APPLICATION NOTE FOR THERMO SCIENTIFIC QE ORBITRAP™ MS

Localization of the Heme groups in proteins

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Introduction

Cytochrome C and myoglobin are proteins that participate in O_2 reduction and dioxygen storage in living organisms, including humans.[1] Both these proteins have the heme group in their structures. Tandem mass spectrometry can be used for the localization of the heme group in similar proteins. For some heme-containing proteins, conventional collisional activation might be too hard method for this task due to easy loss of the heme group. In this work, we show using electron capture dissociation (ECD) method with supplemental activation (EChcD) for localization of the heme group in cytochrome c and myoglobin. The method used here can be used for studying other heme-containing proteins or peptides.

Experimental

Sample preparation

Samples cytochrome c and myoglobin were bought from Sigma-Aldrich and used without further purification. The both samples of 1 µM concentration were dissolved either in a 1:1 (v:v) water-acetonitrile mixture with 0.1% concentration (denatured solution) of formic acid or in water solution of 100 mM ammonium acetate (native solution).

Instrumentation

ECD spectra of both samples were obtained with an ExD cell (e-MSion, Inc.)[2] installed on a Thermo Scientific Q Exactive Orbitrap mass spectrometer (Figure 1). The native solutions of the samples were introduced via custom-made nanospray tip (1.5 micron ID; static nanospray), whereas denatured solutions were 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 analyze the EChcD spectra, Viewer software (e-MSion, Inc.) was used at 10 ppm tolerance for masses.

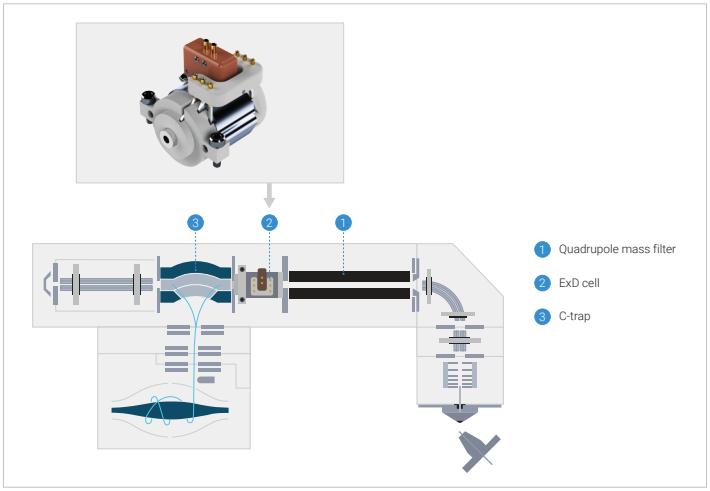

Figure 1. Schematic of the ExD cell in the Thermo Scientific Q Exactive Orbitrap mass spectrometer.

Results and Discussion

Figure 2 shows the ECD spectrum of cytochrome c from denatured solution obtained with HCD supplemental activation 20 V. Rather abundant peak of protonated heme was observed at these experimental conditions. Working with 7+ precursor from native solution and similar supplemental activation (HCD = 20 V) practically did not show loss of the heme group (Figure 3). Both spectra did not produce any fragment ions within cytochrome c structure associated with the heme group (Figure 4). Being connected to two cystines, the heme group prevents fragmentation between two cysteine residues.

Figure 5 shows ECD spectrum of 9+ precursor of myoglobin from native solution. In contrast to EChcD spectrum of native cytochrome c (Figure 3), the same supplemental activation HCD = 20 V applied for the 9+ myoglobin precursor produces quite visible peak of protonated heme (Figure 5). In myoglobin, the heme group is attached to a single histidine residue and its loss is much easier to realize. Nevertheless, the localization of the heme group was nicely confirmed via observation of both *c*- and *z*-ECD fragment ions around position of the heme group (Figure 6).

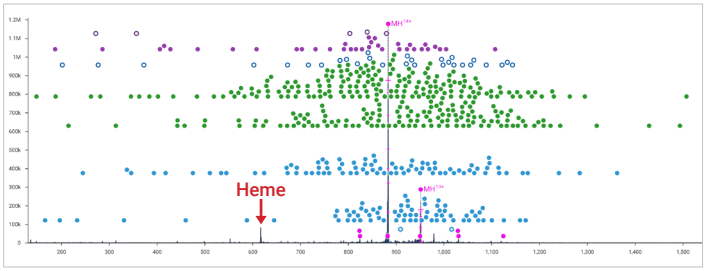

Figure 2. EChcD spectrum of Cytochrome C for 14+ charge state precursor. Blue filled circles show ECD-type *c*- and *z*-fragments, green filled circles are CID-type *b*- and *y*-fragments, purple filled circles are *a*-ions, open circles are secondary *d*- and *w*-ions.

