

II. Top-down ECD fragmentation - flexibility

ECD is an established tool for top-down sequencing and for determining post-translational modifications (PTMs). Proteins of MW up to 200 kDa and non-covalent protein–ligand complexes can be sequenced. Although the combination of ECD and FTICR MS is an appealing approach to study large protein complexes, only recently have results been obtained that show that ECD has the potential to fragment the protein complex without dissociating the subunits.

The high mass resolving power of the FTICR instrument can also improve the confidence in identifying fragment ions in complicated ion mixtures. For example, we reported that a large number of consecutive backbone cleavages occur for the 147 kDa yeast alcohol dehydrogenase (ADH) tetramer upon ECD in a 12 T FTICR mass spectrometer (Fig. 1).

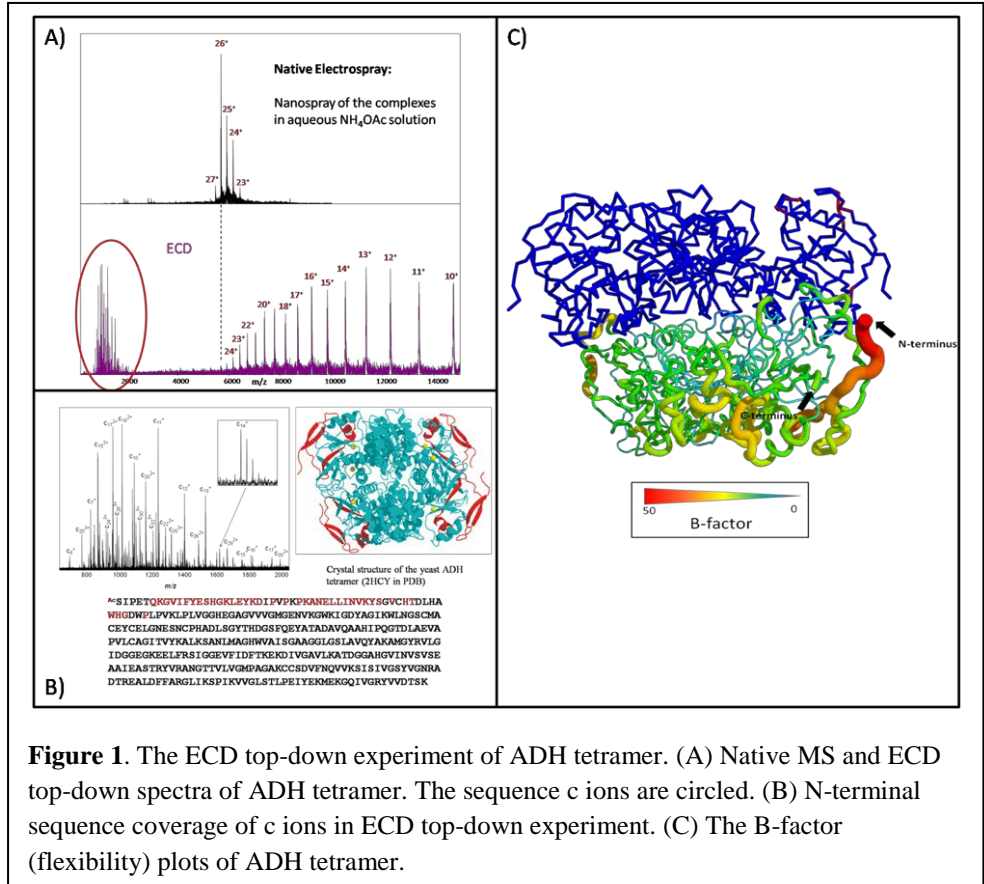


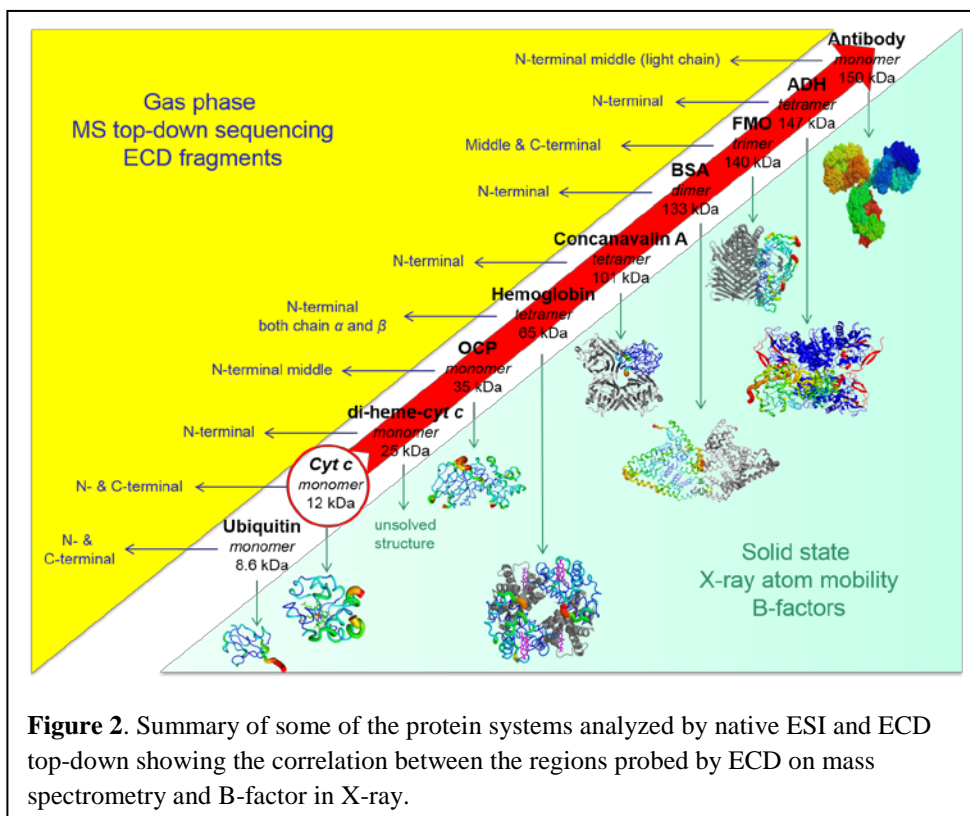
Figure 1. The ECD top-down experiment of ADH tetramer. (A) Native MS and ECD top-down spectra of ADH tetramer. The sequence c ions are circled. (B) N-terminal sequence coverage of c ions in ECD top-down experiment. (C) The B-factor (flexibility) plots of ADH tetramer.

yeast alcohol dehydrogenase (ADH) tetramer upon ECD in a 12 T FTICR mass spectrometer (Fig. 1). The ECD spectrum contains two sets of product ions: those fragment ions of m/z <2000 and the expected array of charge-reduced precursor ions at higher m/z than that of the precursor. Remarkably, no subunit dissociation was observed in the ECD top-down experiment (Fig. 1A). The intact ADH complex is presumably preserved in the gas phase by the many salt bridges that become stronger in the gas phase.

The low m/z fragment ions were searched against the database (NCBI) and 39 c-type ions up to the 55th residue from the N terminus of ADH and N-terminal acetylated serine were identified (Fig. 1B). In this case, fragment ions from the ECD top-down experiment are sufficient in number to identify the protein subunit. The ECD top-down experiment does not disrupt the ADH assembly but rather provides sequence information. Moreover, the origin of fragments is from a flexible region of the protein as determined by the B-factor—a measure of flexibility—in the X-ray crystal structure. The B-factor shows that the N-terminus is free and available for fragmentation, whereas the C terminus is buried (Fig. 1C). N-terminal residues,

