Discovery phosphoproteomics using electron-activated dissociation and high sensitivity on a novel QTOF system

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ABSTRACT

- Phosphorylation is a critical post-translational modification whose measurement is critical for thorough understanding of biological signaling.
- Fast and accurate detection of phosphorylation on a proteome-wide scale is challenging with existing technology.
- Efficient electron-based fragmentation on an LC-timescale is needed.
- A novel QTOF system is evaluated and compared to existing technologies.

INTRODUCTION

While collision induced dissociation (CID) is fast and efficient, electron-based dissociation produces phosphopeptide fragments that retain CID-labile phosphorylation modifications. Unfortunately, traditional electron-based dissociation is both inefficient, resulting in unfragmented or charge-reduced precursor ions, and slow, requiring tens of milliseconds of reaction time. Thus, discovery phosphoproteomics experiments using LC separation have relied on the faster, sometimes less informative CID. A new QTOF platform combines an electron activated dissociation (EAD) reaction cell and a Zeno trap for increased MS/MS sensitivity. This EAD cell produces efficient fragmentation with reaction time of 10-20 milliseconds, enabling data dependent acquisition on an LC timescale. Meanwhile, the Zeno trap increases duty cycle of the ions from 5-25% to more than 90% in the orthogonal extraction region of the QqTOF instrument.

MATERIALS AND METHODS

Sample preparation:
Stable IMR90 human fibroblast that express the inducible ER:RASG12V upon (Z)-4-hydroxytamoxifen (4-OHT), were treated with 100 nM 4-OHT to induce senescence and cultured in a low oxygen incubator at 7.5% (v/v) CO2 and 3% (V/V) O2 for six days before harvesting. Control cells were treated with methanol. Nuclei were purified using a two-step process: nuclear extraction by sonication in HEPES/KOH buffer followed by the lysis of the isolated nuclei. Proteins were digested using trypsin/LysC proteases and phosphopeptides are enriched using the polymer-based metal-ion affinity capture (PolyMAC) spin tip (Tymora Analytical, USA).

LC-MS/MS conditions:
Microflow chromatography was performed using a Waters ACQUITY UPLC M-class system in trap-elute mode with Phenomenex Kinetex 2.6 mm XB-C18 100 Å, 150 x 0.3 µm LC column at 30 ºC and a Phenomenex micro trap column. A 45 min gradient was used with an analytical flow rate of 5 µL/min for all experiments. Samples were trapped for 3 min at 10 µL/min, 100% mobile phase A. All mass spectrometry data was acquired using the ZenoTOF 7600 system (SCIEX). Data was acquired in data dependent mode for peptide workflows using either collision-induced activation or electron-activated dissociation.[1] Zeno trap was activated in all DDA experiments. For CID acquisition, an MS/MS accumulation time of 20 ms was used along with dynamic collision energy implemented for fragmentation. For EAD, a reaction time of 20 ms was used in combination with an MS/MS accumulation time of 50 ms. The electron beam current was set to 3000 nA with 0 eV used for the kinetic energy.

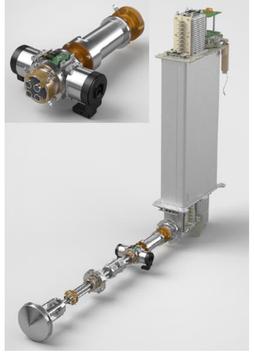


Figure 1. Ion optics in the ZenoTOF 7600 system highlighting the EAD and CID cells (inset)

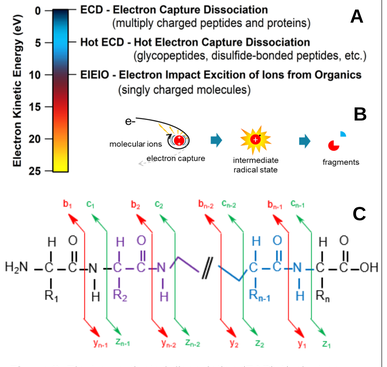


Figure 2. Electron activated dissociation (EAD) A) electron kinetic energy levels, B) mechanism, and C) peptide fragmentation nomenclature.

RESULTS

Tryptic peptides from non-enriched samples were identified using either CID or EAD for fragmentation (Figure 3), resulting in about 39% identified by both approaches. Due to the higher frequency of MS/MS sampling using CID (~50 Hz), the overall number of unique peptide identifications was about 2-fold greater than when using EAD (~20 Hz).

