

Mass Spectrometric Analysis of Gonadotropins and their Glycovariants - A Workshop Beginners.

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Electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) have been introduced relatively recently as 'soft' ionization techniques for mass spectrometry that result in little fragmentation of molecules, allowing accurate measurement of the mass of analytes such as proteins. These techniques have been found to be useful in the analysis of gonadotropins, most experience having been gained to date with hCG and its metabolites

ESI relies on the sample being sprayed in a polar volatile solvent through a capillary into a strong electric field, a process that results in singly and multiply charged gas phase ions. The ESI source can be coupled with various 'unit' mass analyzers operating within a modest upper mass limit, but proteins with a molecular weight often up to approximately 100,000 Da can be detected because of the multiple charges on them. High performance chromatographic systems can be coupled to ESI-MS, typically using a liquid chromatograph (LC/MS)

MALDI uses short intense pulses of laser light in order to vaporize and ionize the sample that is usually embedded in a large excess of a solid matrix to facilitate the desorption/ionization process, and the mainly singly charged ions produced can be assigned using a time-of-flight analyzer (MALDI-TOF MS).

Both LC/MS and MALDI-TOF MS have advantages and disadvantages when it comes to the analysis of gonadotropins, but they should be considered as complementary approaches. Spectra of the glycoforms of intact hCG and structurally related molecules are congested using ESI because each variant will give rise to its own series of multiply charged peaks. As multiply charged ions are not a highly significant feature of MALDI-TOF MS, the presence of hCG, its composite subunits and hCG β -core fragment can be identified in spectra, although the peaks are broad. Glycoforms have been characterized in an hCG β core preparation by reduction of the disulfide bonds, the separated β 6-40 chain being sufficiently low in mass to allow a sufficient discriminating MALDI-TOF MS spectrum. Very informative spectra, either by LC/MS or MALDI-TOF MS, can be achieved by sample preparation incorporating highly specific endoproteinases (known as peptide mass mapping). This approach has been used, for example, to characterize differences in the oligosaccharide distribution on commercial hCG preparations and on isolated nicked β -subunit.

Immunoaffinity extraction of hCG followed by tryptic digestion can confer sufficient specificity and sensitivity for evidential purposes, as exemplified using LC-tandem MS to detect urinary hCG, as low as 5 IU/L. An exciting objective would be to develop for clinical purposes a procedure that avoids complex sample preparation for MS detection and characterization of gonadotropins (and their metabolites) in biological matrices. The application of a MALDI ion source for quadrupole-TOF MS or ion trap MS may provide this capability, especially when combined with on-target immunoaffinity capture as a convenient method for immobilization and purification purposes.