

## Student 15

### MASS SPECTROMETRY OF ACYLATED PEPTIDES

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Acylation of proteins and peptides is associated with signal transduction, membrane function and bacterial pathogenesis. Fatty acyl groups of varying lengths (eg. octanoyl, myristoyl and palmitoyl) have been found to co- and post-translationally modify proteins and peptides such as G-proteins and ghrelin. Acylation sites include N-terminal glycine and lysine, cysteine and serine residues via amino, thioester and ester linkages respectively. Here we examine the fragmentation patterns for naturally occurring and synthetic acylated peptides and proteins using ESI and MALDI tandem mass spectrometry with the aim of developing screening techniques for the analysis of biological samples.

Acylated peptides were synthesised by reaction with octanoyl, myristoyl or palmitoyl chloride. Ubiquitin and bovine carbonic anhydrase were reacted with anhydrides or N-hydroxysuccinimidyl esters.

Ghrelin and the synthetically-acylated peptides including substance P, glutathione and eledoisin were analysed by MALDI-TOF, MALDI-Q-o-TOF and ESI-Q-o-TOF mass spectrometry. It was found that acylated peptides required slightly higher collision energies when compared with the corresponding non-acylated peptide in the ESI-MS/MS analysis. It was also found that higher collision energies were required with increasing chain length of the acyl moiety. Characteristic product ions, corresponding to acylated immonium ions were readily identified in MALDI and ESI tandem mass spectra. MALDI-PSD in contrast did not always yield characteristic immonium ions. These characteristic immonium ions were used to confirm the sites of acylation in synthetically acylated ubiquitin and bovine carbonic anhydrase following enzymatic digestion and LC-MS/MS analysis. This work will be extended to the examination of biological tissues for the presence of acylated peptides and proteins.