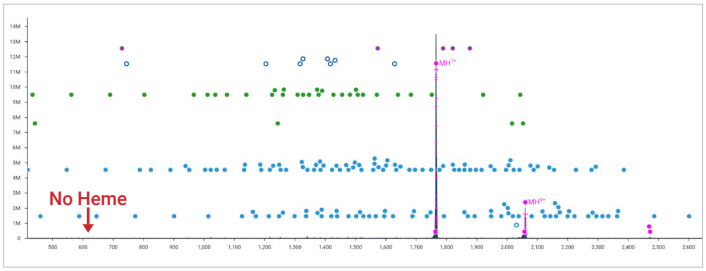

Figure 3. EChcD spectrum of Cytochrome C for 7+ charge state precursor. Blue filled circles show ECD-type *c*- and *z*-fragments, green filled circles are CID-type *b*- and *y*-fragments, purple filled circles are *a*-ions, open circles are secondary *d*- and *w*-ions.

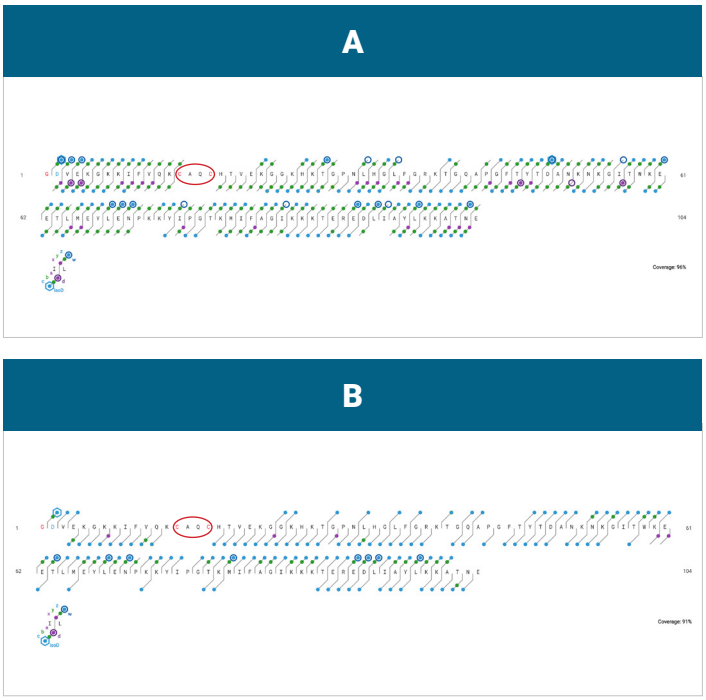

Figure 4. Sequence coverage determined by Viewer from EChcD spectrum of cytochrome C from 14+ precursor (A) and 7+ precursor (B). Red circles show positions for the heme group in cytochrome c.

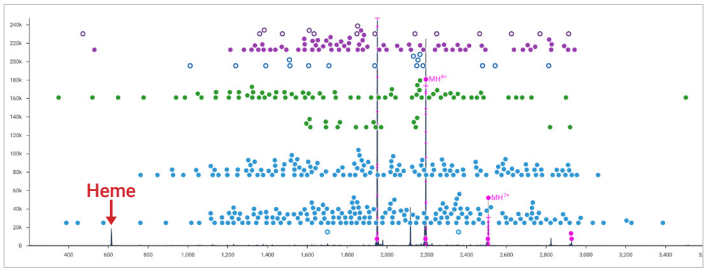

Figure 5. EChcD spectrum of myoglobin for 9+ charge state precursor. Blue filled circles show ECD-type *c*- and *z*-fragments, green filled circles are CID-type *b*- and *y*-fragments, purple filled circles are *a*-ions, open circles are secondary *d*- and *w*-ions.

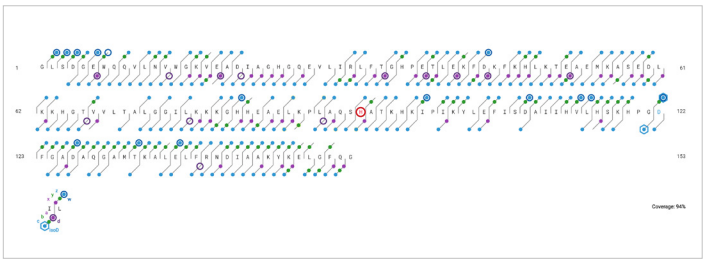

Figure 6. Sequence coverage determined by Viewer from EChcD spectrum of myoglobin in Figure 5. Red circle shows position of histidine residue where the heme group is attached.

References

1. Collman, J.P., Boulatov, R., Sunderland, C.J., Fu, L. (2004) Functional analogues of cytochrome c oxidase, myoglobin and hemoglobin, *Chem*. Rev. 104, 561-588.
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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