Onset Energy Lower for SID
**CID of Large Molecules works but not Understood**

**GroEL chaperonin**
Carol Robinson, Cambridge

Structure and reaction cycle of the bacterial chaperonin GroEL.

Chaperonin homepage: [http://blog02.xmscsa.edu/~seale/Chap/chap.html](http://blog02.xmscsa.edu/~seale/Chap/chap.html)

**GroEL: MS of intact 14mer**

*E. coli* GroEL

\[ M_{\text{calc.}} = 800,758 \]
\[ M_{\text{exp.}} = 805,700 \pm 80 \]

nano-ESI, 10μM in aqueous ammonium acetate (100mM) pH7
GroEL: effect of source pressure

*E. coli GroEL*  
14mer

Source pressure

GroEL: MS/MS

CID of GroEL 14mer at different ion accelerations (30 bar Ar)
Care needed in ionization

reduced charge

“normal” charge NH₄OAc

supercharged
GroEL: Influence of charge on fragmentation pattern

+70 vs +50 ??

GroEL +50 CID and SID

(a) CID

14-mer

(b) SID
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Ion selection and activation in the ICR
IRMPD, SORI, ECD

HDX in the ICR cell
Comparison of Collision-Induced Dissociation and Infrared Multiphoton Dissociation in the Structural Determination of Oligosaccharides

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Infrared Multiphoton Dissociation of O-Linked Mucin-Type Oligosaccharides


Jinhua Zhang, Katherine Schubothe, Bensheng Li, Scott Russell, and Carlito B. Lebrilla

Department of Chemistry and School of Medicine, Biochemistry and Molecular Medicine
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Infrared Multiphoton Dissociation (IRMPD)

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Comparisons between CID and IRMPD of mucin-type oligosaccharides

CID of neutral oligosaccharide XT-1415 (positive mode)

MS/MS only produced high abundance for high mass fragments

IRMPD of neutral oligosaccharide XT-1415

The fragment ions ranging from the parent ion to the last fragment (m/z 228) are readily observed


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**Why does IRMPD yield more extensive fragments?**

CID deposits energy only into the precursor ions
IRMPD excites both precursor and fragment ions
Conclusion

Compared to CID, IRMPD has advantages:

- No collision gas needed, faster
- No multistage (MS^n) needed
- Easy and good control of energy deposited
- The fragmentation efficiency of IRMPD increases with the increasing size of oligosaccharides

IRMPD provides an attractive alternative to CID in the structural elucidation of oligosaccharides

SORI-RE of oligosaccharide Alditol


UV Photodissociation instrument set-up


Orbitrap with photodissociation in the HCD cell


Photodissociation energy deposition

Electron Capture Dissociation

\[
[M + nH]^{n+} + e^- \rightarrow [(M + nH)^{(n-1)+*}]^* \rightarrow \text{fragments}
\]

Electron Capture Dissociation

- Multiply charged (ESI) ions and also their fragments can be reduced by low energy electrons
- Electrons produced by a conventional heated filament outside the FTMS magnet opposite to the ESI source (10^{-5} torr Ar for cooling, < 0.2 eV)
- Mostly c and z type ions are formed
- Gentle fragmentation, good for detecting post-translational modification sites (e.g., phosphorylation, glycosylation, sulfation, gamma-carboxylation)
- Disadvantage: reduced charge state may require extended m/z ion analyzer range


Comparison of ECD and SORI-CAD in FTICR

- c and z ion series
- no loss of water, phosphate groups or phosphoric acid

Cleave Peptide Bonds without Cleaving Sugar

ECD for Peptide Bonds, IRMPD for Sugar
Gas-phase reactions of peptide cations with anions
(ion/ion chemistry)

\[ \text{charged products} \]

\[ \downarrow \text{MS} \]

1. proton transfer
\[ (M + 3H)^{3+} + A^- \rightarrow (M + 2H)^{2+} + HA \]

2. electron transfer
\[ (M + 3H)^{3+} + B^{2+} \rightarrow (M + 3H)^{2+} + B \]

3. anion attachment
\[ (M + 3H)^{3+} + C^- \rightarrow (M + 3H + C)^{2+} \]
Instrument Configuration for ETD

Linear Trap With End Segments:
Axial Confinement With DC Potentials

Axial Confinement With DC Potentials
Trapping is Charge Sign Dependent
Axial Confinement With DC Potentials
Trapping is Charge Sign Dependent

Simion Simulation of Dipole Excitation Field

Segmented Trap

Unsegmented Trap
Procedure for ETD

Step 1: Move M/z Isolated Cations Precursor Ions Front Section
Step 2: Anion Injection
Step 3: End Lens RF On and Ion/Ion Reaction
Step 4: End Lens RF Off and Ready to scan

ASMS Fall Workshop 2006 John E. P. Syka
Ion Activation in MS/MS

Linear Change in Internal Energy Characteristic of SID

Activation Can be “Energy-Sudden” or “Slow Heating”

keV CID, SID

Multiple Collision CID


Energy Deposition in SID vs. CID

Energy Deposition in SID vs. CID


Instrument Modification for SID

Galhena A et al., Anal. Chem., 2008, 80, 1425-1436
SID Design

Gold with F-SAM surface

From Quad
Precursor

To Hexapole Collision Cell
Fragments

Galhena A et al., Anal. Chem., 2008, 80, 1425-1436

SID Design

Gold with F-SAM surface

From Quad
Precursor

To Hexapole Collision Cell
Precursor

Instrument capable of CID with SID apparatus installed

Galhena A et al., Anal. Chem., 2008, 80, 1425-1436
Asymmetric Charge & Mass Partitioning

Monomer accounts for $\frac{1}{4}$ the total mass, but carries away $\frac{1}{2}$ the charge!

Jurchen and Williams, Robinson, Smith, Heck, Klassen

Problem

CID (collision with gas) gives

monomer plus \((n-1)\)-mer

doesn’t provide full sub-structure
Why use SID vs CID for large complexes?

SID

Single collision
Sudden energy deposition

CID

Multiple collisions
Multi-step activation

Cooks and coworkers

Why use SID vs CID for large complexes?

SID

Access pathways not available by CID?

CID

97

98
Multi-Step CID vs. Single Step SID

Multi-Step Activation (CID)

Single-Step Activation (SID)

(A) Unfolded

(B) Folded


(CsI)$_n$Cs$^+$ and (CsI)$_n$Cs$_2^{++}$

10 or 100 mg/mL CsI in 50% MeOH or 50% ACN
(CsI)$_4$Cs$_2$I$_2$ $\rightarrow$ CID

CID

SID

= Cs

= I

Cytochrome C MS/MS CIS and SID

Cytochrome C MS/MS CIS and SID


MS/MS of Transthyretin Homotetramers

M = monomer
D = dimer
T = trimer
Q = tetramer

CID
CE = 1350 eV
Asymmetric mass and charge partitioning
MS/MS of Transthyretin Homotetramers

M = monomer
D = dimer
T = trimer
Q = tetramer

CID
CE = 1350 eV
Asymmetric mass and charge partitioning

SID
CE = 759 eV
Mass and charge more equally distributed

SID: Variation with Energy

ΔV = 30 V
CE = 450 eV

ΔV = 50 V
CE = 750 eV

ΔV = 90 V
CE = 1350 eV

M = monomer
T = trimer
D = dimer
Q = tetramer
Onset Energy Lower for SID

NIH, NSF, Waters, UK EPSRC, OSU