Analysis of Phosphoprotein Using Proteomic Approach

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In order to perform the intrinsic functions, most proteins need to be post-translationally modified. Therefore, analysis of post-translational modification is important to determine the *in vivo* state of proteins. Recently, modifications such as phosphorylation are frequently detected by MS-based analysis. However, phosphoproteins are generally difficult to analyze by MS for several reasons. They are negatively charged whereas ESI is generally performed in the positive mode. Phosphoproteins or phosphopeptides are not observed as intense peaks, especially in the presence of other nonphosphorylated peptides owing to ionic suppression. Therefore, global analysis of protein phosphorylation needs various methods such as 2-DE, phosphatase treatment and affinity purification, in addition to MS-based techniques. In this presentation, we outline several methods for enrichment of phophorylated proteins and peptides. In addition, we introduce some example that actually has been identified using affinity purification with anti-phosphotyrosine antibody and MS/MS analysis.