

P038 Development of novel mass spectrometry methodology to detect post-translational modifications in oxidative stress and disease.

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Oxidative stress is a factor in inflammatory diseases, where immune cells release oxidants that can damage biomolecules, including proteins. At present there are no reliable methods which can identify the specific sites of damage to individual proteins in complex mixtures such as clinical samples. Chlorination and nitration are examples of oxidative modifications commonly used as markers of damage by myeloperoxidase and nitric oxide-derived oxidants. The generation of indicative immonium ions, such as the chlorotyrosine immonium ion (m/z 170), on fragmentation of peptides in a mass spectrometer, could allow us to detect these modifications and identify the damaged protein in complex samples. However, studies using LC-MS² showed that ions isobaric to these immonium ions exist in unmodified peptides, so this method is prone to false positives. To overcome this, we have optimised the use of a nano-LC/MS³ mass spectrometry method involving the dissociation of immonium ions to give fragments specific for oxidized residues such as chlorotyrosine or nitrotyrosine. Lysozyme has been used as a model protein to develop the methodology, then more complex mixtures were used to validate the method. The method has also been validated by comparison with western blotting, and has proved able to identify diagnostic fragments for chlorotyrosine in complex mixtures of proteins subjected to oxidative stress. The application to other oxidative modifications such as hydroxytryptophan is currently being investigated.