

Analysis of Phosphopeptides using AXIMA-CFR

Information provided by : Toshifusa Toda, Ph.D.; Tokyo Metropolitan Institute of Gerontology, Proteomics Collaboration

Protein phosphorylation plays important roles in various types of cell function regulation, including intracellular signal transduction, microtubule formation, etc., and research into the roles of protein phosphorylation in cell replication, malignancy, and aging have been reported.

Conventionally, phosphopeptide analyses have been performed by methods such as Western blotting using antibodies specific for phosphorylated peptides, labeling using radioisotopes and dephosphorylation by phosphatase, but now, detailed analysis can easily be performed using AXIMA-CFR.

Neutral Loss Analysis by MALDI-PSD-TOF/MS

Neutral loss by PSD (Post-source decay) refers to the reduction of mass due to β elimination (cis-elimination). This occurs when a peptide is irradiated with high energy laser, causing instability and ionization of the peptide. When a serine (S) or a threonine (T) residue is phosphorylated, it is known that there occurs a neutral loss of -80 Da and -98 Da (Fig. 1), respectively, which can be used as an indicator to detect phosphorylated proteins.

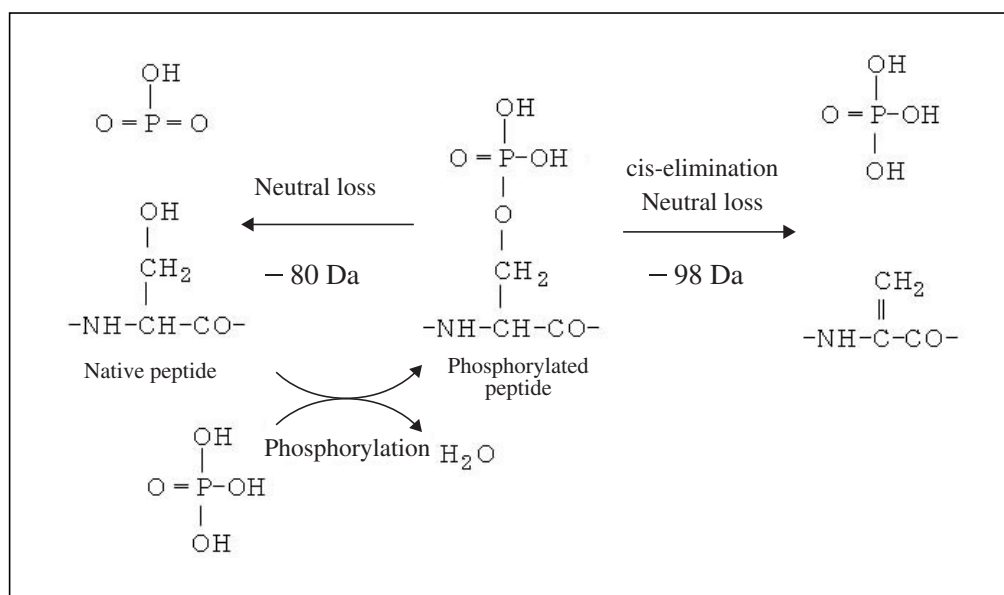


Figure 1 Neutral Loss in Phosphorylated Peptides

Neutral Loss Analysis by MALDI-PSD-TOF/MS

In-gel digestion of bovine α -casein was performed using trypsin, and 1 μ L of the resulting peptide solution was mixed with 1 μ L of a 10mg/mL α -cyano-4-hydroxycinnamic acid (α -CHCA) matrix solution. The prepared sample was then applied to the AXIMA 384-well sample plate. First, normal MALDI-TOF-MS was performed with the Ion Gate in the OFF mode, and a Peptide Mass Fingerprint (PMF) was obtained (1 of Fig 2). Next, the Ion Gate was turned ON, and with each mass peak taken as the parent ion, neutral loss analysis was performed by post-source decay (PSD) (Fig. 2, 2 - 10). In the case of trypsin digestion of bovine α casein, a neutral loss of -80 Da and -98 Da were observed for the 1660.96 Da peptide (6 in Fig. 2).

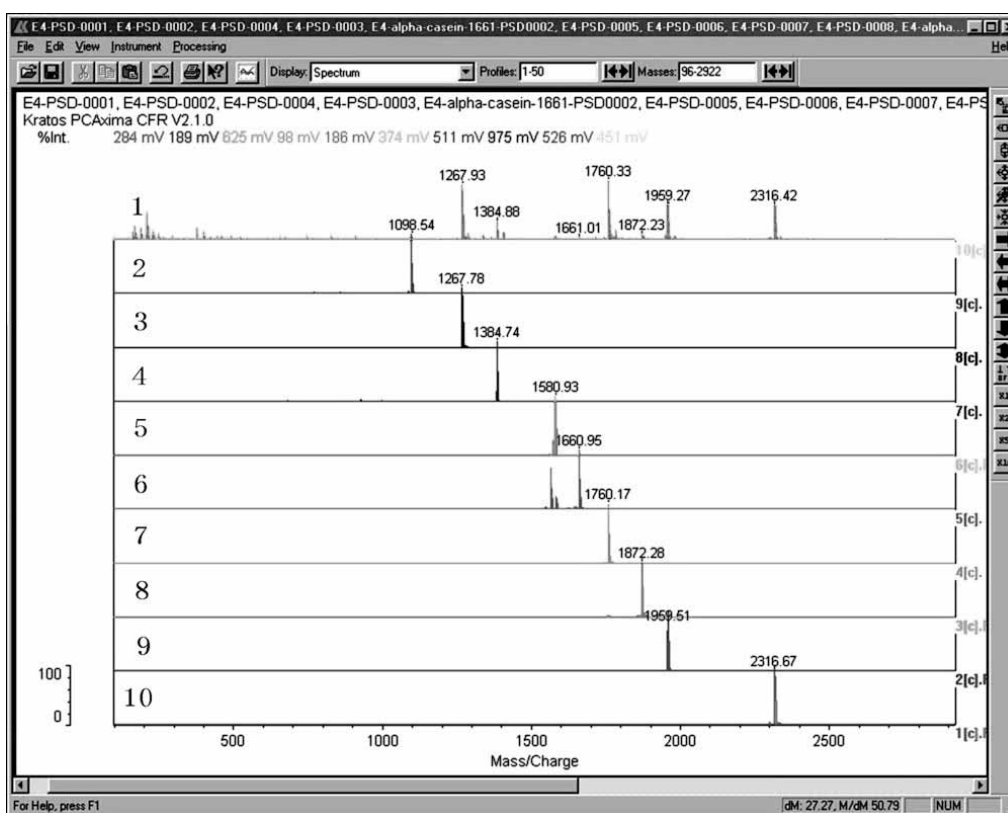


Figure 2 Neutral Loss Analysis of Bovine α -Casein Trypsin Digest using AXIMA-CFR. For the 1660.96 Da peptide, a database search revealed that a serine residue in VPQLEIVPNSAEER was phosphorylated.