Activation and spectroscopy of mass and charge selected ions

Alexandre Giuliani
Synchrotron SOLEIL & INRAE
Motivations

• Profit from the capacity of modern ionization sources to place virtually anything in the gas phase.
Motivations

• Profit from the capacity of modern ionization sources to place virtually anything in the gas phase.
• Spectroscopy on charged species: is it relevant?
Motivations

• Profit from the capacity of modern ionization sources to place virtually anything in the gas phase.
• Spectroscopy on charged species: is it relevant?

The isoelectric point (pI), is the pH at which a particular molecule carries no net electrical charge.

Figure 1. Number of proteins in the UniProtKB/Swiss-Prot database versus pI value, retrieved with the TagIdent tool (http://www.expasy.ch/tools/tagident.html) in June 2010.
Motivations

• Profit from the capacity of modern ionization sources to place virtually anything in the gas phase.
• Spectroscopy on charged species: is it relevant?

The isoelectric point (pI), is the pH at which a particular molecule carries no net electrical charge.

Most of the proteins are charged in solutions
→ Nucleic acids, fatty acids ...
Motivations

• Profit from the capacity of modern ionization sources to place virtually anything in the gas phase.
• Spectroscopy on ions

• Control over the target:
  - mass and charge selected species
Motivations

ESI-MS of PEG 4100

Intensity

0 1000 2000

m/z

500 1000 1500 2000

+4
+3
+5
Motivations

ESI-MS of PEG 4100

ESI-MS of PDMS. The arrow indicates the monoisotopic ion of \([\text{PDMS}_{25}^{\text{+Na}}]^+\)
Motivations

ESI-MS of PEG 4100

ESI-MS of PDMS. The arrow indicates the monoisotopic ion of [PDMS$_{25}$+Na]$^+$

ESI-MS of a protein interacting with its ligand. Mixture of stoichiometries (1•0 à 1•8) and charge states (6+ à 8+).
Experimental setup

Experimental setup
SRMS2 @ DESIRS

DESIRS beamline, SOLEIL
http://www.synchrotron-soleil.fr/Recherche/LignesLumiere/DESRS

Action spectroscopy

A. Milosavljević, C. Nicolas, J. Lemaire, C. Dehon, R. Thissen, J.-M. Bizau, M. Réfrégiers, L. Nahon and A. Giuliani,
Action spectroscopy

A. Milosavljević, C. Nicolas, J. Lemaire, C. Dehon, R. Thissen, J.-M. Bizau, M. Réfrégiers, L. Nahon and A. Giuliani,
Action spectroscopy

Precursor selection

A. Milosavljević, C. Nicolas, J. Lemaire, C. Dehon, R. Thissen, J.-M. Bizau, M. Réfrégiers, L. Nahon and A. Giuliani,
Action spectroscopy

Precursor selection

Normalized ion abundance of $[M+nH]^{(n+1)+}$

A. Milosavljević, C. Nicolas, J. Lemaire, C. Dehon, R. Thissen, J.-M. Bizau, M. Réfrégiers, L. Nahon and A. Giuliani,
Action spectroscopy

A. Milosavljević, C. Nicolas, J. Lemaire, C. Dehon, R. Thissen, J.-M. Bizau, M. Réfrégiers, L. Nahon and A. Giuliani,
Outline

• VUV activation of oligosaccharides

• Serine dimer
Outline

• VUV activation of oligosaccharides

• Serine dimer
Nomenclature from Domon et Costello (1988)
Nomenclature from Domon et Costello (1988)
Nomenclature from Domon et Costello (1988)
Limitation of collision activation

DP3Me3

Z₂ or C₂
Limitation of collision activation

DP3Me3

![Chemical structure diagram]

Water loss

Methanol loss

Relative Abundance

Z₂ or C₂
Limitation of collision activation
Limitation of collision activation

DP3Me3

\[ \text{CH}_3\text{O} - \text{C} \rightarrow \text{OCH}_3 \]

\[ \text{O} \rightarrow \text{O} \cdot \text{OCH}_3 \]

\[ \text{OH} \rightarrow \text{OH} \cdot \text{OH} \]

