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APPLICATION 13

Protein Oxidative Modification Analysis using AXIMA-CFR

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Protein oxidation is thought to be one of the causes for aging of cell, neurological injury due to reflow following cerebral ischemia, arteriosclerosis and other vascular pathologies due to oxidative stress. Therefore, analysis of protein oxidation is considered to become increasingly important in the field of research directed at preventing aging and various aging related disorders. Protein oxidation occurs at various amino acid residues, among which methionine is considered to be one of the most susceptible. Since oxidation of methionine occurs easily even in the test tube, this phenomenon has been conventionally disregarded as physiologically meaningless. However, since many cells contain enzymes that repair proteins by reducing methionine sulfoxide residues, the product of methionine oxidation, it suggests that methionine oxidation does indeed occur in cells and that its continuous reduction is critical for the meintenance of cell function. Our research group found that the occurrence of proteins containing oxidized methionine increases in brain tissue of aged mice and cultured cells subjected to oxidative stress, and part of this data have been reported in *Applied Genomics and Proteomics 2003*: 2 (1), 43-50.
Oxidized methionine in peptides is detectable by PMF analysis using AXIMA-CFR as a mass increase of 16 Da in the result of database search, and it can be further confirmed by an easily observable reduction of 64 Da in neutral loss analysis using MALDI-PSD-TOF/MS.

