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ELECTROSPRAY IONIZATION-MASS SPECTROMETRY AND TANDEM MASS SPECTROMETRY

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Electrospray ionization coupled with mass spectrometry (ESI-MS) has its roots 20 years ago with the pioneering experiments by Malcolm Dole(1) and has been recently rejuvenated by the work from the laboratory of John Fenn, who first published elegant results of electrospray ionization mass spectra of large molecules with molecular weights up to 40 kilodaltons and demonstrated the unusual propensity for multiple charging produced by this soft ionization technique(2). The ability to produce multiply charged molecular ions for biomolecules allows mass spectrometers to analyze compounds with molecular weights (MW) much in excess of the instrument's mass (m) limit, but within the mass-to-charge (m/z) range of most conventional mass spectrometers. In fact, proteins with molecular weights in excess of 130,000 daltons have been successfully analyzed by ESI-MS with a quadrupole mass spectrometer of limited m/z range (1700) in our laboratory.

The mechanism for desorption of multiply charged molecular ions from highly charged liquid droplets is presently unclear. However, an ion evaporation model in which Coulombic explosion of rapidly evaporating liquid droplets of high charge density results in smaller sized charged droplets and continues this sequence until solute field-assisted desorption occurs is generally accepted. For peptide and proteins, we had previously observed(3) a crude correlation between the maximum positive (multiply protonated) charge state observed in our electrospray ionization mass spectra with the total number of basic amino acid residues (i.e., arginine, lysine, and histidine) in the primary sequence and, to some extent, the N-terminal amino group. For example, glucagon (MW 3483) possesses 2 arginines, 1 lysine, 1 histidine, and the N-terminal amino group; its ESI mass spectrum shows the $(M + 5H)^{5+}$ as the highest multiply charged species. However, the correlation for hen egg white lysozyme, and protein with 18 basic amino acids, is poor; the $(M + 14H)^{14+}$ is the highest charge state molecular ion observed. Little information is known regarding the effect of tertiary structure (either solution phase or gas phase) on the multiple charging phenomenon of electrospray ionization.

We have compared the ESI mass spectra of large proteins with and without reduction of its disulfide bridges. By cleavage of cysteine-cysteine bonds and thereby affecting the tertiary structure of the protein, a dramatic increase in the number of positive charges is observed. An ESI mass spectrum of egg white lysozyme shows the $(M + 20H)^{20+}$ as the highest charged species with the addition of 1,4-dithiothreitol (DTT). Disulfide-cleavage reactions may allow the protein to relax into more extended conformations, allowing "buried" basic amino sites to be more fully protonated. Over 175 positive charges are resolved in the mass spectrum for the albumin dimer molecule with the addition of DTT, an increase of over 50 charges relative to the unreduced form.

Highly charged ions desorbed from the droplets are sampled through a nozzle-skimmer orifice to be detected by the quadrupole mass spectrometer. The typical applied nozzle voltage for positive ion analysis is +200V with the skimmer at ground potential. Solvent clustering with the analyte is substantially reduced by collisions within this region. Increasing collision energies also yields significant fragmentation of large peptide and proteins.

Singly and multiply charged sequence ions can be detected. Collisionally activated dissociation of selected multiply charged molecular ions for large peptides with MW > 3000 Da can easily be performed with a triple quadrupole MS to obtain structural information, with singly and multiply charged b and y type sequence ions generally dominating the spectra.

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