Improved Top-Down Sequence Coverage on an Orbitrap Fusion Lumos Tribrid Mass Spectrometer by Ion-Ion Proton Transfer (IIPT) Reactions Subsequent to ETD and UVPD

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ABSTRACT

Improved sequence coverage for top-down protein standards is demonstrated by applying IIPT reactions subsequent to activation by ETD or UVPD on all or a selected portion of the MS^2 fragment ion population.

INTRODUCTION

Electron and photon based activation methods have proven to be indispensable tools for the analysis of peptides and proteins. Electron Transfer Dissociation (ETD) and Ultra Violet Photo-Dissociation (UVPD) have been demonstrated to provide unique sequence information, and, in conjunction with recent advances in proteome fractionation and top-down informatics, have facilitated intact protein characterization. However, as the molecular weight of protein precursor ions increases, so too does the number of possible product ions, and considerable spectral congestion occurs in the vicinity of the precursor ion m/z. In this region, the combination of low product ion signal-to-noise ratios and overlapping isotopic clusters greatly complicate both manual and computerized charge state and sequence ion assignment.

MATERIALS AND METHODS

Sample Preparation

Apoymoglobin from equine skeletal muscle, lyophilized powder, Sigma-Aldrich A8673. 1 pmol/µL in 50/50 MeOH/H_2O w/ 0.1 % Formic Acid.

Carbonic Anhydrase II from bovine erythrocytes, lyophilized powder, Sigma-Aldrich C2522. 1 pmol/µL in 50/50 MeOH/H_2O w/ 0.1% Formic Acid.

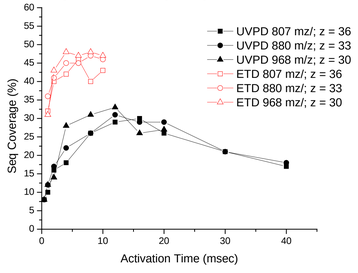
Test Method(s)

Anderson et al. reported increased top down sequence coverage for apomyoglobin when applying IIPT reactions subsequent to ETD. The improvement in sequence coverage was attributed to a reduction in product ion spectral density via a dispersion of the fragment ion population over a greater m/z range.[1] Here we demonstrate the potential for IIPT to increase top-down sequence coverage by exposing multiple 10-500 m/z windows of UVPD or ETD product ion populations to varying degrees of IIPT. The proton transfer reagent used for these studies is perfluoroperhydrophenanthrene (623 m/z, $C_{14}F_{24}$) delivered from a from a separate reagent inlet into the ETD glow discharge reagent ion source. The reaction q during the ion-ion reaction was 0.4 unless otherwise noted.

Data Analysis

MS/MS and MS/MS/MS spectra were deconvoluted using the Hardklör[2] or Xtract algorithms, and searched against their respective sequence using ProSight Lite software with a 10 ppm mass tolerance.

Figure 1. Carbonic Anhydrase sequence coverage by MS^2 ETD and UVPD activation on a Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer. Experimental conditions are 100 µScans, 120k OT resolution, 1e6 precursor target, and 5e6 ETD reagent target.



RESULTS

Examination of MS^2 Spectra Reveals High Complexity

The challenge presented to charge assignment algorithms in top-down MS^2 spectra is highlighted below in the zoom portion of Figure 2. One can see that the large number of accessible dissociation channels in conjunction with the tendency of fragment ions to cluster near each other due to a roughly uniform charge distribution on the protein, leads to very rich regions in the product ion spectra.

Figure 2. Carbonic Anhydrase [M+34H]$^{34+}$ (854 m/z) MS^2 ETD spectrum with zoom region showing high complexity and many overlapping and unresolved isotopic distributions in the region near the precursor. Experimental conditions are 2 msec activation time, 1e6 precursor target, 5e6 reagent target, 100 µScans, 120k OT resolution.

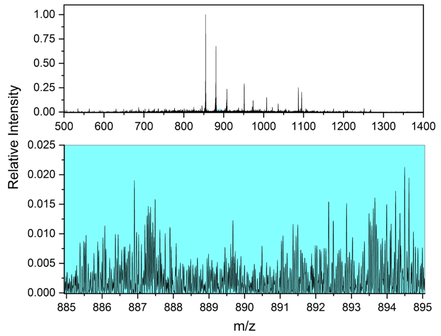


Figure 3. Pseudo MS^3 spectrum of the 10 Th window shown in Figure 2, demonstrating that the S/N ratio in the isolated region is approximately 100.

