Posttranslational Modifications in an Insect Cell-Free Protein Synthesis System and Their Identification by MALDI-TOF MS

T. Ezure$^1$, T. Suzuki$^1$, M. Shikata$^1$, E. Ando$^1$, T. Utsumi$^2$, S. Tsunasawa$^{1,3}$

$^1$Clinical & Biotechnology Business Unit, Analytical & Measuring Instruments Division, Shimadzu Corporation, Kyoto, Japan
$^2$Applied Molecular Bioscience, Graduate School of Medicine, Yamaguchi University, Yamaguchi, Japan
$^3$Institute for Protein Research, Osaka University, Osaka, Japan

We have established a cell-free protein synthesis system (Transdirect insect cell) derived from Spodoptera frugiperda 21 insect cells [1]. This cell-free system has high protein productivity, and therefore it is expected to be sufficient to perform gene expression analyses including not only the measurement of enzymatic activity and western blotting, but also investigation of posttranslational modifications. In this study, several posttranslational modifications in the insect cell-free protein synthesis system were confirmed and identified by MALDI-TOF MS [2, 3, 4].

One significant posttranslational modification is the formation of disulfide bonds. This plays a very important role in both the biological activity and stabilization of native protein structures. Human lysozyme (h-LYZ), which contains four disulfide bonds were expressed in the insect cell-free protein system. h-LYZ was expressed in a soluble and active form under non-reducing conditions after addition of reduced glutathione , oxidized glutathione , protein disulfide isomerase. Analysis of the disulfide bond arrangements by MALDI-TOF MS showed that disulfide linkages identical to those observed in the wild-type proteins were formed.

Protein $N$-myristoylation and prenylation are the important lipid modifications of proteins, and they play crucial roles in regulating reversible protein-membrane and protein-protein interactions. Epitope-tagged truncated human gelsolin (tGelsolin) and human rhoC, which are natural $N$-myristoylated...
and geranylgeranylated protein respectively, were synthesized using the insect cell-free protein synthesis system with or without addition of a specific substrate for each protein modification, such as myristoyl-CoA, farnesyl pyrophosphate, and geranylgeranyl pyrophosphate. Analyses of these proteins by MALDI-TOF MS indicate that the insect cell-free protein synthesis system, as is the case with the rabbit reticulocyte lysate system, possesses $N$-myristoyltransferases and prenyltransferases.

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