Water loss

Methanol loss

X, Y, Z fragments shift by 2 Da

Limitation of collision activation
Limitation of collision activation

- Difficulty to achieve a complete labelling
- Cost!
- Double fragmentation

X, Y, Z fragments shift by 2 Da
Limitation of collision activation

- Difficulty to achieve a complete labelling
- Cost!
- Double fragmentation

X, Y, Z fragments shift by 2 Da

Water loss
Methanol loss
Limitation of collision activation

DP3Me3

Before labelling by $^{18}$O

After labelling by $^{18}$O
Limitation of collision activation

Before labelling by $^{18}$O

After labelling by $^{18}$O

2Da shift
No shift
Limitation of collision activation

Before labelling by $^{18}$O

After labelling by $^{18}$O

$\text{DP3Me3}$
VUV activation vs CID

DP3Me3

Neutral loss

Neutral loss

VUV activation vs CID
VUV activation vs CID

- More intracyclic fragments
- No neutral losses
- No double fragmentation

Interfering peaks from the beam
VUV activation vs CID

- More intracyclic fragments
- No neutral losses
- No double fragmentation

Interfering peaks from the beam

Neutral loss

Neutral loss

• More intracyclic fragments
• No neutral losses
• No double fragmentation

Interfering peaks from the beam
"RULES":

- Systematic series of fragments (X, Y, Z) from reducing end and (A, B) from non-reducing end
  - More intracyclic fragments
  - No neutral losses
  - No double fragmentation

Interfering peaks from the beam

Neutral loss

Interfering peaks from the beam
DP5Me3 sequencing

DP5Me3

\[ \text{C=O} \quad \text{O} \quad \text{C=O} \quad \text{O} \quad \text{C=O} \quad \text{O} \quad \text{C=O} \quad \text{O} \quad \text{C=O} \quad \text{O} \]

\[ \text{HO} \quad \text{O} \quad \text{C=O} \quad \text{O} \quad \text{C=O} \quad \text{O} \quad \text{C=O} \quad \text{O} \quad \text{C=O} \quad \text{O} \]

\[ \text{Me} \quad \text{Me} \quad \text{Me} \quad \text{Me} \]

\[ \text{SY0487} \]

\[ \text{C:\Users...DATA_BRUTE\SY0487} \]

\[ \text{23/06/2012 23:19:31} \]

\[ \text{F38 HGB69 945 VUVPD 18eV 3000ms 4min} \]

\[ \text{SY0487} \]

\[ \# \]

\[ \text{1-23} \]

\[ \text{RT: 0,00-3,91} \]

\[ \text{AV: 23} \]

\[ \text{NL: 1,07E3} \]

\[ \text{T: ITMS + p ESI Full ms2 945,20@cid0,00 [260,00-1000,00]} \]

\[ \text{300 350 400 450 500 550 600 650 700 750 800 850 900} \]

\[ \text{x10} \quad \text{x20} \quad \text{x50} \quad \text{x100} \]

\[ \text{m/z} \]

\[ \text{945,2} \]

\[ \text{Relative Abundance} \]

\[ 0 \quad 10 \quad 20 \quad 30 \quad 40 \quad 50 \quad 60 \quad 70 \quad 80 \quad 90 \quad 100 \]

\[ \text{300 350 400 450 500 550 600 650 700 750 800 850 900} \]

\[ \text{Relative Abundance} \]

\[ 0 \quad 10 \quad 20 \quad 30 \quad 40 \quad 50 \quad 60 \quad 70 \quad 80 \quad 90 \quad 100 \]

\[ \text{m/z} \]

\[ 945,2 \]

\[ \text{313,0} \quad \text{335,0} \quad \text{389,2} \quad \text{407,2} \quad \text{435,2} \quad \text{443,2} \quad \text{479,0} \quad \text{500,9} \quad \text{519,2} \quad \text{533,2} \quad \text{561,2} \quad \text{594,9} \quad \text{583,2} \quad \text{625,2} \quad \text{644,9} \quad \text{660,9} \quad \text{737,2} \quad \text{725,2} \quad \text{801,1} \quad \text{814,1} \quad \text{826,8} \quad \text{855,1} \quad \text{885,2} \]

\[ \text{x10} \quad \text{x20} \quad \text{x50} \quad \text{x100} \]
DP5Me3 sequencing
DP5Me3 sequencing
DP5Me3 sequencing
DP5Me3 sequencing

