

S002 High-resolution mass spectrometry and inactivation of protein targets during oxidant stress

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Mass spectrometry with or without pre-analysis peptide fractionation can be used to decipher the residues on proteins where oxidative modifications caused by peroxynitrite, singlet oxygen and electrophilic lipids have occurred. Peroxynitrite nitrates tyrosine and tryptophan residues on the surface of actin. Singlet oxygen, formed by UVB light's interaction with tryptophan, leads to the oxidation of neighboring cysteine, histidine, methionine, tyrosine and tryptophan residues. 4-hydroxynonenal (4HNE) inactivates in a concentration-dependent manner human bile acid CoA: amino acid N-acyltransferase (hBAT) and the cytosolic brain isoform of creatine kinase (BB-CK). Dose-response analysis of the modifications to a protein reveals the modifications associated with change in function. Fourier Transform-Ion Cyclotron Resonance Mass Spectrometry using gas phase fractionation and/or nanoLC-ESI-MS identified 14 HNE modifications to hBAT and 17 to BB-CK. However, at 4HNE concentrations in the physiological range, two members of the catalytic triad of hBAT (Cys235 and His362) were modified; for BB-CK, all four members of the active site formed 4HNE adducts. In summary, future *in vivo* studies should carefully assess the critical sites that are modified rather than using antibodies that measure all of them.