Comparison of Gas Phase Stability Diagrams of Fish Parvalbumins Using Different Dissociation Techniques

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ABSTRACT

IgE-mediated reactions to fish allergens represent the most frequent cause of food allergy. Fish parvalbumins are very abundant in fish tissue and are the major fish allergen that is contain in the sarcoplasmatic reticulum. These proteins share physicochemical properties that distinguish them from nonallergens, Among those properties, these proteins are usually thermostable, resistant to low pH, to the activity of proteases, etc. All together translates in sufficient gastric stability to reach the intestinal mucosa where absorption and sensitization (development of atopy) can occur. Similar properties are shown in the gas phase when these proteins are ionized their stability makes them very difficult to fragment and to provide proper sequence coverage. Here, we explore their fragmentation patterns and their resistance to be broken down in the gas phase. To this end, a modified Orbitrap Fusion Tribrid mass spectrometer capable of using five different dissociation techniques was used and applied to the characterization of three different fish species that cause allergic reactions of different severities in fish-allergic individuals. Sequence coverage obtained from the MS/MS spectra of the purified proteins showed an agreement with their overall secondary structure contents. In the case, of salmon parvalbumins, one of the most allergenic proteins in fish, very poor fragmentation spectra were obtained when HCD, ETD, EThCD were used. Howeverm UVPD was able to provide a proper MS/MS spectra at 40 milliseconds activation time.

MATERIALS AND METHODS

Reference samples from commercial fish species were included in the work. Protein extraction was carried out by mechanically homogenizing 1 g of muscle. Water soluble proteins were centrifuged, the supernatant heated at 70 ºC for 5 min and centrifuged again. Soluble proteins were diluted 1:1000 with 0.1 formic acid in water and the directed injected into an LC-MS system. Samples were injected directly via autosampler into the MAbPac™ RP 2.1X100mm column hyphenated to a Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™. The mass spectrometer acquisition consisted on a Parallel Reaction Monitoring (PRM) method consisting of targeting a specific proteoform either several charge states or a single charge state. MS/MS acquisition was performed using ETD, EThcD, HCD or UVPD fragmentation at 120K@m/z 200. Raw files were analyzed using Proteome DiscovererTM 2.2 using the ProsightPDTM and Protein CenterTM nodes. A fish database was created containing the major parvalbumin protein sequences.

WORKFLOW

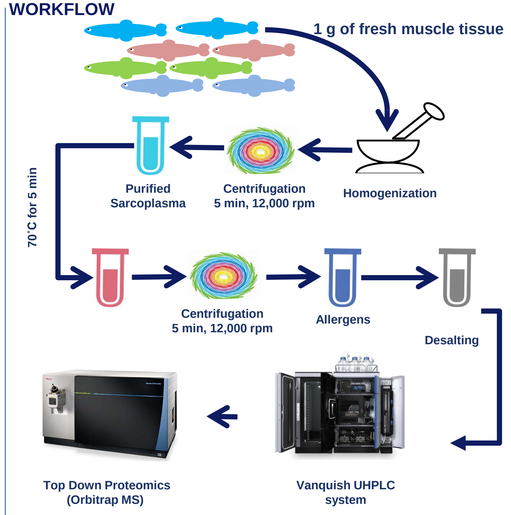

FIGURE 1. General overview of the analytical workflow with fish muscle samples. The thermo-stable proteins, β-parvalbumins are separated by LC before top-down MS.

RESULTS

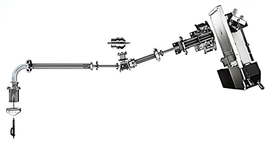

FIGURE 2. Schematic of different hardware parts including UVPD source (far right) on the Orbitrap Fusion Lumos

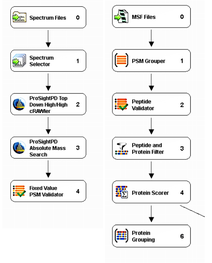

FIGURE 3. Processing and Consensus workflows for top-down proteomics analysis in Proteome Discoverer™ 2.2 using ProsightPD™ node.

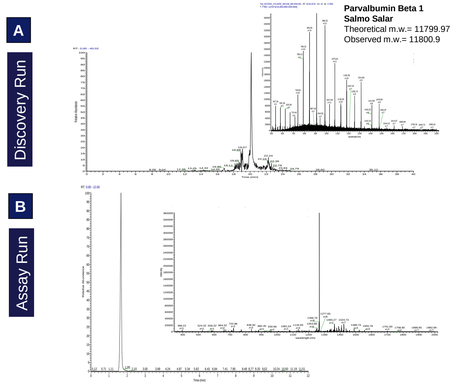

FIGURE 4. Analytical Strategy for Parallel Reaction Monitoring with Orbitrap Fusion Lumos Tribrid MS. First, a discovery LC-MS run allowed us to identify the target proteoforms (A). A target assay is built (B)

