Influence of Ion Storage Location and Multiple Fill/Laser Pulse Sequence on Top-down UVPD Using an Orbitrap Fusion Mass Spectrometer

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Overview

Purpose: Evaluation of performance characteristics of UVPD on the Thermo Scientific[™] Orbitrap Fusion[™] Tribrid[™] Mass Spectrometer.

Methods: Top-down protein characterization with UVPD capable Orbitrap Fusion MS

Results: Multiple laser pulses and multiple ion fills coupled with multiple laser pulses results in higher top-down sequence coverage using UVPD.

Introduction

Ultraviolet photo-dissociation (UVPD) has been demonstrated as an effective means for generating sequence informative polypeptide product ions. Improvement in sequence coverage and decreased charge state dependence on sequence coverage and product ion yield are chief reasons for choosing UVPD^[1] over any other single dissociation technique available for intact protein interrogation. Here we utilize the flexible configuration of the Orbitrap Fusion to enable UVPD reactions within any of its three ion storage regions, the low and high pressure cells (LPC and HPC respectively) of the quadrupole linear ion trap analyzer and the collision cell (HCD cell or lon Routing Multipole, IRM). Multiple ion packet fill/laser pulse sequences are evaluated for improvement in S/N and protein sequence coverage.

Methods

Sample Preparation

Apomyoglobin was used as received (Sigma Aldrich). Myoglobin was diluted in ultrapure water to a stock concentration of 100 μ M. A final dilution step brought the working solution to a concentration of 0.5 μ M in spray solution (H₂O:Methanol:Acetic Acid; 49:49:2).

Mass Spectrometry

A 193-nm ArF excimer laser (Coherent ExciStar XS) was coupled to an Orbitrap Fusion[™] Mass Spectrometer to enable UVPD. Laser optics utilized for coupling instruments resembles the configuration described by Brodbelt *et al.* A blank flange behind the (LPC) was replaced with a UV transparent window flange for laser access. This laser has an energy feedback circuit to enable fixed energy laser pulses within a certain range (0.4-60 mJ/pulse). Pulse width is 5 ns (FWHM). The site of irradiation has been explored among the three ion storage devices bore sighted to the beam. A variety of combinations of laser pulse sequences and (multiple) ion injection events were explored for performance optimization .

Data Analysis

All .raw files were analyzed using Hardklor^[2] for peak deconvolution. Database searches were carried out using Prosight Lite^[3]. Some of the data presented was processed using embedded Lua directly on the Orbitrap Fusion MS instrument computer.

FIGURE 1. UVPD interface with Orbitrap Fusion MS. UVPD can be conducted in all three locations: HPC, LPC, and IRM (HCD).



Results

UVPD reaction location

The site of UVPD was explored to discern whether any performance advantage is conferred via UVPD location. Sequence coverage and number of matching fragment ions was surveyed in the LPC and HPC. Laser beam alignment proved critical for optimized results in each location.

Multiple fill/ laser pulse sequence

Various instrument operation modes using combinations of ion injection events combined with laser pulse sequences have been explored. Sequence coverage was ultimately utilized to determine which configuration offered a benefit to top-down analysis. In addition, preliminary results illustrating proof of principal concept behind RF based laser irradiation protection are shown.

FIGURE 2. Diagram representing the experimental sequence of events regarding ion injection events and laser pulse events: A) Single ion injection/single laser pulse experiment, B) Single ion injection followed by N laser pulses, C) N ion injection events (single fill target/N) which equate to a single ion injection event followed by N laser pulses.









Figure 4. UVPD fragmentation pattern produced through irradiation in the HPC with 5 laser pulses of 0.6 mJ each. Inset spectra reveal the product ion peak density produced through this process. Sequence coverage map shows extensive coverage throughout the protein sequence.



Figure 5. (Left) Apomyoglobin [M+19H]¹⁹⁺ was used to study sequence coverage as a function of pulse energy in both the LPC and the HPC. (Right) Number of matching fragment ions are also reported as a function laser pulse energy. These data are preliminary results in an effort to characterize the best location for UVPD on the Orbitrap Fusion MS platform. Fine adjustment to laser alignment was performed for each device without breaking vacuum through monitoring the depletion of precursor.



Figure 6. Product ion coverage maps from two selected experimental data points from Figure 5 (0.2 and 1.0 mJ). The HPC exhibits a much slower roll-off in sequence coverage as a function of laser energy than the LPC. Also, sequence coverage overall is relatively stable across a wide energy range in the HPC. However, smaller fragment ions are observed as the energy is increased (both HPC and LPC) as illustrated by the absence of product ion coverage throughout the middle of the protein sequence at 1.0 mJ. This is as expected if multiple product ion generations are produced during a single laser pulse.

