Technical bulletin
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Enrichment and identification of phosphopeptide using Phos-Pep™

Introduction

Phos-pep™ was designed to ensure the charactrization of phosphopeptides. Investigators to characterize the phosphoproteins by mass spectrometry hampered by the low abundance of phosphoprotein and the suppression of ionization of phosphopeptide in mass spectrometry resulting in failure to obtain sufficient signals. Phos-Pep™ facilitates the isolation and enrichment of phosphopeptide from complex mixtures of trypsin digest of phosphorylated proteins.

Strategy

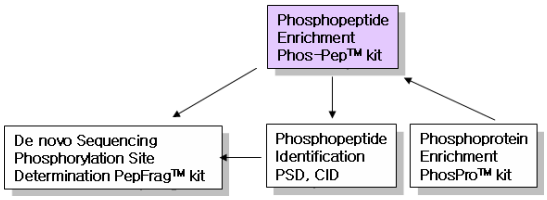


Fig.1. Phosphopeptide enrichment using Phos-Pep™ in conjunction with MALDI-TOF-based CAF sequencing or MS/MS for identification of phosphopeptide and phosphorylation site determination.

Materials & Methods

Materials

Phos-pep™kit contents

Solution A
Solution B
Washing solution : ammonium acetate stock solution
Dissolving solution : 1% phosphoric acid
Phosphopeptide standard : beta casein tryptic digest 10μg

Procedure Summary

1. Selective binding of phosphpeptide
2. Precipitation of phosphopeptide complex
3. Washing phosphopeptide aggregates
4. Dissolving of phosphopeptide aggregates
5. Desalting and concentration
6. Characterization of phosphopeptide

Results and Discussion

Phosphopeptide enrichment from beta casein

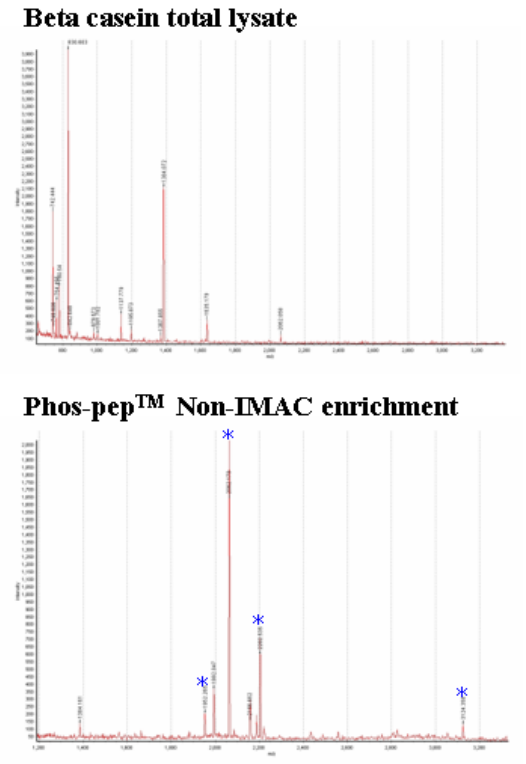


Fig.2. MALDI-TOF mass spectrum of beta casein trypsin digest(upper) and enriched phosphopeptide from beta casein trypsin digest using Phos-Pep™ (lower). Blue asterisk represent the enriched phosphopeptides.

Phosphopeptide identification

Beta casein was used to examine the capacity of Phos-Pep^{TM}, non-IMAC phosphopeptide enrichment kit. Beta casein (purchased from Sigma, Cat.No.C6905) was digested with trypsin and phosphopeptide was enriched using Phos-Pep^{TM} and peptide map was measured. Phosphopeptide which was not shown in total digest of beta casein(Fig.2.) was detected in enriched fraction(Fig.2. lower panel, Fig.3. A). Among these phosphopeptide, mono phosphopeptide(2062) and tetra-phosphopeptide(3124) was originated from beta casein. Some phosphopeptide, 1660, and 1952, seems to be originated from contaminated alpha casein, because this phosphopeptide is a major component of commercially available alpha casein phosphopeptide(see Fig.4.). Some peptides, ranging 2884 to 3054, seems to be the derivatives of tetra-phophopeptide of 3124, which have differential mass according to the status of the number of phosphorylation site. Some of phosphopeptide was identified by PDS of MALDI-TOF by detecting the mass loss of phosphoric or phosphorous acid (Fig.3.).