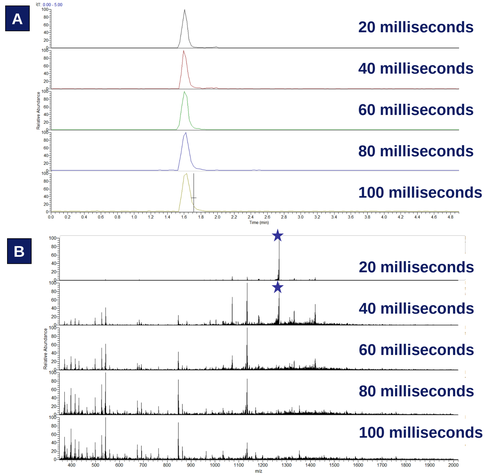

FIGURE 6. (A) Representative chromatogram for 5 different PRM experiments using purified parvalbumins from salmo salar. Activation time was sequentially increased to find the optimum fragmentation spectra. The target ion was a charge +9 at m/z 1268 (B) Representative spectra for each of the different activation energies. Star indicates precursor ion.

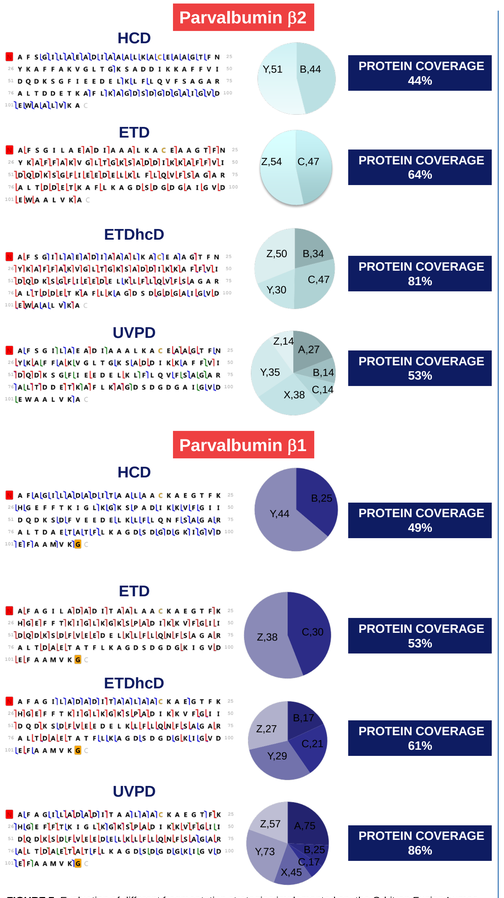

FIGURE 7. Evaluation of different fragmentation strategies implemented on the Orbitrap Fusion Lumos Tribrid MS for research purposes. Parvalbumin β2 (top figure) or Parvalbumin β1 (bottom figure) were fragmented using HCD, ETD, ETDhcD and UVPD fragmentation modes/ Data was analyzed with Prosight Light at 10ppm mass tolerance. The candidate sequence was Merluccius Paradoxus (P86768) from UniProt.

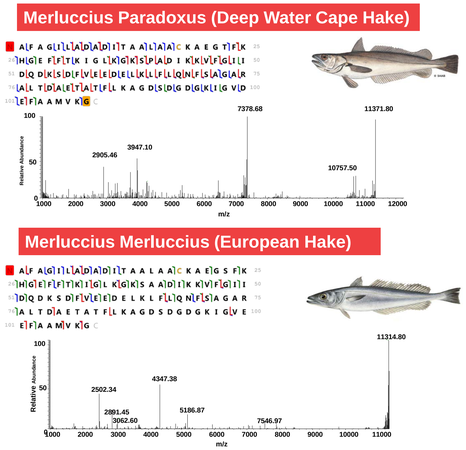

FIGURE 8. Characterization and identification of two Hake species with MS, Top Merluccius Paradoxus and bottom Merluccius Merluccius.

CONCLUSIONS

- Successfully purified fish allergens in minutes
- Parvalbumin protein isoforms were identified successfully using a combination of fragmentation techniques.
- Parvalbumin β1 and β2 were identified from the Hake fish Merluccius Paradoxus within 4.1 and 1.8 ppm mass difference. Difference protein sequence coverage was obtained for each of them, most likely due to the different structure that produces a variable allergenicity among Parvalbumin β1 and β2
- 81% protein coverage of Parvalbumin β2 was achieved by using ETDhcD fragmentation,
- 86% protein coverage using UVPD fragmentation in the case of Parvalbumin β1 from hake. .

REFERENCES

1. Carrera M., Canas B., Gallardo J.M., Journal of Proteomics, 2012, 75, 3211-3220

TRADEMARKS/LICENSING