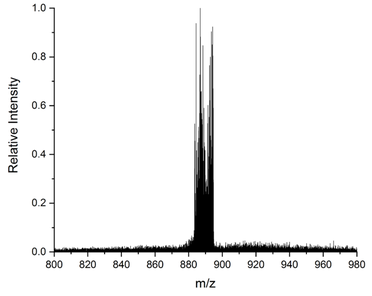
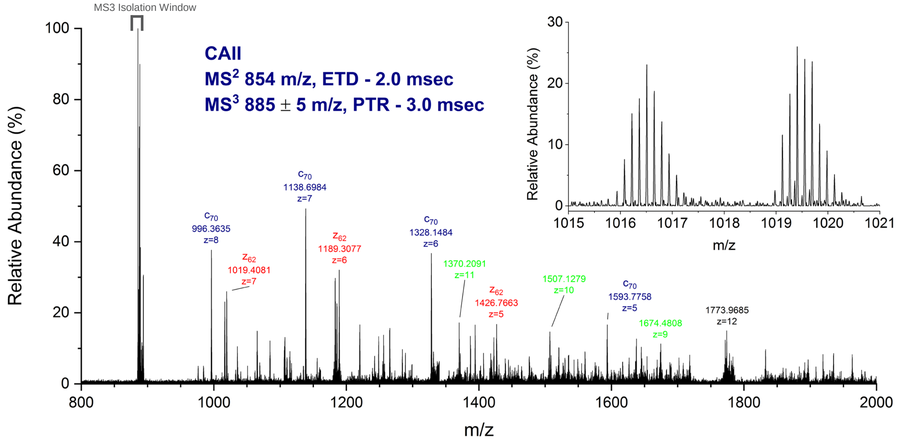


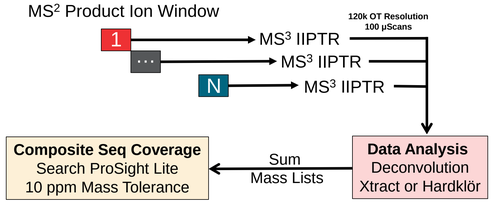
Figure 4. Carbonic Anhydrase IIPT MS^3 spectrum of a 10 Th isolation window of ETD MS^2 product ions. The reduction in spectral peak density resulting from the distribution of the product ion population over a wide m/z range leads to isotopically resolved clusters and easy charge state assignment.



The MS^3 Experiment - Concept

The experimental concept examines the change in top-down sequence coverage resulting from subjecting multiple windows of the MS^2 product ion population to IIPT reactions in a manner described by Figure 5. The number of MS^2 product ion windows expanded and their respective widths are varied to find conditions where the spectral peak density is sufficiently reduced to allow for sequence ion assignment.

Figure 5. Experimental workflow.



Composite Sequence Coverage - Results

Figures 6 and 7, and Table 1, show the composite sequence coverage obtained by summing together the mass lists acquired by expanding individual MS^2 product ion windows using IIPT reactions. In all cases the sequence coverage and the number of assignable fragments are increased relative to the MS^2 only experiment.

Figure 6. Carbonic Anhydrase ETD composite sequence coverage obtained by summing together 21, 10 Th MS^2 product ion windows exposed to IIPTR. 68% sequence coverage and 700 fragments identified.

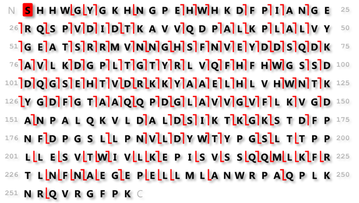


Figure 6. Carbonic Anhydrase UVPD composite sequence coverage obtained by summing together 21, 10 Th MS^2 product ion windows subjected to IIPTR. 59% sequence coverage and 464 fragments identified.

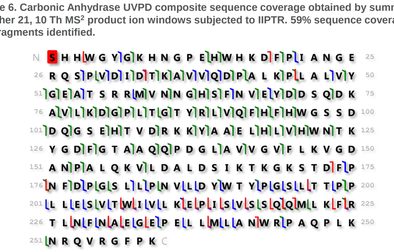


Table 1. Carbonic Anhydrase sequence coverage as a function of the number and width of the MS^2 product ion windows interrogated by IIPT reactions.

ETD (msec)	PTR (msec)	Num MS^3 Windows	Win Width	MS^3 Iso MR	Sequence Coverage (%)	Num Assigned Fragments
3	MS^2 Only	0	0	0	54	157
2	3	21	10	750-950	63	300
0.4, 1, 2, 3	3	21	10	750-950	75	933
3	15	1	1000	500-1500	62	180
3	10	2	500	500-1500	70	248
3	10	4	250	500-1500	70	345

CONCLUSIONS

- IIPT reactions increase the spectral peak capacity by decreasing isotopic cluster overlap
- Increased attainable sequence coverage on top down standards by ~ 30%
- For optimal gains, IIPT reactions are best done on multiple segments of the MS^2 product ion population, the width of which will depend on the MS^2 spectral peak density.

REFERENCES

1. Anderson LC, English MA, Wang WH, et. al. "Protein derivatization and sequential ion/ion reactions to enhance sequence coverage produced by electron transfer dissociation mass spectrometry" *IJMS* 2015;377:717-624.
2. Hoopman MR, Finney GL, and MacCoss MJ. "High-speed data reduction, feature detection, and MS/MS spectrum quality assessment of shotgun proteomics data sets using high-resolution mass spectrometry" *Anal Chem* 2007:Aug 1;79(15):5620-5632.

TRADEMARKS/LICENSING

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