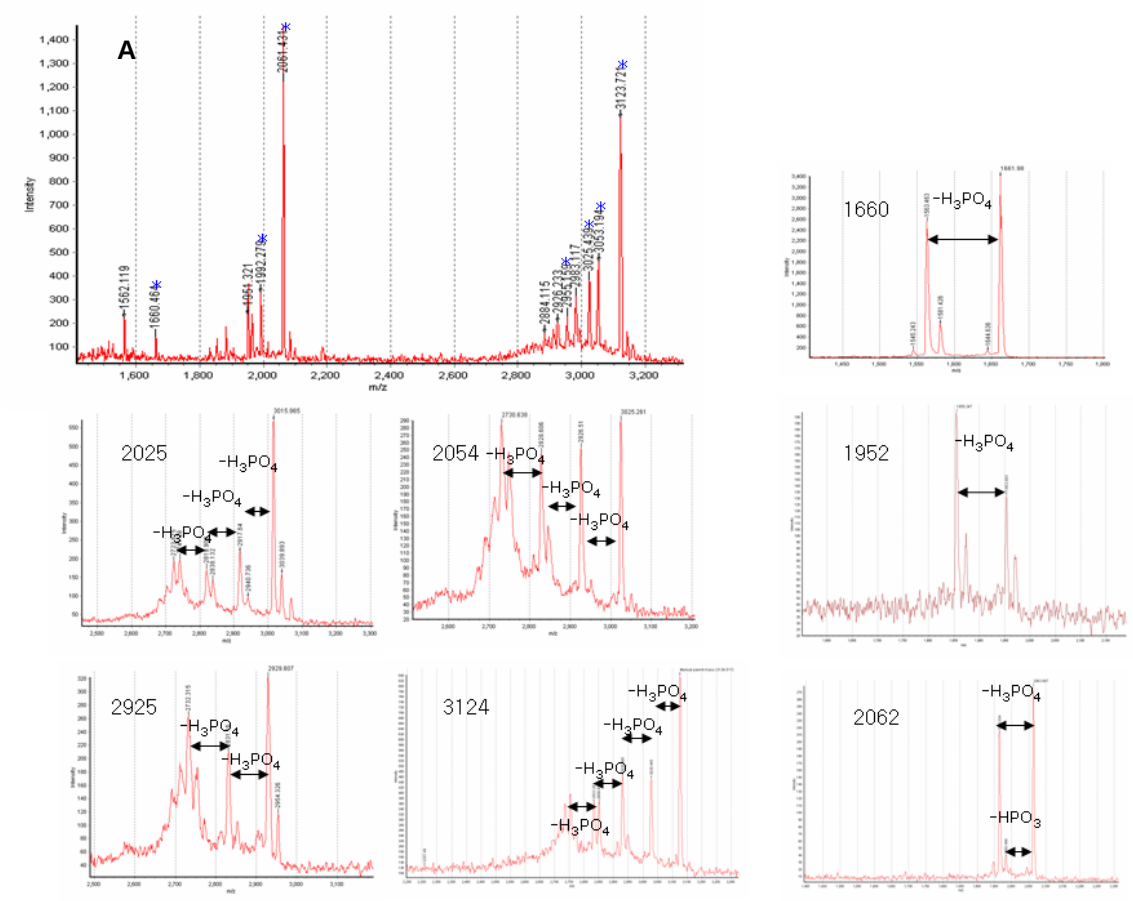


Fig.3. Identification of phosphopeptide enriched from beta casein trypsin digest by PSD(post source decay) using MALDI-TOF. Panel A represents the MALDI-TOF spectrum of enriched phosphopeptide from beta casein trypsin digest. Blue asterisk represent the enriched phosphopeptides. Rest of the seven spectrums represent the PSD spectrum of enriched phosphopeptide.

