SHIMADZU

Application News

MALDI-TOF Mass Spectrometry No.B19

Identification of Proteins by LC-MALDI System (2) [Analysis of Carbonylated Proteins]

Using the AXIMA MALDI-TOF-MS system for end-point analysis of two-dimensional electrophoresis, we analyzed carbonylated proteins in the CA1 area of a monkey hippocampus affected by ischemia-reperfusion.
It is known that reactive oxygen species, or oxygen radicals (ROS), negatively impact a great many diseases, including cancer, cardiac disease and cerebral stroke, which are the leading causes of death among the Japanese, in addition to such lifestyle diseases as diabetes and arteriosclerosis. Reactive oxygen species are known to include hydrogen peroxide, the superoxide anion radical, and the hydroxyl radical, etc., and all of these cause non-physiological posttranslational modifications in nucleic acids, lipids, proteins and other types of biological molecules.
Protein carbonylation is a type of protein damage resulting from oxidative stress in cells, and these carbonylated proteins are used as markers for oxidative stress to proteins. Oxidative carbonylation occurs when an aldehyde is formed on the side chains of amino acids such as arginine and lysine, which comprise proteins (Fig. 1).

It is suggested that selective neuronal cell death occurs in the hippocampus CA1 area due to transient cerebral ischemia, resulting in memory impairment.
Here, using a sample consisting of the hippocampus CA1 area affected by transient cerebral ischemia, two-dimensional electrophoresis was conducted, and spots thought to be carbonylated proteins were excised and analyzed by LC-MALDI. The results confirmed the carbonylation of the 469th arginine of the Heat shock-70 kDa protein 1 (Hsp70-1), which plays a role in regulating cell death (Fig. 2, 3).

