APPLICATION NOTE FOR THERMO SCIENTIFIC QE ORBITRAP™ MS

Localization of Multiple Phosphorylation Sites in the case of αS-Casein

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Introduction

Casein phosphorylation is a post-translational modification (PTM) catalyzed by kinase enzymes when phosphate groups are attached to specific amino acids[1]. The labile nature of phosphorylation modifications makes it very difficult to study them with traditional tandem mass spectrometry (MS/MS) techniques using collision-induced dissociation (CID). Electron capture dissociation (ECD) is known as a soft MS/MS technique with ability to preserve labile PTM during fragmentation. The present work shows using ECD method for localization of phosphorylation in the case of αS-casein.

Experimental

Sample preparation

Phosphorylated αS-casein from bovine milk was bought from Sigma-Aldrich and used without further purification. The αS-casein sample of 1 µM concentration was dissolved in a 1:1 (v:v) water-methanol mixture with 0.1% concentration of formic acid.

Instrumentation

ECD spectra of the αS-casein were obtained with an ExD cell (e-MSion, Inc.)[2] installed on a Thermo Scientific Q Exactive Orbitrap mass spectrometer (Figure 1). The sample was introduced through direct infusion at a flow rate of 5 µL per minute. The autotune feature in the ExDControl software (e-MSion, Inc.) was used to optimize the ExD cell voltage profiles for transmission and for ECD fragmentation, both with the filament heating current set to 2.2 A. To tune for transmission, Thermo-Fisher calibrant solution was used, whereas tuning for ECD the peptide standard Substance P and bovine ubiquitin were used. To preserve phosphorylation in the α-casein sample, ECD spectra with low HCD supplemental activation (1 and 5 V) were recorded. To analyze the EChcD spectra, Viewer software (e-MSion, Inc.) was used at 10 ppm tolerance for masses.

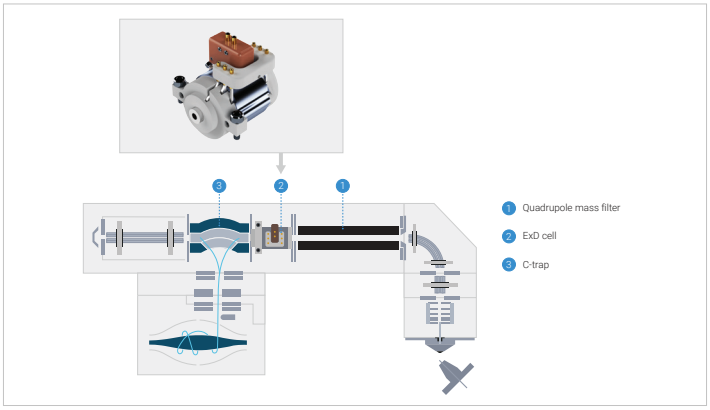

Figure 1. Schematic of the ExD cell in the Thermo Scientific Q Exactive Orbitrap mass spectrometer.

Results and Discussion

Figure 2 shows the ECD spectrum of α-casein obtained with HCD supplemental activation 5 V. All eighth phosphate groups attached to some serine residues were identified in the spectrum (see some of them in Figure 3) with total sequence coverage 95% as was determined by Viewer (Figure 4).

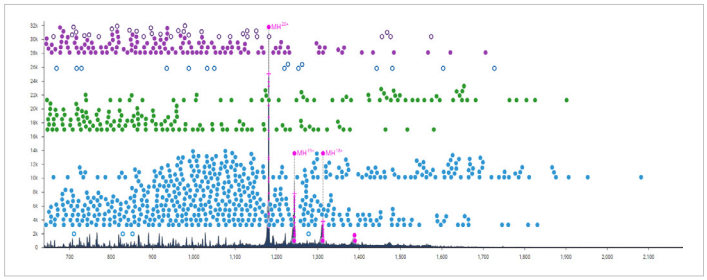

Figure 2. EChcD spectrum of α-casein for 20+ charge state precursor. Blue filled circles show ECD-type *c*- and *z*-fragments, green filled circles are CID-type *b*- and *y*-fragments, purple filled circles are *a*-ions, open circles are secondary *d*- and *w*-ions.

