MASS SPECTROMETRIC CHARACTERIZATION OF STRUCTURALLY MODIFIED PEPTIDES AND PROTEINS

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The burgeoning use of mass spectrometry in proteome analyses is based principally on the molecular mass profiling and partial sequencing of unmodified peptides derived from enzymatic digestion of proteins. There are three situations, however, in which the analysis of structurally modified peptides and proteins is desirable or essential:

- for the characterization of post-translational modifications
- where peptide derivatization is beneficial to improve analytical properties
- in the study of interactions between proteins and small molecules, of endogenous or exogenous origin

Examples of analytical strategies for each of these situations are described here. In the area of post-translational modifications, the determination of the location and extent of protein phosphorylation is frequently critical to the elucidation of protein function. The frequently minor extent of phosphorylation at specific sites, combined with the common observation of reduced response factors during mass spectrometric analyses, suggests an analytical strategy combining selective enrichment and specific detection of phosphorylated peptides in protein digests. In this laboratory, the combination of immobilized metal ion affinity chromatography (IMAC) and precursor ion scanning during electrospray/tandem MS has been used in the analysis of several phosphorylated proteins of biological significance.

Peptide derivatization may be used to enhance response factors during conventional MS analysis and to promote and direct fragmentation during tandem MS. Conversion of lysine to homoarginine residues, for example, leads to substantial enhancements of response during MALDI MS, attributable both to increased ion yield and greater ion stability. Selective fragmentation of peptides following collisional activation may be achieved by N-terminal conversion to Edman-type derivatives: highly favoured cleavage of the N-terminal residue is observed.

As an example of the characterization of proteins modified by reaction with small molecules, we have studied the structural modification of the protein constituent (apoprotein B-100, Mr ca. 500 kDa) of low density lipoprotein (LDL) by interaction with a major products of lipid oxidation, 4-hydroxynonenal. In vitro oxidation of LDL gives rise to adducts at histidine residues; interestingly, such modification appears to be abolished when oxidation of LDL takes place in the presence of high density lipoprotein.