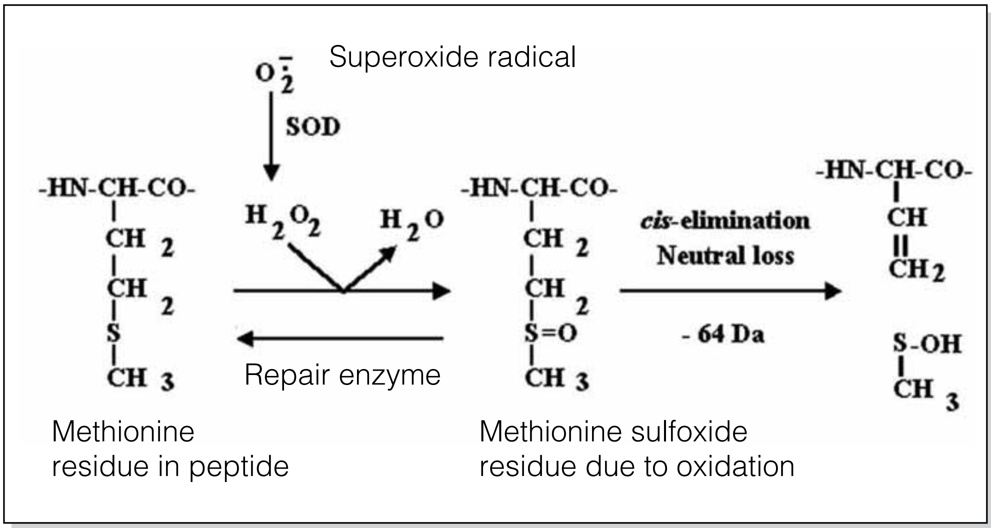

Figure 1 Neutral Loss of Oxidized Methionine Peptide

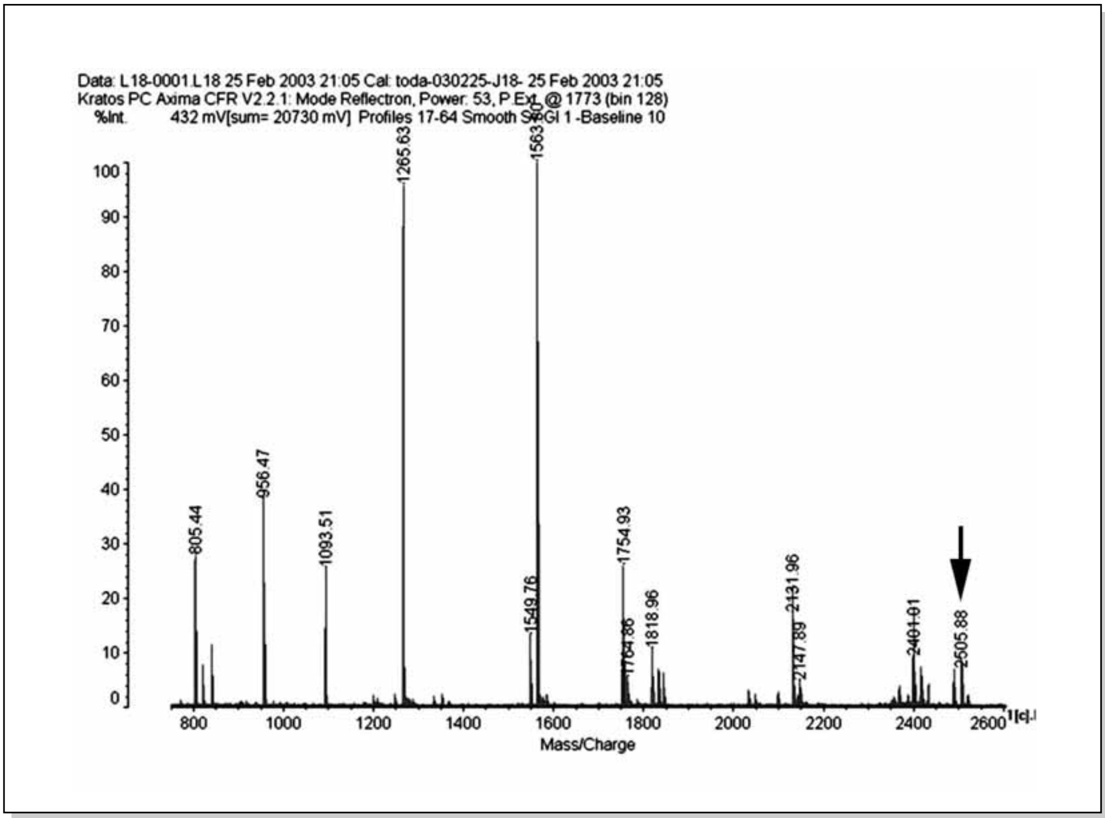

Figure 2 MALDI-TOF/MS Analysis of Trypsin-Digested Calmodulin from Aged Mouse Hippocampus using AXIMA-CFR. The PMF database search result suggested oxidized methionine in peptide indicated by arrow.

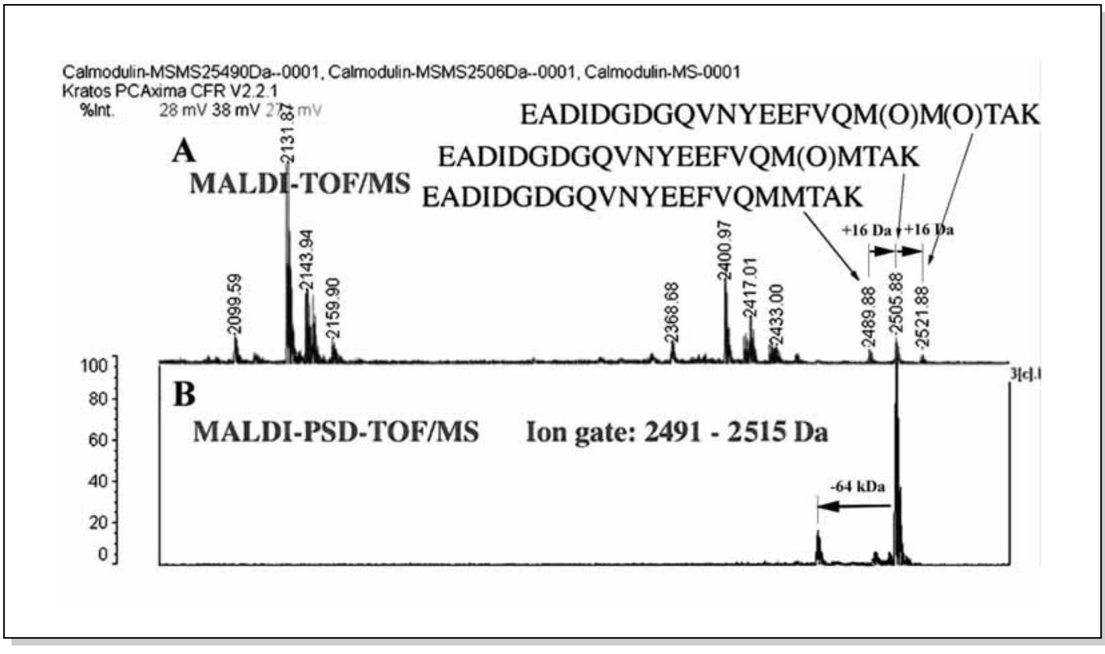

Figure 3 Neutral loss analysis of 2505.88 Da peptide by AXIMA-CFR suggesting methionine oxidation. Loss of -64 Da mass was seen using PSD (Post-source decay), confirming methionine oxidation.