

Chemical Method for Dephosphorylation of Phosphopeptides

Phosphorylation of proteins is a ubiquitous posttranslational modification, which plays a key role in various cellular processes, such as growth, metabolism, proliferation, motility, differentiations, and tumorigenesis. It is estimated that about 1/3 of the proteins in mammals are phosphorylated. To understand these essential roles in cells, it is necessary to locate the sites and to determine the stoichiometry of the phosphorylation.

Analyses of protein phosphorylation have been performed using various biochemical and genetic approaches. Among these, combination of phosphatase treatment and MS analysis gives a clear evidence for phosphorylation where the mass shift owing to loss of phosphate group (80 Da or multiples thereof) is seen after the phosphatase treatment. But “enzyme reaction” is often incomplete, reflecting substrate structure dependence. Also, care should be taken to avoid contamination of a peptide sample during sample preparation, with substances that could inhibit the activity of the phosphatase used. At this point, we introduce a fast, versatile, and simplified dephosphorylation method employing hydrofluoric acid (50%) or hydrogen fluoride-pyridine (70%).

1) We investigated dephosphorylation using hydrogen fluoride – pyridine (70%).

Three types of model peptides are used, namely:

- (A) WAGGDApSGE
- (B) Ac-IpYGEF-NH₂
- (C) GFETVPEpTG-NH₂

All of the phosphorylated peptides were dephosphorylated in 1 hour in an ice bath. In each set of the spectra (A, B, and C), the lower shows the spectrum of the starting phosphorylated peptide, and the upper shows the post-reaction spectrum (Fig.1). MS data acquisition was performed using AXIMA-CFR in positive reflectron mode.

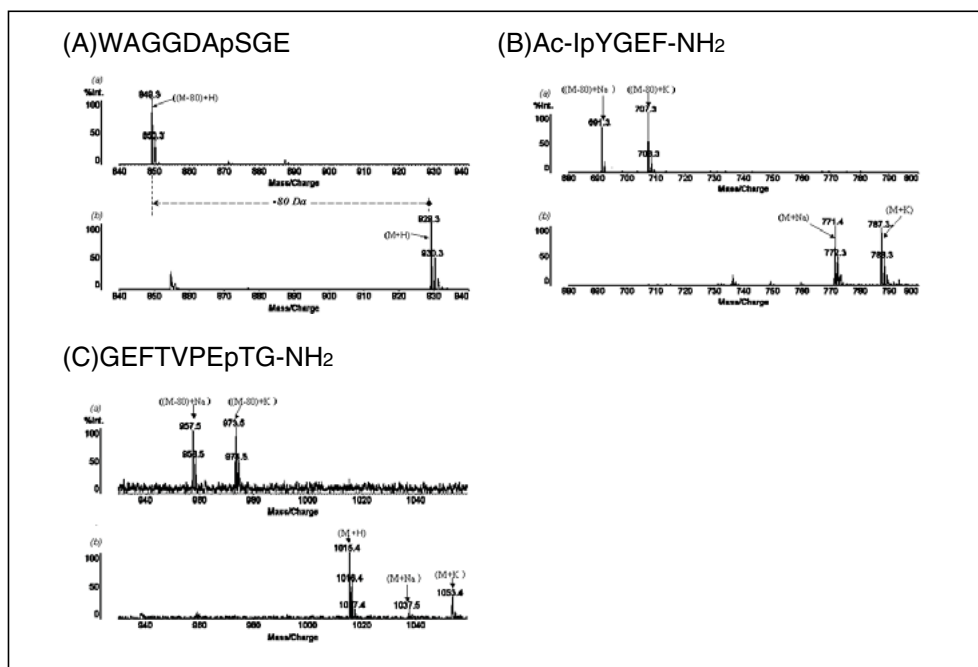


Figure 1

2) We investigated dephosphorylation of phosphopeptides with tryptic digests of α -casein using a 50% aqueous solution of hydrofluoric acid. After enzymatic digestion followed by enrichment column (Ga IMAC column), the phosphopeptide signals (1661.2, 1952.3) were observed in the mass spectrum. Then the enriched sample was treated with aqueous hydrofluoric acid (50%) for 3 hours in an ice bath. In each set of spectra (A and B), the lower shows the spectrum obtained before treatment, and the upper shows post-treatment spectrum (Fig.2). MS data was acquired using AXIMA-CFR in positive reflectron mode, just as in experiment 1).

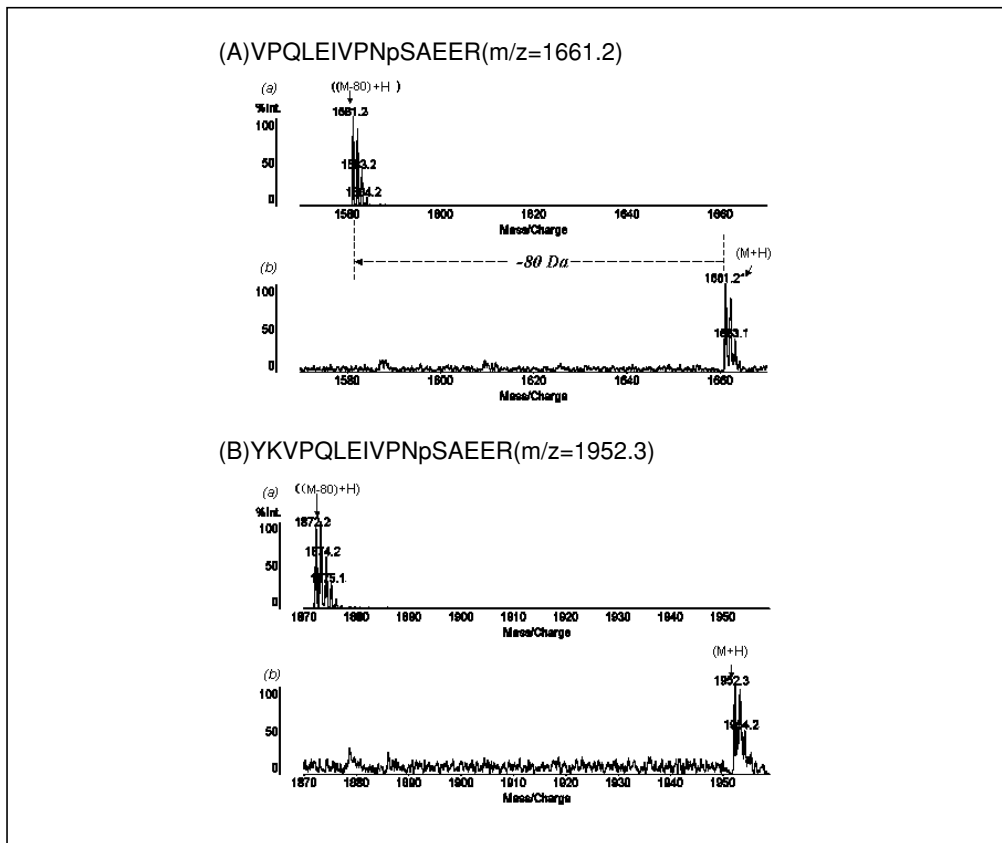


Figure 2

References : H.Kuyama, C.Toda, M.Watanabe, K.Tanaka, and O.Nishimura,
 “ An efficient chemical method for dephosphorylation of phosphopeptides ”
 Rapid Commun. Mass Spectrom., 2003;17:1493-1496.