[S3-2] [4/20/2001(Fri) 9:55-10:20 / Hall A]

Identification of Protein Modifications in Cellular Signaling Using Proteomics

Kong-Joo Lee

Division of Molecular Life Sciences and Collge of Pharmacy
Ewha Womans University, Seoul, Korea

Genomic technologies have enabled rapid accumulation of information from complex biological systems over the last two decades. The complete DNA sequence is now known for many organism and the informational database obtained from genome sequencing projects has provide the base for the specification of proteome - the protein complement of genome. Genomic functions can be infered from the analysis of gene structure and gene expression profiles because proteins are the functional molecules of an organism. Integrated technologies including protein separation, identification, characterization and information manage system are essential to analyze the proteins in complex cellular matrix.

This presentation will focus on the strategies of proteome analysis using sample preparation, 2-dimensional gel electrophoresis, processing of protein spots and identification of proteins and posttranslational modifications by peptide fingerprinting and amino acid sequencing with PSD (post source decay) using MALDI-TOF-MS, In this work, we applied this technology for the determination of post-translational modifications after various stresses, including oxidation, phophorylation and proteolytic cleavage.

Nucleoside diphosphate kinase (NDPK, Nm23) has been implicated as a multifunctional protein. However, the regulatory mechanism of NDPK is poorly understood. We have examined the modification of NDPK in oxidative stresses. We found that oxidative stresses including diamide and H2O2 treatment cause disulfide cross-linking of NDPK inside cells. This cross-linking was reversible in response to mild oxidative stress, and irreversible to strong stress. This suggests that disulfide cross-linked NDPK may be a possible mechanism in the modification of cellular regulation. To confirm this idea, oxidative modification of NDPK has been performed *in vitro* using purified human NDPK. H2O2 inactivated the nucleoside diphosphate (NDP) kinase activity of NDPK by producing intermolecular disulfide bonds. Disulfide cross-linking of NDPK also dissociated the native hexameric structure into a dimeric form. The oxidation sites were identified by the analysis of tryptic peptides of oxidized NDPK, using MALDI-TOF MS. Intermolecular cross-linking between Cys109-Cys109, which is highly possible based on the X-ray crystal structure of NDPK-A, and oxidations of 4 methionine residues were identified in H2O2-treated NDPK. This cross-linkng was confirmed using mutant C109A (NDPK-AC109A) which had similar enzymatic activity as a wild NDPK-A. Mutant NDPK-AC109A was not cross-linked and was not easily denatured by the oxidant. Therefore, enzymatic activity and the quaternary structure of NDPK appear to be regulated by cross-linking with oxidant. These findings suggest one of the regulatory mechanisms of NDPK in various cellular processes.

Heat shock induces the various processes including inhibition of protein synthesis, production of heat shock protein (HSP) and induction of thermotolerance. However, the molecular mechanisms of this process have not been well understood. Here we have identified the differentially phosphorylated proteins in tyrosine residues by heat shock and between control RIF-1 and their thermotolerant derivatives, TR-RIF-1 cells, using in-gel digest peptide fingerprinting using MALDI-TOF MS and semi-quantified using 2D-gel image analysis after Western analysis using antibody specific for phospho-tyrosine. We have identified 17 proteins shown discernible differences in phosphorylation by heat shock in a recovery time dependent manner: From the differential phosphorylations between control and thermotolerant cell lines,

we suggest that cellular thermotolerance can be regulated by changing the phosphorylation levels in various proteins.

The results obtained from proteomics were confirmed by biochemical methods. The relationships between both methods were reliable to accept. The developed integrated proteome technologies are very useful to understand the biological phenomena at molecular level by identifying the new molecules and their modifications in various cellular processes, and the obtained valuable results can be applied for biotechnology including medical and pharmaceutical industry.