

**S006** Mass spectrometry to detect and identify post-translational modifications of protein

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Modification of proteins by oxidation, glycooxidation and lipoxidation reactions is associated with the pathogenesis of many chronic diseases. When sugars, lipids or their oxidation products react with protein, the protein adducts formed are termed advanced glycation, glycooxidation and lipoxidation end products (AGE/ALEs). Formation of AGE/ALEs on protein is not uniform, but is site-directed. For example, our previous studies on ribonuclease (RNase) indicated that Lys-41 is the primary site of early glycation and carboxymethylation, Arg-39 is the main site of reaction with glyoxal, and Met-29 is the principal site of methionine sulfoxide formation. In our latest work, we incubated RNase with methylglyoxal (MGO) under physiological conditions. We compared the single combined spectra extracted from the full-scan MS data of the tryptic digests from native and modified proteins to detect several ions that were unique in the modified RNase. One of these ions was a 32-amino acid peptide containing a modified Arg-85 residue. Sequential digestion of MGO-modified RNase by endoproteinase Glu-C and trypsin yielded peptides that were amenable to sequencing analysis. The dihydroxyimidazolidine and hydroimidazolone derivatives were the main adducts formed, with minor amounts of the tetrahydropyrimidine and argpyrimidine derivatives. MS experiments were performed by varying the source voltage and collision energy to yield dehydration and decarboxylation products of the tetrahydropyrimidine-containing peptides and dehydration of the dihydroxyimidazoline-containing peptides and to confirm structures.