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Top-down Sequencing of Glycoproteins

Sandrine Bourgoin-Voillard, Nancy Leymarie and Catherine E Costello
Center for Biomedical Mass Spectrometry, Departments of Biochemistry, Biophysics and Chemistry, Boston University School of Medicine, Boston, MA 02118-2646

The possibility for protein characterization using top-down approaches emerged with the advances of high resolution mass spectrometers and the diversity of activation modes available: collision-induced dissociation (CID), infrared-multiphoton dissociation (IRMPD) electron-capture dissociation (ECD) and electron-transfer dissociation (ETD). Nevertheless, the top-down approach is rarely used for glycoproteins and the region containing the glycan modification(s) has not been well defined. Hence, this work was undertaken to evaluate the capacities of the top-down approach to explore maximizing the depth of information on the glycoprotein RNase B: sequencing coverage, glycan structure, glycosylation site. The effect of the glycan on the protein fragmentation pattern was also investigated by comparing fragment patterns of RNase B, which contains 124 amino acids and has high mannose glycoforms ($GlcNAc_2Man_{5-9}$) at a single site (Asn34), and its corresponding non-glycosylated protein RNase A. The experiments were performed on a 12-T Qh/FTICR hybrid mass spectrometer (SolariX, Bruker) using both vibrational (CID/IRMPD) and radical activation (ECD/ETD) with/without pre- or post-activation (CID or IRMPD, respectively). The fragments generated by vibrational and radical modes on both the glycosylated and non-glycosylated proteins yielded complementary sequencing information and more extensive sequence coverage for the radical modes. An improvement of the sequence coverage was observed when a pre-dissociation-activation event (pre-$AIEXD_{(CID)}$) was used for radical activation modes. Therefore, the coverage efficiency of the activation modes used for RNase B was ranked as follows: pre-$AIECD_{(CID)}$ > ECD > pre-$AIETD_{(CID)}$ ≥ post-$AIECD_{(IRMPD)}$ > ETD > post-$AIETD_{(IRMPD)}$ >> CID > IRMPD. The same classification was observed for RNase A, except that the post-$AIECD_{(IRMPD)}$ activation mode yielded the highest sequence coverage. Overall, the several activation modes evaluated in this work yielded complementary sequence information and the combination of them made it possible to obtain final sequence coverages of 90% RNase A and and 86% for RNase B, while the maximum coverage obtained from one experiment was 71% and 69% for RNase A and B, respectively.

Our investigation on the glycoprotein showed also that both vibrational and radical activation modes mostly preserved the glycan attachment, and that the electron-based methods were more advantageous than CID. Comparison of the fragmentation patterns of the glycoprotein (RNase B) and its corresponding protein (RNase A) indicated that the presence of the glycan favored fragmentation around the glycosylation site, presumably since the presence of the glycan motif induces a higher degree of protonation at the Asn carbonyl site by modification of the three-dimensional structure or its proton affinity. These approaches are being extended to glycoproteins containing complex glycans.