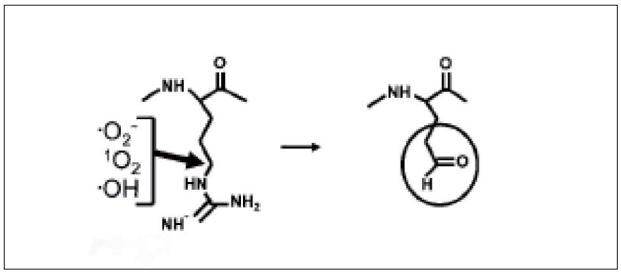


Fig. 1 Carbonylation of Arginine

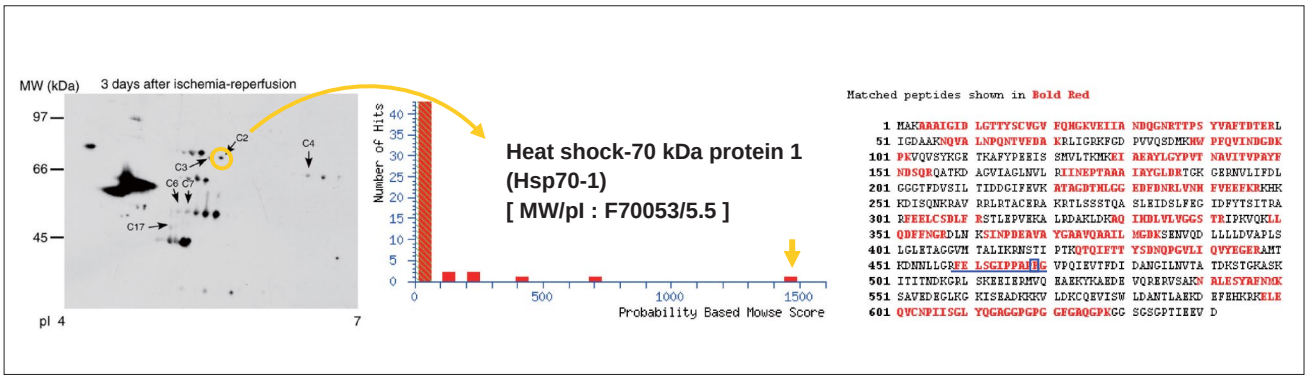


Fig. 2 Results of Identification of spot C2 (Hsp70-1) by LC-MALDI

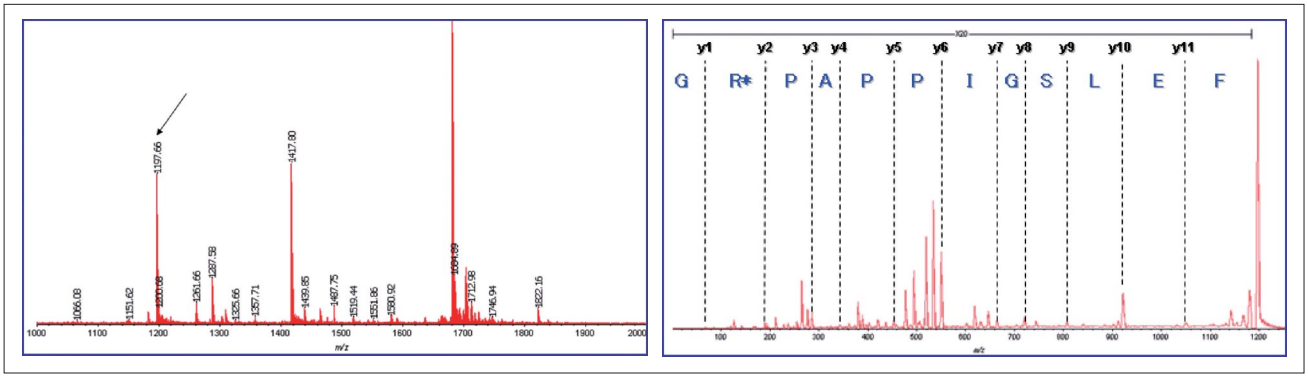
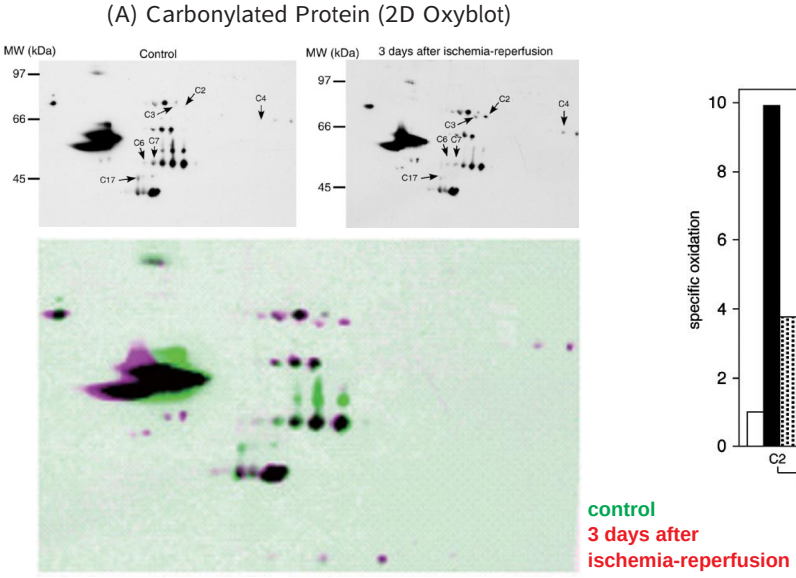


Fig. 3 Mass Spectra of Hsp70-1
(A) Spectrum of the Tryptic Digest of Hsp70-1
(B) MS/MS Spectrum of *m/z* 1197.66 (FELSGIPPAPR*G:R*, Carbonylated Arginine)

After extracting proteins from the CA1 area of a monkey hippocampus prior to, and 3, 5 and 7 days following transient cerebral ischemia, the proteins subjected to oxidative stress were labeled using 2,4-dinitrophenylhydrazine (DNPH), and separated by two-dimensional electrophoresis. In addition, Western blotting was performed using anti-DNP antibodies, and the carbonylated proteins were detected (Fig. 4 (A)).
In addition, we checked for variations in all proteins using 2D-DIGE (Fig. 4 (B)). The results confirmed remarkable changes in 6 spots, and that these changes were due to carbonylated proteins. PMF analysis conducted on these 6 spots confirmed that carbonylation had affected 4 types of proteins (Fig. 5).
In addition, after performing In-gel digestion of the C2 spot, we conducted MS/MS automatic measurement using the LC-MALDI system. The results confirmed that the 469th arginine was carbonylated.

The Axima Performance system for two-dimensional electrophoresis used here is an extremely effective tool for posttranslational modification analysis, as demonstrated in these results of analysis of carbonylated proteins as one type of oxidative stress to proteins.



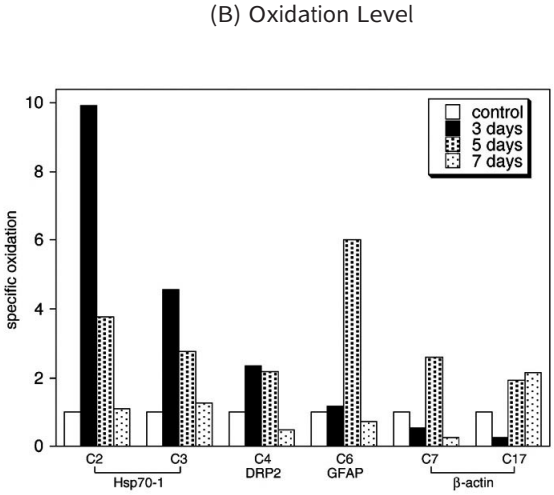


Fig. 4 (A) 2D Oxyblot (Control and Day 3 Post-Ischemic CA1 and Image Analysis (Using Progenesis PG200)
(B) Specific Oxidation Levels of Identified Proteins at 3, 5, and 7 Days After Ischemia-Reperfusion Insult

Spot	Protein Name	% Coverage	Theoretical Molecular Mass (Da)/pI
C2	Heat shock-70 kDa protein 1 (Hsp70-1)	22.6	70053/5.5
C3	Hsp70-1	29.0	70053/5.5
C4	Dihydropyrimidinase-like 2 isoform 2	28.2	73583/5.9
C6	Glial fibrillary acidic protein	37.0	47412/5.2
C7	β-Actin	39.7	41737/5.3
C17	β-Actin	32.3	41737/5.3

Fig. 5 Identification of Carbonylated Proteins

[References]
The data presented here was acquired through joint research with Associate Professor Shinji Oikawa of the Department of Medicine, Mie University.

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