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NEW METHODS AND INSTRUMENTATION FOR THE CHARACTERIZATION OF BIOPOLYMERS USING ELECTROSPRAY IONIZATION-MASS SPECTROMETRY

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The technique of electrospray ionization (ESI) has significantly extended the ability to characterize large molecules by mass spectrometry. Proteins to at least 200,000 D can be transferred intact to the gas phase and molecular weights determined with precisions as high as 0.001% if individual charge states can be resolved. The ESI-MS can also serve as a near ideal interface and detector for capillary column separations (i.e., packed capillary HPLC and capillary electrophoresis), providing a basis for highly efficient sample utilization. Using capillary electrophoresis (CE)-MS, injection quantities in the low attomole (10^{-18} mole) range can be detected for smaller polypeptides using selected ion monitoring, and separation efficiencies as high as $5 \cdot 10^5$ theoretical plates have been realized. We have recently shown that the use of small 5 μm i.d. capillaries allows CE-MS with scanning detection for proteins for injection of 600 attomoles.

An important property of ESI-MS is its production of multiply charged molecules, a phenomenon which has the result of allowing the efficient collisional dissociation of very large molecular ions. Dissociation studies can be conducted at relatively high pressures (~ 700 torr), which we term thermally induced dissociation, in the interface (~1 torr), or in a low pressure collision cell (~10^{-3} torr ; i.e., conventional MS/MS). For example, it is possible to obtain sequence related information for proteins as large as albumin (~66 kD) from only a few picomoles of material using a triple quadrupole mass spectrometer. These techniques allow the extension of peptide sequencing for larger species than previously feasible and, in conjunction with the capillary electrophoresis or LC separation of enzymatic digests, may allow faster and more sensitive protein sequencing methods.

An important recent application of ESI-MS is for the identification and detection of specific noncovalent associations in solution. The preservation of such associations upon ESI and transfer to gas phase, requires gentle interface conditions. For noncovalently associated species, the interface conditions are generally selected to minimize heating and collisional activation, but still provide adequate desolvation. If the associated species have similar charge polarity, the greatest stability in the gas phase would be anticipated for low charge states (higher *m/z*). The careful application of these approaches demonstrates the unique capability of ESI-MS for the study of noncovalent complexes. Perhaps the most important and widely studied noncovalent complex from a biological viewpoint is that of double stranded DNA having the classical double helix structure. All previous attempts to observe this complex by ESI-MS (and other MS techniques) have failed. We have examined a range of such complexes using a quadrupole mass spectrometer an *m/z* range extending to 45,000. Figure 1 shows the low resolution mass spectrum obtained for a double stranded 20-mer duplex (D). The sequences for the complementary strands (I and II) are shown in the Figure along with the molecular weightsfor the individual oligonucleotides. The sequences were chosen to allow unambiguous determination of the specificity of complex (i.e., nonspecific dimers of the same strands would be easily resolved). At higher *m/z* (>2000) only the duplex species consisting of the complementary oligonucleotides is observed.

High resolution is often necessary to realize high mass measurement accuracy for large biopolymers, due to contributions arising from sample impurities or dissociation. We have recently developed a new (FT-MS) instrument designed to realize both high sensitivity and high resolution. Figure 2 shows an initial result from this instrument, demonstrating both the high resolution resolution and useful dynamic range obtainable. Initial results have obtained resolutions of >100,000 for myoglobin with mass measurement accuracies of <2 ppm from subpicomole quantities of protein.