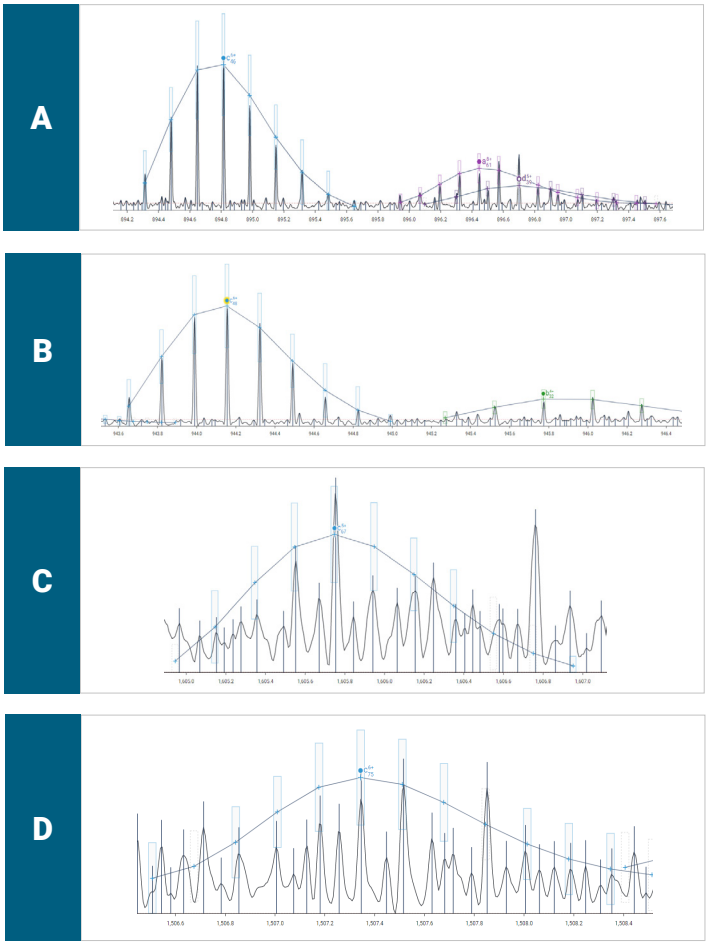

Figure 3. Some ECD-fragments with phosphoserines: c_{46}^{6+} (A), c_{48}^{6+} (B), c_{67}^{5+} (C), c_{75}^{6+} (D).

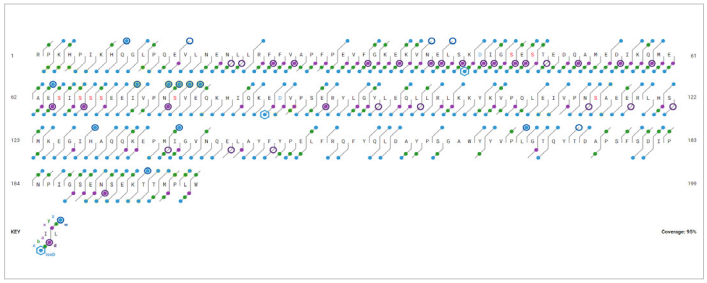

Figure 4. Sequence coverage determined by Viewer from EChcD spectrum of αS-casein in Figure 2. Serines with phosphate groups are shown in red.

References

1. Bijl, E., van Valenberg, H.J.F., Huppertz, T., van Hooijdonk, A.C.M., Bovenhuis, H. (2014) Phosphorylation of aS1-casein is regulated by different genes, J. *Dairy Sci. 97* (11), 7240-6.
2. Shaw, J. B.; Malhan, N.; Vasil'ev, Y. V.; Lopez, N. I.; Makarov, A.; Beckman, J. S.; Voinov, V. G. Sequencing Grade Tandem Mass Spectrometry for Top–Down Proteomics Using Hybrid Electron Capture Dissociation Methods in a Benchtop Orbitrap Mass Spectrometer. *Anal. Chem.* **2018**, *90* (18), 10819–10827. https://doi.org/10.1021/acs.analchem.8b01901.

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MARK-Th-007 • R001 • June 2022