

Using a Fragmentation Database to Derive VUV Photodissociation Selection Rules and Interpret Peptide Spectra

Xiaohui Liu, James P. Reilly

Chemistry Department, Indiana University

Overview:

- + Photofragmentation generates a series of x-type ions that can be used for peptide sequencing.
- + Photofragmentation rules need to be determined for *de novo* sequencing.

Introduction:

Peptide *de novo* sequencing is performed to derive peptide sequences without referring to a database. 157 nm laser light generates a series of x-type fragments in matrix-assisted laser ionization tandem time-of-flight (MALDI-TOF) mass spectrometer. A *de novo* sequencing algorithm has been developed combining x-type ions from photodissociation and y-type ions from postsource decay[1]. The x/y pairs are used to derive peptide sequences. However, some x-type or y-type fragments are missing from the spectra and the search results from *de novo* sequencing do not contain complete sequence information. An abundance of fragments produced by 157 nm photodissociation are not considered in *de novo* sequencing algorithm. Peptide libraries are synthesized and photofragmented to analyze the photodissociation rules to improve the sequencing algorithm. The following discussion derives general photodissociation selection rules.

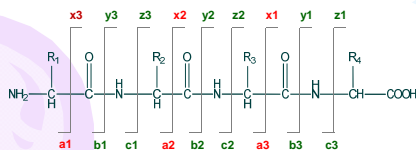
Experiment:

Peptide libraries from 9 to 12 amino acids were synthesized. The peptides were separated using 2D-nano LC and spotted onto the MALDI plate by an Eksigent Spotter. The samples were analyzed by an ABI 4700 MALDI TOF-TOF instrument. Photofragmentation and PSD spectra were collected. Both Mascot Search and *de novo* sequencing were used for data analysis.

Discussion:

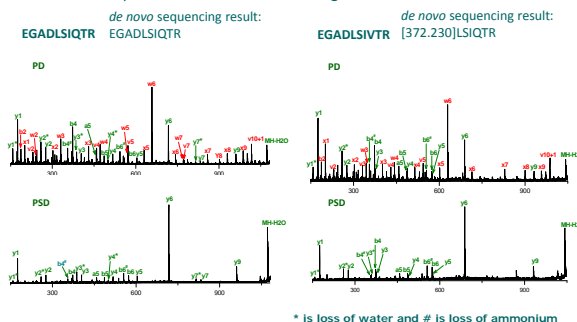
157 nm photofragmentation:

- singly charged precursor produces x-, a-, w-, v-type fragments



de novo Sequencing^[1]

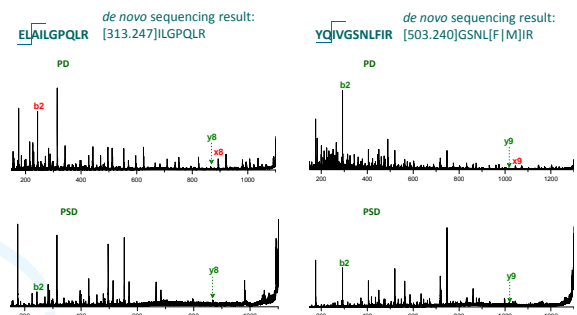
- Photodissociation (PD) produces x-type fragments.
- Postsource decay (PSD) produces y-type fragments.
- Identification of x/y pairs confirms x-type ions.
- x-type ion spacing derives peptide sequences.
- N-terminal sequence of EGADLSIVTR is missing.



* is loss of water and # is loss of ammonium

b₂-X_{N-2} pairs:

- 640/642 photofragmentation spectra contain b₂ ions.
- Some peptides having x_{N-2} but no y_{N-2} fragments are not sequenced.
- 568/642 photofragmentation spectra have b₂-x_{N-2} fragment pairs.
- 167/642 photofragmentation spectra have x_{N-2}-y_{N-2} fragment pairs.
- XT-XX...XX has strong y_{N-2} fragments
- Mass of b₂+x_{N-2} is 26.9865 Da heavier than precursor ion mass.