Specificity and sensitivity of phosphopeptide enrichment

Heterogeniety of phosphorylation site in peptide sequence could affect the efficiency of phosphopeptide enrichment. We examined the specificity of Phos-Pep™ kit for phosphopeptide from known phosphoprotein as a model peptide, such as alpha casein, ovalbumin, pepsin and phosvitin. As shown in Fig.4., Phos-Pep™ isolated the phosphopeptide from most of the tryptic digest of model protein used and characterized as a phgosphopeptide by PSD using MALDI-TOF. The sensitivity of capability of Phos-Pep™ was also tested. From one microgram trypsin digest of beta casein, which is equivalent to 45 pmole of each digested peptide, to 175 fmole of 256-fold diluent, the capacity of enrichment was tested(Fig.5.)

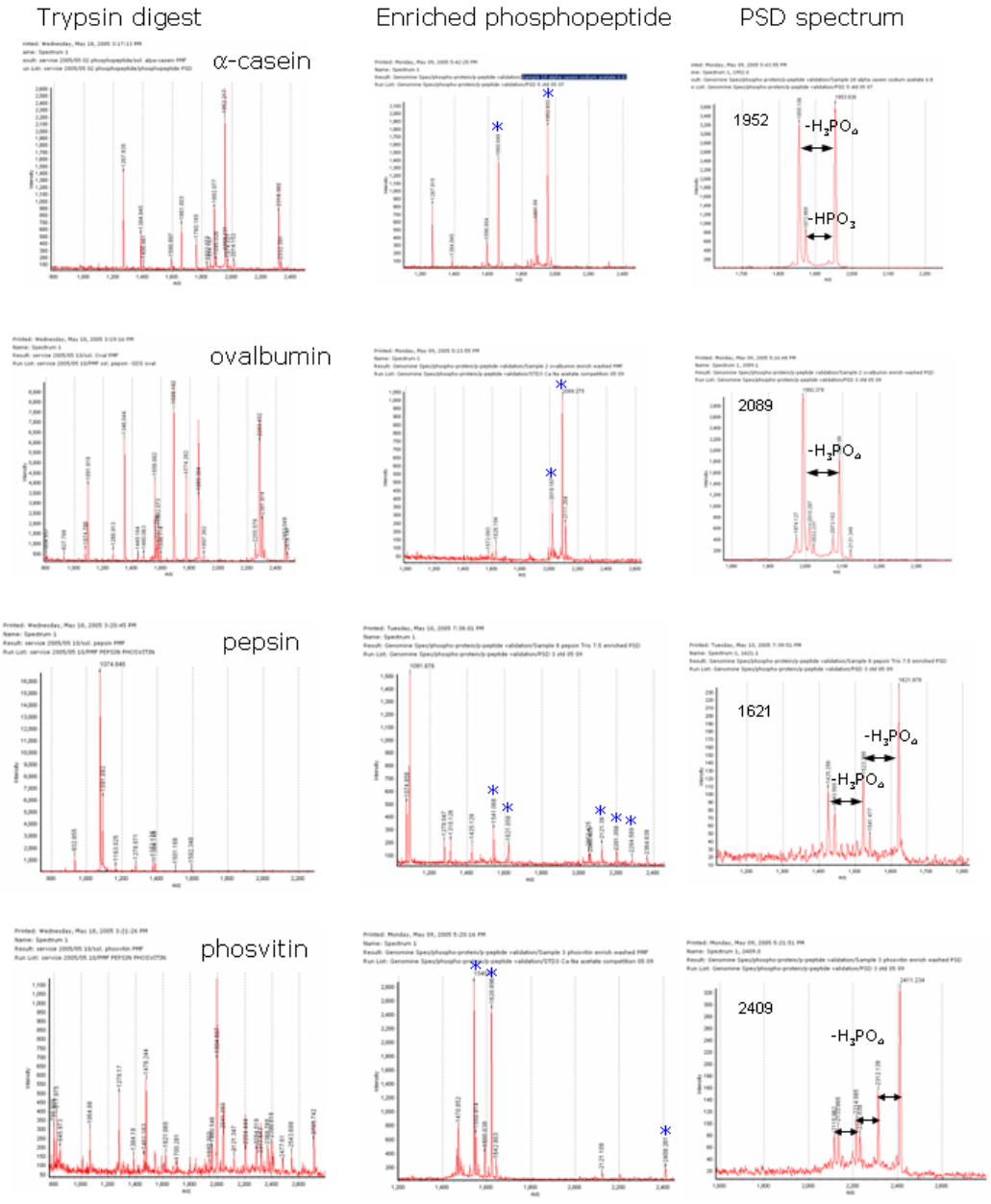