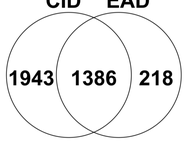


Figure 3. Venn diagrams for global peptide ID for non-enriched D6-MeOH IMR90 and D6-4OHT IMR90 cells detected by CID vs EAD.

Phosphopeptide-enriched samples of both control and treated samples were analyzed by LC-MS/MS using either CID or EAD as shown in Figures 5 and 6. While CID produced the larger number of phosphopeptide identifications, the localization of the phosphorylation site is more confident based on the EAD data.

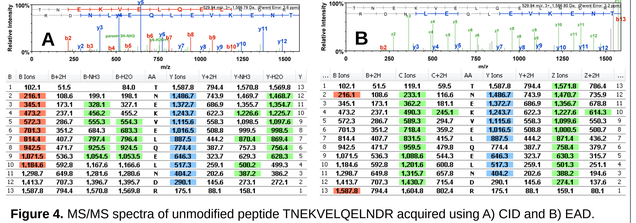
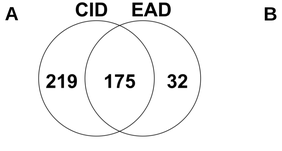


Figure 4. MS/MS spectra of unmodified peptide TNEKVELQELNDR acquired using A) CID and B) EAD.



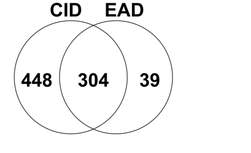


Figure 5. Venn diagrams for phosphopeptide ID for phosphopeptide-enriched A) D6-MeOH IMR90 and B) D6-4OHT IMR90 cells detected by CID vs EAD.

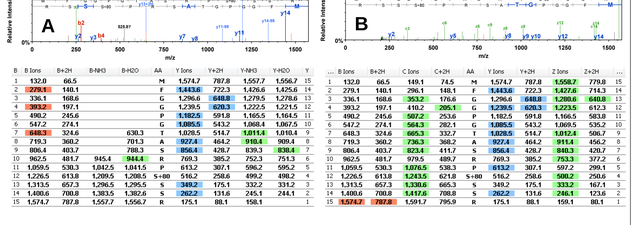


Figure 6. MS/MS spectra of phosphopeptide MFGGPGTASRPpSSSR acquired using A) CID and B) EAD. EAD fragmentation provided more complete coverage across the peptide sequence including the phosphorylation site providing more confident site assignment.

Consistent with the global peptide results, phosphopeptide identifications from enriched sample were about 2-fold higher for CID compared to EAD (Figure 5). Nevertheless, confident localization of phosphorylation site was more robust using EAD as illustrated in Figure 6.

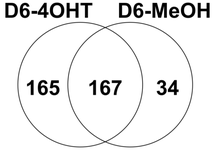


Figure 7. Venn diagram of total unique EAD phosphopeptide identifications from treated (D6-4OHT) vs control (D6-MeOH) cells.

Accession	Protein name
RU17_HUMAN	U1 small nuclear ribonucleoprotein 70kDa
SNIP1_HUMAN	Smad nuclear-interacting protein 1
NFIC_HUMAN	Nuclear factor 1 C-type
NU214_HUMAN	Nuclear pore complex protein Nup214
NOP58_HUMAN	Nucleolar protein 58
TPR_HUMAN	Nucleoprotein TPR

Table 1. Unique nuclear proteins found only in treated (D6-4OHT) cells.

A number of phosphorylated proteins were identified uniquely by EAD in the treated (D6-4OHT) cells (Table 1). These initial results provide potential targets for further investigation into their role in oncogene induced senescence.

CONCLUSIONS

Phosphorylation is a key post-translational modification for understanding of biological signaling and plays important role in cellular senescence. Precise mapping of site-specific phosphorylation is the key to understand biology of senescence. As demonstrated in this study, phosphopeptides can now be monitored using fast and efficient electron-based fragmentation. While not as fast for global proteome discovery, electron-based fragmentation on an LC-timescale is demonstrated. Based on these initial experiments, EAD provides complementary information to CID for phosphopeptide identification and potentially more robust localization information.

REFERENCES

1 T. Baba *et al. Anal.Chem*. 2014

TRADEMARKS/LICENSING