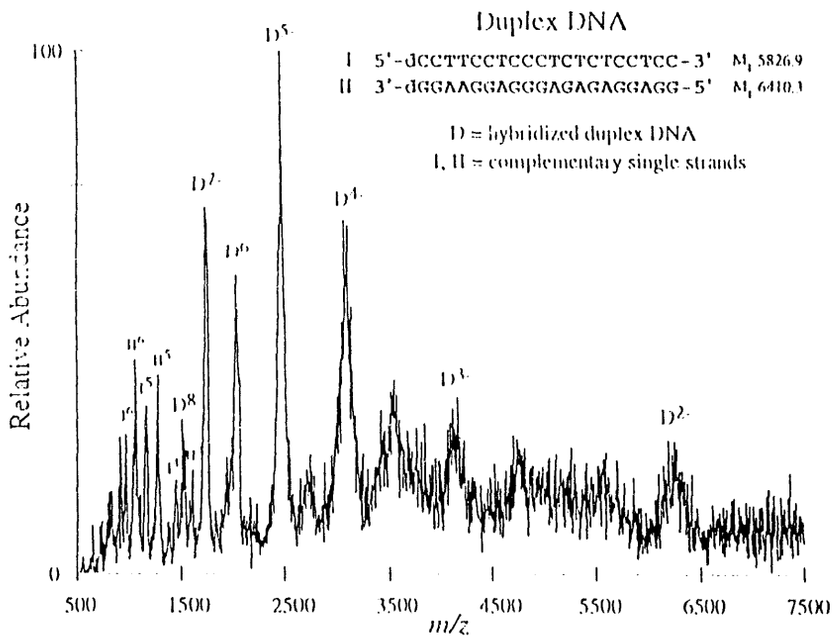

Figure 1. Low resolution ESI-Mass Spectrum obtained with an extended range quadrupole instrument for a double stranded oligonucleotide (sequences noted). At low *m/z* (<1500) ion arise primarily from the individual oligonucleotides, (I and II) while at higher *m/z* (>2000) the complementary double stranded complex (D) is dominant.

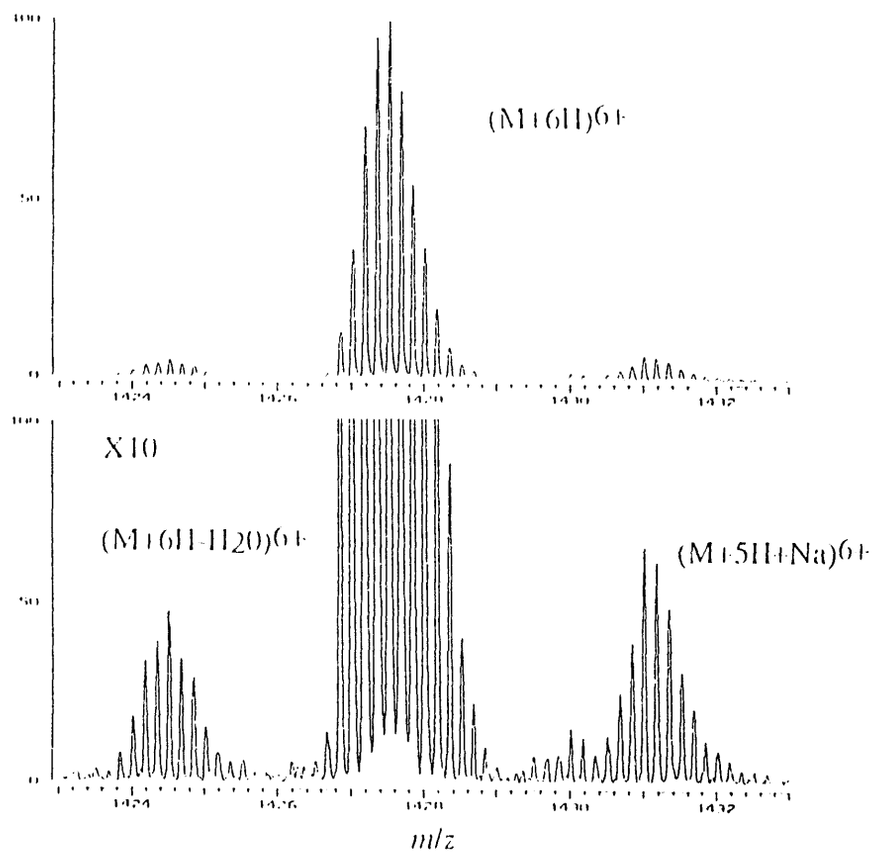

Figure 2. High resolution FT-MS of the $(M+5H)^{5+}$ charge state of ubiquitin showing peaks due to a sodium adduct and loss of water. The mass spectrum was obtained from a single injection and trap cycle, followed by a single broadband (full spectrum) acquisition.

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