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

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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.

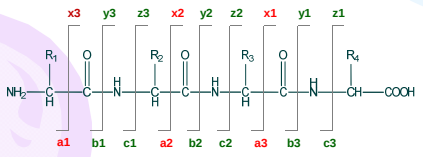
Experiment:

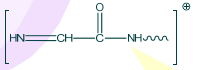
Peptide libraries from 9 to 12 amino acids were synthesized. The peptides were separated using 2D-nano LC and spotted onto the MALDI plate byan Eksigent Spotter. The samples were analyzed by an ABI 4700 MALDI TOF-TOF instrument. Photofragmentation and PSD spectra were collected. Both Mascot Search and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

Discussion:

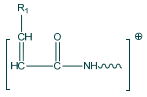
157 nm photofragmentation:

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





v-type ion

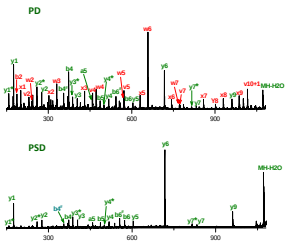


w-type ion

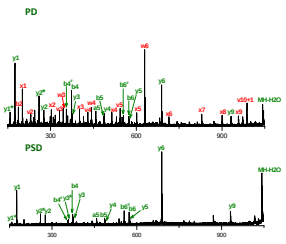
de novo Sequencing[1]

- 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.

de novo sequencing result: EGADLSIQTR



de novo sequencing result: [372.230]LSIQTR

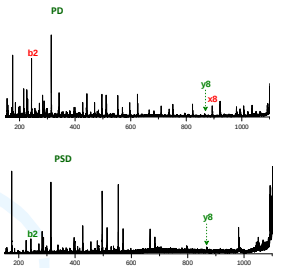


* is loss of water and # is loss of ammonium

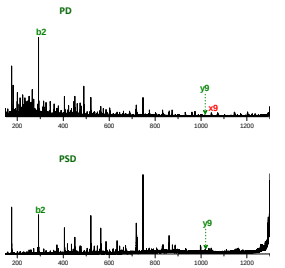
b_2-X_{N-2} pairs:

- 640/642 photofragmentation spectra contain b_2 ions.
- Some peptides having x_{N-2} but no y_{N-2} fragments are not sequenced.
- 568/642 photofragmentation spectra have b_2-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 b_2+x_{N-2} is 26.9865 Da heavier than precursor ion mass.

de novo sequencing result: [313.247]ILGPQLR



de novo sequencing result: [503.240]GSNL[F|M]IR



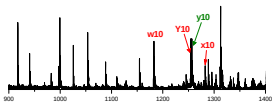
b_3-X_{N-3} pairs:

- b_3 fragment exists in all other spectra from photodissociation except XTX-HXX…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 b_3-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 b_3+x_{N-3} is 26.9865 Da heavier than precursor ion mass.

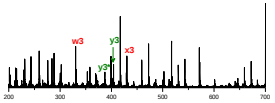
Y-type fragments:

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

YQEHLSNPFER

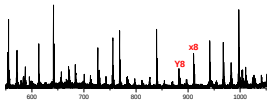


ETADLEIQTR

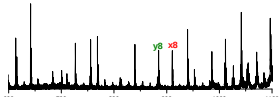


- XG-AXX…XX has Y_{N-2} fragment.
- No Y-type fragment for other XX-AXX…XX.

EGAILSIVLR



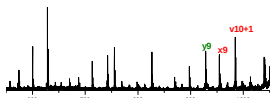
ETAILSIVLR



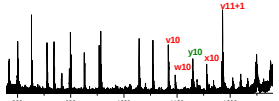
N-terminal effect:

- 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.

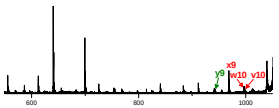
EGAILGIQTR



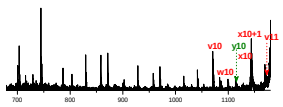
YTEVGPNPFIR



NGADLGIQLR



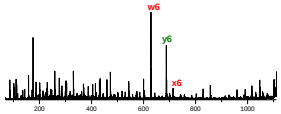
ATIVGPTLFIR



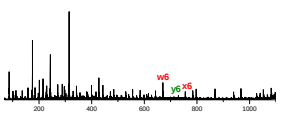
D-L effect:

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

EGMDLGIQTR



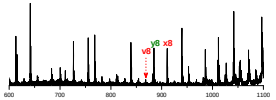
ELAILSIQLR



v ion of Alanine:

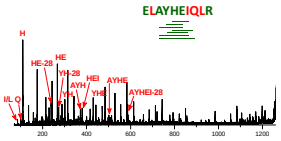
- v ion of Alanine tends to be weak in MS/MS spectrum.
- There is usually no v ion of Alanine in high mass range.

ETAILSIVLR

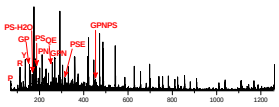


Internal fragments& immonium ions:

ELAYHEIQLR



YQEVGPNPSER



Conclusion:

★ Identification of b_2-x_{N-2} and b_3-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 Chemsitry Vol. 82, N0. 2, 2010