up to 55th in the polypeptide sequence, are not involved in the interface of ADH complex and ready for ECD fragmentation. This trend is consistent based on protein and protein complexes summarized in Figure 2.

Two advantages make the dissociation methods attractive complements for native MS of protein complexes. First, the sequence identification of subunits from protein complexes may be obtainable using top-down approaches. In a native MS experiment, the MW of a complex is directly measured, but sequence identification still relies on traditional bottom-up proteomics, whereby the protein complex is denatured



and analyzed using a combination of SDS-PAGE, proteolytic digestion, and LCMS analysis to provide the sequence information. Thus, elucidating the nature and stoichiometry of a protein complex requires two independent experiments, native MS and bottom-up LCMS proteomics. The application of ECD (and possibly ETD) to the protein complex ion can generate sequence-specific fragmentation and allow integration of protein identification with native MS in a single experiment. The second advantage is that some structural analysis of the protein complex is achievable (e.g., location of flexible regions of the protein assembly). Other activation approaches (e.g., CID) identify subunit interactions among protein complexes by dissociating the protein complexes. Furthermore, by using Q-TOF and IM technology, the architecture of protein complexes can be established based on the dissociation pathways and from information from IM. ECD now joins the other dissociation methods, CID, BIRD, IRMPD and SID as reviewed above and in other publications, in providing new sources of information about protein complexes.

References

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