Combining Bottom-Up Proteomics with High-Accuracy FTICR MS Intact Protein Mass Measurements for Intact Protein Analysis

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Bottom-up proteomics (analyzing peptides that result from protein digestion) is not ideally suited to the discovery of modified proteins. Top-down proteomics (subjecting intact proteins to gas-phase dissociation) is appropriate for the study of modified proteins, but under-sampling becomes an issue when combined on-line with separations. In this work, we describe the combination of bottom-up analyses with intact protein analyses for the characterization of modified proteins. Fractionation at the intact protein level was employed to reduce complexity. Bottom-up measurements were used to identify the subset of proteins that were present in each fraction; these identifications were then used in combination with high-accuracy Fourier-transform ion cyclotron resonance (FTICR)-mass spectrometry (MS) intact protein mass measurements to achieve protein and modified-protein identifications. The relative performance of size exclusion chromatography (SEC) fractionation in combination with on-line reversed-phase liquid chromatography (RPLC)-FTICR-MS was compared with the combination of RPLC fractionation with capillary isoelectric focusing (CIEF)-FTICR-MS.