High Sensitivity PTM Characterization in Complex Cell Lysates Using Trapped Ion Mobility

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Introduction

Post-translational modification of proteins represents an essential mechanism that regulates the function and abundance of proteins and is critical to a wide variety of cell processes such as signal transduction, cell development and mitosis. Post-translationally modified peptides are often present in low abundance and many are isobaric, differing only by the site of the modification and hence represent a significant analytical challenge. Here we investigate the use of trapped ion mobility (TIMS) for the identification and characterization of tyrosine phosphorylation, lysine acetylation, and lysine ubiquitination (K-ε-GG) on peptides immunoenriched from mouse tissue.

Methods

Post-translationally modified peptides were prepared from mouse liver protein tryptic digests using three different PTMScan® kits (Cell Signaling Technology), each enriching a specific class of PTM.

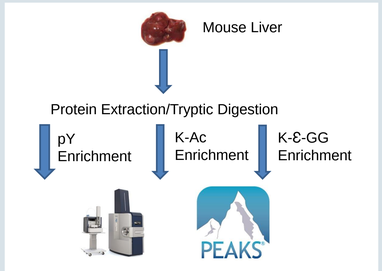

Fig. 1. Mouse Liver protein tryptic digests enriched for three different PTMS. The resulting samples were analyzed on the timsTOF Pro. Data were processed with PEAKS X

Initial Sample Amount	Unique pY Peptides	pY Peptide PSMs	Unique K-Ac Peptides	K-Ac Peptides PSMs	Unique K-ε-GG Peptides	K-ε-GG Peptide PSMs
150 ug	63	164	3465	6178	517	1589
1.5 mg	363	894	7075	16973	2783	5912
15 mg	782	3094	8804	24613	7199	16476

Fig. 2. High sensitivity PTM identification of three different post translational modifications from mouse tissue obtained from three different initial sample amounts.

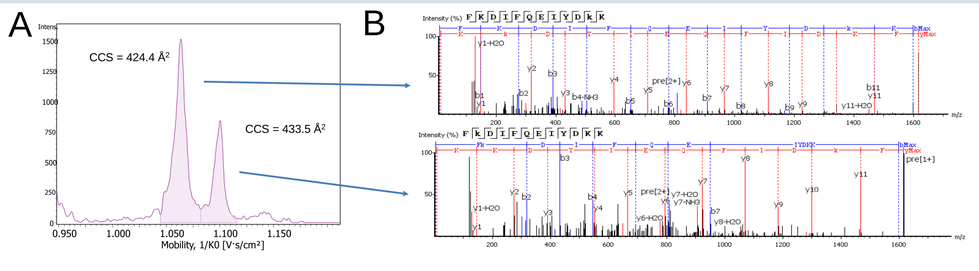

Fig. 3. High resolution TIMS resolves coeluting, isobaric peptides differing only by the site of modification. A) Extracted Ion Mobilogram for 951.537 m/z at retention time 43.01 min shows clearly resolved isomers and corresponding CCS values. Each results in a unique MSMS spectrum. B) MSMS spectrum matching peptide FKDIFQEITDK(42.02)K (upper spectrum) and FK(42.02)DIFQEITDKK (lower spectrum). Each peptide differing only by the site of acetylation. Resolving power approx. 120.

Phosphotyrosine (PTMScan® Phospho-Tyrosine Rabbit mAb (P-Tyr-1000) Kit #8803), acetyl-lysine(PTMScan® Acetyl-Lysine Motif [Ac-K] Kit #13416), and the ubiquitin remnant K-ε-GG (PTMScan® Ubiquitin Remnant Motif (K-ε-GG) Kit #5562).

The resulting extracts were separated by nano HPLC (nanoElute, Bruker) on a 250 mm x 75 µm, 1.6 µm column (IonOpticks, Australia). 50 min gradients at 400nL/min were analyzed on a trapped ion mobility Q-TOF (timsTOF Pro, Bruker Daltonics) operating in PASEF mode. 200ms of ions were accumulated in the first TIMS analyzer and eluted based on their mobility in the second analyzer using 200 ms ramp time. Data were processed in PEAKS X software (Bioinformatics Solutions Inc).

Results

Sensitivity

PTMScan® antibody enrichment kits (CST) successfully enriched phosphotyrosine, acetylated lysine and lysine ubiquitinated peptides. The increased peak capacity from the extra dimension of separation provided by TIMS and increased sequencing speed of the PASEF method enables very large numbers of PTM identifications from small sample amounts. This enabled 164 pY, 1589 K-ε-GG, and 6178 K-Ac modified peptides to be identified from only 150 ug of initial material (Figure 2). With 15 mg of tissue the number of modified peptides increased to 3094, 16476 and 24613 peptides respectively.

Isobaric Modification Separation

The very high mobility resolution of the TIMS analyzer on the timsTOF Pro enabled separation of coeluting, isobaric peptides differing only by the position of the PTM on the peptide (Figure 3). This is achieved while maintaining high sensitivity and a very high duty cycle. Allowing isobaric peptide separation in complex samples.

Additionally, relative quantitative information can be obtained from the area under the mobility peak for each positional isomer. In the example in Figure 3 the peptide with the acetylation on residue 11 is 1.8 times higher in terms of peak area than the same peptide with the acetylation on residue 2. As Trapped Ion Mobility also provides true, reproducible CCS values this provides an additional identification metric when comparing results between samples.

Conclusions

- PTMScan antibody enrichment provides very efficient, focused enrichment of modified peptides from tissue samples.
- The outstanding peak capacity and sensitivity of the timsTOF Pro make it very well suited for the identification of low abundance post translational modifications from small sample amounts.
- High resolution TIMS allows localization of PTMs on co-eluting, isobaric peptides. True CCS values provide additional identification metric across samples.

timsTOF Pro