	0.2 mJ	1.0 mJ
НРС	N G L S D G E W Q Q V LÌN VÌW G K V EÌAÌDÌ IÌAÌGÌHÌG 25	N G L S D G E W QÌQ VÌLÌNÌVÌW G K VÌEÌAÌDÌIÌAÌG HÌG
	26]Q]E]V L I]R]L]F]T G]H]P]E]T L]E]K]F]D]K]F]K]H]L]K 50	20]Q]E[V]L]I]R]L]F]T]G]H]P]ET L]E[K]F]D[K]F]K]H]L]K
	^{5⊥}]T E]A]E]M]K A]S E]D L K]K]H]G[T V V]L]T A L]G G I ⁷⁵	TEA]EMKA]SEDLKKH]GTVVLTALGGI
	⁷⁶ ι κ κ κζο]Η Η]Ε <mark>[Α</mark> [Ε ι Κ】Ρ <mark>]</mark> ι]Α[Ο S <mark>]</mark> Η Α Τ[Κ[Η <mark>]Κ</mark> [Ι]Ρ ¹⁰⁰	⁷⁶ L K K K G H H E A E L K P L A Q S H A T K H K I P
	101 LIKLYLL ELFLISD ALI IHLVLLHISKHUPGDLFGA 125	101 LIKUYLLEEF ILS DIALI ILHUVLUHSUKHPGDEFGA
	126 DIALQLG AMLT KLALLLELLLERNDLIALAK YLK ELG 130	126 DIA QIGIA M TIKALI LELI FIRIN DI I A A K YIK E L G
	151 FQGC	151 FQGC
	N G L S D G E W Q Q V L N V W G K V E A D I A G H G 25	N G L S D G E W Q QÌV L NÌVÌWÌG K VÌE AÌDÌIÌAÌG HÌG
	N GLSDGEWQQVÌLNVÌWGKV EÌAÌDÌIÌAÌGHG ²⁵ 24 QEÌVLÌIÌRÌLÌFÌTÌG <mark>ÌH</mark> ÌPÌEÌTLÌE <mark>Ì</mark> KFÌDÌKÌFÌKÌHÌLÌK ∞	N G L S D G E W Q QÌV L NÌVÌW <mark>Ì</mark> G K VÌE AÌDÌIÌAÌG HÌG ²°ÌQÌEÌVÌLÌI RÌLÌFÌT G HÌP E T L E K F D K F K H L K
	N GLSDGEWQQV]LNV]WGKVE]A]D][]A]GHG 25 24 QE]V[]]]R][]F]T]ĞH[P]E]T[]E]KF]D]R]F]R]H]L]K 50 3-]]T]EA]E]M KA]SE]DLKKH]G]TVV[]TALGGI 75	N GLSDGEWQQÌVLNÌVÌWÌGKVÌEÀÌDÌ ÌAÌGHÌG ?ª]QÌEÌVÌLÌIRÌLÌFÌTGHÌPETLEKFDKFKHLK ∴ TEAEMKASEDLKKHGTVVLTALGGI
LPC	א נו ג ג ג ג ג ג ג ג ג ג ג ג ג ג ג ג ג ג	N G L S D G E W Q QÌV L NÌVÌWÌG K VÌE A ÌDÌ IÌ ÀÌG HÌG ≫ÌQÈIVÌLÌI RÌLÌFÌT G HÌP E T L E K F D K F K H L K № Т E A E M K A S E D L K K H G T V V L T A L G G I № L K K K G H H E A E L K P L A Q S H A T K H K I P
LPC	N GLSDGEWQQV]LNV]WGKVEÌAD]1 A]GHG ²⁵ ²⁸ QE]VL]1]R[L]F]T]G[H]P]E]TL]E]KF]D[K]F]K H]L]K ²⁵ ²¹]T[EA]E]M[KA]SE]DLKKHÅ]G[TVVL]TALGGI ²⁵ ²⁸ LKK[K]GHHEAELKKHÅ]CVQKACKL]F ¹⁰⁵ ²⁰]L[K[V]LEIF]L[SDA]L[L]HVLH]SKHÅ[G]D[F]G]D[G]GA ¹⁵⁵	N G L S D G E W Q QÌ V L NÌ VÌ WÌG K VÌ E AÌDÌ I ÀIG HÌG ³¹ QÌ EÌ VÌ LÌ I RÌ LÌ FÌ T G HÌ P E T L E K F D K F K H L K ³¹ T E A E M K A S E D L K K H G T V V L T A L G G I ³² L K K K G H H E A E L K P L A Q S H A T K H K I P ³³¹ I K Y L F I S D A I I N VÌ L H S KÌH P IGLP I GÌA
LPC	א כוגס כניע קעיע און איז	N G L S D G E W Q QÌ V L NÌ VÌNG K VÌ E AÌDÌ Ì À ÌG HÌG ²¹ QÌEÌVÌ LÌ RÌ LÌ ÌT G HÌP E T L E K F D K F K H L K ²³ T E A E M K A S E D L K K H G T V V L T A L G G Ì ²⁴ L K K K G H H E A E L K P L A Q S H A T K H K I P ¹²⁵ I K Y L E F I S D A I I H VL H S KIHÌ P(GÌG Ì GÌA ²¹⁶ DIAIQÍGÌ M TIKAÌL I E LIFRIJNI Ì T I A KIH P (GÌ D FÌGÌA

Figure 7. A) Laser energy was varied in 0.1 mJ/pulse steps and the precursor intensity monitored. The insets illustrate the extent of fragmentation achieved at each energy level. As expected for a first-order process, depletion of precursor is an exponential decay with energy delivery. B) Semi-log plot with exponential fit indicating linear increase in total energy with increasing energy.







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FIGURE 9. Experiment indicates proof of principal for protection of ions from irradiation of the laser. This will enable a direct mechanism for over-fragmentation control in UVPD experiments. A single frequency resonant dipolar excitation was applied varying the amplitude to bring the precursor ion out of the path of the laser. As the excitation amplitude is increased the onset of CID is observed. A) UVPD specific and CID specific product ions are monitored from apomyglobin [M+24H]²⁴⁺.



Conclusion

- UVPD capable Orbitrap Fusion MS system can efficiently fragment polypeptide ions to produce informative product ions
- UVPD experiments were successfully conducted in the HPC and LPC
- Proper control over laser pulse energy will be required to ensure reproducibility and robust product ion generation
- Protection of product ions generated early during multiple laser pulse experiments will be required for control of over-fragmentation

References

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