Fig.4. Phosphopeptide enrichment from trypsin digest of phosphoprotein

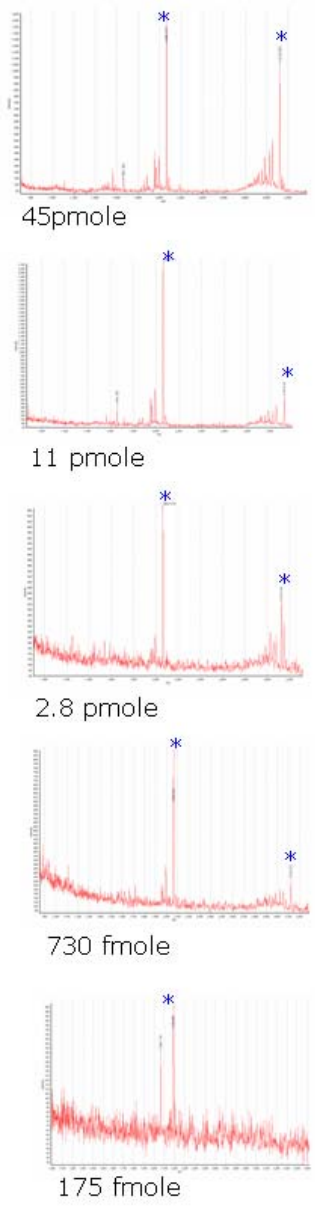


Fig.5. Sensitivity of enrichment of phosphopeptides.

Phosphorylation site determination from enriched phosphopeptide

Beta casein 2062 phosphopeptide CAF sequencing

FQpSEEQQQTE

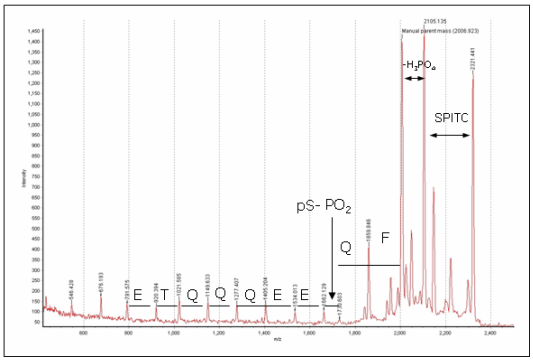
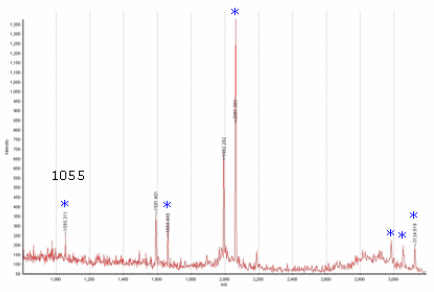


Fig.6. Determination of phosphorylation site of beta casein phosphopeptide enriched using Phos-Pep™ followed by chemical assisted fragmentation (CAF).

Phosphopeptide enrichment from beta-casein trypsin digest mixed with pTyr synthetic phosphopeptide



PSD spectrum of 1055(m/z)
(**pTyr-Asp-Leu-Leu-Leu-Glu**)

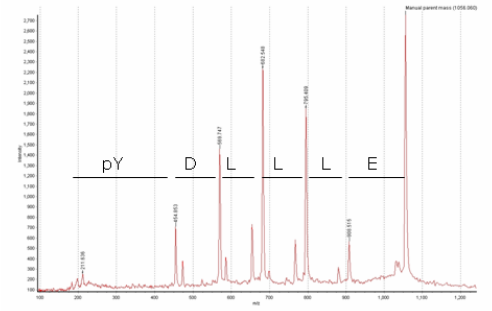


Fig.7. Enrichment and phosphorylation site determination of phosphopeptide containing phosphotyrosin amino acid residue.