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Implementation of in-source pH modulation for flexible top-down analysis

Top-down analysis of denatured, highly charged proteins often yields greater sequence coverage than native charge states. However, the abundance of individual precursors under denaturing conditions is limited because of signal dilution amongst a wide charge envelope. Further, the resulting MS/MS spectra are often highly congested, making it difficult to confidently assign fragmentation signals above noise levels. To address this, we utilized the Agilent dual AJS electrospray ion source to perform in-spray acid/base reactions, enabling precise control over charge distribution. We also explore how acidified nebulizer gas can be utilized to expand top-down capabilities for native proteins containing cofactors.

Charge reduction was achieved by infusing base into the reference nebulizer of the dual spray AJS source. Adjusting gas flow or base infusion rate enabled controlled shifts in charge state distributions. For example, denatured carbonic anhydrase shifted from a dominant 32+ to 22+ precursor, with a two-fold increase in intensity after reduction. Similar shifts were observed for aldolase (39kDa) and enolase (46kDa). These charge-reduced precursors were fragmented using electron capture dissociation in the Agilent ExD cell, resulting in improved sequence coverage. Aldolase increased from 38% to 46% and enolase increased from 23% to 39%. Ion mobility studies showed that charge-reduced precursors retained extended structures, however, folding/unfolding behavior was found to be protein size and charge-dependent. CIU was also used to study dynamic structural changes.

In-spray acidification involved introducing acidified gas into the main nebulizer, enabling online hydrolysis of cofactors such as heme in native myoglobin. This allowed simultaneous top-down fragmentation of both holo and apo forms. Fragment evidence for heme binding was visualized using ExDViewer, with top-scoring sequences aligning with known binding sites. Ion mobility revealed structural differences between holo and apo myoglobin. Combining these in-spray acid/base reactions with top-down mass spectrometry enhances protein characterization by providing complementary structural and sequence information.

User consent

yes

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