Exploring the Mechanisms behind Disease using Mass Spectrometry

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Mass spectrometric study of oxidant damage

Generation of endogenous oxidizing species have been implicated in the pathogenesis of a wide variety of diseases from cancer to Alzheimer's Disease to Inflammatory bowel diseases. Antioxidant compounds may therefore be useful in treating or preventing these diseases. However, designing an optimal treatment strategy is complicated by uncertainty over the relevance of oxidant pathways studied *in vitro* to diseased tissue. To better explore this area we have developed mass spectrometric approaches capable of identifying the specific chemical modifications of biomolecules produced by different oxidant pathways. For example, myeloperoxidase (MPO) in white blood cells is used to generate reactive oxidants such as hypochlorous acid (HOCl). We have therefore identified 3-chlorotyrosine, 5-chlorocytosine, and 5-chlorouracil as specific products of oxidation by myeloperoxidase and used gas chromatography/mass spectrometry to measure these compounds in tissue from humans and animal models of disease. We have also developed stable isotope dilution techniques for quantitation of 3-nitrotyrosine, a specific marker of nitrating oxidants found in human and animal tissue. The combination of chemical studies, cell culture, transgenic and mass spectrometric analysis of tissue has allowed us to define not only what oxidants are present *in vivo* but also how they are produced. Currently, we are emphasizing development of on-line analysis by tandem mass spectrometry combined with high pressure liquid chromatography. This methodology will be used both to study oxidation of intact polypeptides and to identify the specific proteins that are targets of oxidation *in vivo*. Efforts are made to understand the ion chemistry involved in mass spectrometric identification of these compounds and to apply them to collaborative research in cancer and other inflammatory diseases.

Proteomics

Mass spectrometry also presents the opportunity for proteomic analysis of all proteins expressed by a cell or tissue. Combining 2-dimensional gel technology with peptide sequencing by tandem mass spectrometry and computerized genomic databases allows changes in protein expression patterns to be analyzed in cultured cells, animal models of disease, and human tissue. This ability to measure protein expression in a screening assay is very complementary to gene chip expression profiling, which measures RNA transcription. We have used this approach to identify the preferred bacterial targets of nitration by white blood cells. We have also utilized 2D gel technology to examine the effect of a high fat diet on the vascular tissue. As exemplified by our studies with oxidized matrix metalloproteinases, proteomic analysis also offers the ability to detect site-specific post-translational modification of proteins during experimental manipulation or in disease states. In the future, the use of proteomic analysis in collaborative efforts between bioanalytical chemists and biologists will result in a greatly improved understanding of disease pathogenesis and treatment.