DP5Me3

DP5Me3 sequencing
DP5Me3 sequencing

DP5Me3

![Diagram of DP5Me3 structure]

**Relative Abundance vs. m/z**

- B3: Δ -176
- B4: Δ -208

**Peaks at**
- m/z 285.0
- m/z 335.0
- m/z 313.0
- m/z 371.2
- m/z 389.2
- m/z 407.2
- m/z 435.2
- m/z 443.2
- m/z 479.0
- m/z 500.9
- m/z 519.2
- m/z 533.2
- m/z 561.2
- m/z 594.9
- m/z 597.2
- m/z 625.2
- m/z 644.9
- m/z 660.9
- m/z 737.2
- m/z 801.1
- m/z 814.1
- m/z 826.8
- m/z 855.1
- m/z 885.2

**RT:** 0.00-3.91
**AV:** 23
**NL:** 1.07E3

**T:** ITMS + p ESI Full ms2 945.20@cid0.00 [260.00-1000.00]
DP5Me3 sequencing
DP5Me3 sequencing

DP5Me3

DP5Me3 +
DP5Me3 sequencing

[Diagram of DP5Me3 sequencing with molecular structures and mass spectra]
Liquid chromatography coupling

Red alga

Digestion par
une agarase +
une porphyranase

SEC

ESI   Ion Trap

+1Me
Liquid chromatography coupling

Digestion par une agarase et une porphyranase

ESI Ion Trap

Intracluster bond formation

• VUV activation of ligosaccharides

• Intracluster bond formation: the case of serine dimer
LE-CID of protonated serine dimer

→ Cluster evaporation
→ Fragments of serine
× Peptide bond formation expected at m/z 193
Intracluster bond formation was never observed using LE-CID.

- Heating of the cluster leading to statistical products (evaporation more likely than ICBF)

Ser+SerH⁺

H₂O release

CO₂ release

M06-2X / 6-31++G(d,p) molecular dynamics
Intracluster bond formation

\[ \text{Serine} + \text{Serine} + \text{H}^+ \rightarrow \text{diserine} + \text{H}_2\text{O} \]

- Excited states dynamics which can possible results in different, non statistical products
  → ICBF has been reported at 157 nm irradiation (JACS 2011, 133, 15834)

- Ability to deposit a well defined amount of energy into the system

- Tunable source: identify the excited states involved
LE-CID versus Photon activation

ICBF region
Peptide bond formation

→ m/z 193 low abundant (<1%) below 7 eV
→ PBF is present at higher energy
→ PBF is present at higher energy (above 10 eV)
Fragmentation of diserine is a two steps process:
- either PBF followed by fragmentation
- or fragmentation followed by bond formation
Peptide bond formation

PBF sterically hindered

Peptide bond formation

- States \( S_2, S_3, S_4, S_5 \) and \( S_{12} \) relaxes into stable minima that could further evolve towards PBF.

- PBF in the ground state \( S_0 \) implies an energetic barrier of 2.48 eV, compared to 0.5–1.6 eV from the excited states.

Conclusions

- VUV activation of oligosaccharides
  - Better suited than CID
  - Brilliance of SR makes it compatible with LC
- Intracluster bond formation
  - Evidence for peptide bond within a cluster
Acknowledgements

SynchrotronSOLEIL
Laurent Nahon
Aleksandar Milosaljevic
Matthieu Réfrégiers

Bar-Ilan University, Israel
Ori Licht
Yoni Toker

Normandie Univ, Caen
Patrick Rousseau

Universidad Autónoma de Madrid, Spain
Darío Barreiro-Lage
Lara Martínez-Fernández
Sergio Díaz-Tendero

LAMBE, Evry
William Buchmann

INRAE
Hélène Rogneau
David Ropartz
Francis Canon

Thank you for your attention