

Analytical Mass Spectrometry: Top Down MS/MS with Electron Capture Dissociation

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If the molecular weight from a DNA-predicted protein sequence differs from the measured mass value of the protein's molecular ions, posttranslational modifications and/or sequence errors are indicated. In the top down mass spectrometry approach, the measured molecular ion is dissociated, and these fragment masses are matched against those predicted from the protein sequence to restrict the locations of the modifications. Cleaving the ion's interresidue bonds on either side of the modified amino acid makes possible its exact location. For carbonic anhydrase (29 kDa), cleavages at 250 of its 258 interresidue locations have been achieved with the new technique of electron capture dissociation (ECD). "Plasma ECD", in which the electrons are introduced into the collision gas followed by the ions, has characterized 183 cleavages in a single carbonic anhydrase spectrum, clearly indicating two sequence errors in this long-studied protein. A P4H enzyme gave a MW value low by 135 Da; MS/MS clearly showed the absence of the predicted N-terminal Met (-131 Da) and located two S-S bonds (-2 Da each) between 4 of its 5 Cys residues.

The fact that ECD does not cleave tertiary noncovalent bonds has provided details of the unfolding and folding kinetics of gaseous cytochrome c and ubiquitin ions. The ECD data, combined with that from H/D exchange and collision cross sections, have lead to postulations of conformational structures for the 5+ to 13+ ubiquitin ions at 25 – 175 C. This approach appears promising also for the structural characterization of intermolecular noncovalent protein complexes in both solution and gas phases.
