Enhanced top-down MS/MS of small proteins using multiple precursor *m/z* isolation/fragmentation

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Novel Aspect: Simultaneous CID of multiple charge states for Top-Down afford increased sequence coverage

Introduction (109)

Top-down mass spectrometry is a fast and efficient method to analyze proteins for sequencing, variant identification and characterization of post-translational modifications. However, due to gas phase folding and charge localization, in order to obtain complete information it is often necessary to isolate and fragment more than one precursor charge state from the ESI-MS charge envelope. This adds time to the experiment and limits this approach for full automation; i.e. for top-down analyses on an LC-MS/MS time scale. Here we explore and present preliminary results featuring the unique multiple precursor *m/z* isolation MS/MS capabilities of the Q Exactive mass spectrometer (Thermo) for improved sequencing capability using biologically relevant target proteins.

Method (121)

Feasibility studies focused on sequencing hemoglobin variants from patient samples. Whole blood was diluted 1:250 in water and added in a 1: 1 ratio to acetonitrile containing 0.2% formic acid. Top-down analysis was performed on sickle cell (beta chain Glu6Val) hemoglobin. Beta chain charge states were fragmented by HCD in separate *m/z* windows and in a “multiplex” fashion with multiples of charge states via accumulation in the HCD collision cell and subsequent HCD fragmentation of these ions. Five different charge states of the beta chain were investigated for this purpose. Fragment ion mass spectra were deconvoluted using Xtract software (Thermo) and the resulting fragment masses were analyzed using BUPID-Topdown (Boston University Protein Identifier-Topdown), a custom-programmed software algorithm written in-house.

Preliminary results (276)

Our laboratory has a vested interest in developing top-down MS/MS as a high-throughput methodology to obtain 100% sequence coverage of proteins for the characterization of sequence variants, including those related to hemoglobinopathies, and PTMs, including those related to oxidative stress. While we have explored alternative means of fragmentation, CID remains the choice for top-down sequencing, albeit with its inherent limitations mentioned above. The Q Exactive mass spectrometer offers the possibility to perform automated collisional activation on multiple charge states of precursors at the same time thus hinting at the use of top-down for routine protein and PTM characterization. Our initial results indicated that from the charge

envelope of the intact form of the beta chain of human hemoglobin, five different charge states could be “multiplexed” or sequentially accumulated in the HCD collision cell to undergo simultaneous HCD. These summed ions MS/MS were observed to yield fragment ion mass spectra representative of all charge states HCD fragment ion tandem mass spectra obtained independently. The advantage of “multiplexing” the charged states was a considerable savings in time and the data obtained offered more complete sequence coverage than what could be obtained by using a single charge state. By using a mass selection window wide enough to accommodate the mass shifts accompanying most amino acid substitutions this approach could prove a very useful tool for the detection and characterization of protein variants such as in hemoglobinopathies and low mass shift PTMs observed on proteins subjected to oxidative stress.

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