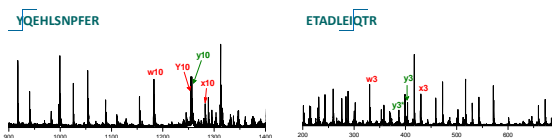


b₃-X_{N-3} pairs:

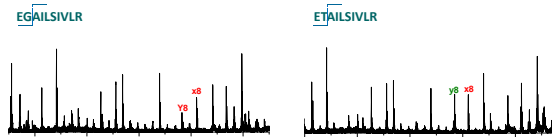
- b₃ fragment exists in all other spectra from photodissociation except TX-XXX...XX and XXM-DXX...XX sequences.
- Some peptides having x_{N-3} but no y_{N-3} fragments are not sequenced.
- 569/642 photofragmentation spectra have b₃-x_{N-3} fragment pairs.
- 219/642 photofragmentation spectra have x_{N-3}-y_{N-3} fragment pairs.
- XXE-XX...XX has strong y_{N-3} fragments
- Mass of b₃+x_{N-3} is 26.9865 Da heavier than precursor mass.

Y-type fragments:

- X-QXX...XX has Y_{N-1} fragment.
- No Y-type fragment for XX...XX-QXX.

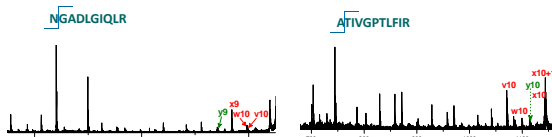
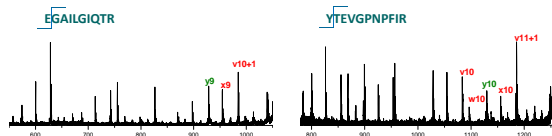


- XG-AXX...XX has Y_{N-2} fragment.
- No Y-type fragment for other XX-AXX...XX.



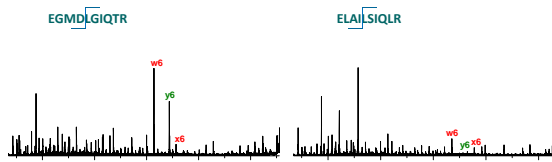
N-terminal effect:

- Y-XX...XX and E-XX...XX facilitate formation of y_{N-1} and v_{N+1} fragments.
- N-XX...XX and A-XX...XX suppress formation of y_{N-1} and v_{N+1} fragments.



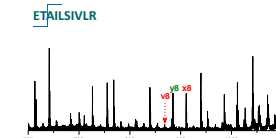
D-L effect:

- Leucine has dominant w-type fragment if glutamic acid is on its N-terminal.
- Other residues do not have this effect.

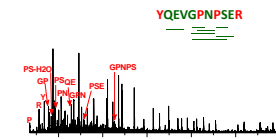
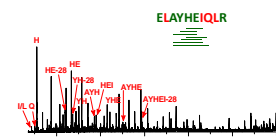


v ion of Alanine:

- v ion of Alanine tends to be weak in MS/MS spectrum.
- There is usually no v ion of Alanine in high mass range.



Internal fragments & immonium ions:



Conclusion:

- ★ Identification of b₂-X_{N-2} and b₃-X_{N-3} fragment pairs facilitates *de novo* sequencing.
- ★ X-QXX...XX and XG-AXX...XX sequences have Y-type ions.
- ★ Y-XX...XX and E-XX...XX facilitate formation of y_{N-1} and v_{N+1} fragments.
- ★ Leucine has dominant w-type fragment if glutamic acid is on its N-terminal.
- ★ Presence of immonium ions and internal fragments confirms the peptide sequence from *de novo* sequencing.

Reference:

Zhang L.Y., Reilly J.P., Analytical Chemistry Vol. 82, No. 2, 2010