Phosphopeptide Enrichment using Sachtopore®-NP Titanium Dioxide

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We report a rapid highly selective enrichment procedure utilizing 2,5-dihydroxybenzoic acid (DHB) to enhance the selective enrichment of phosphorylated peptides on a titanium dioxide micro-column. This unique technique dramatically increases the selectivity, and thus sensitivity, of enrichment purification of phosphorylated peptides from complex mixtures of non-phosphorylated and phosphorylated peptides.

Introduction

Historically, researchers have been unable to fully realize the benefits of mass spectrometry as an analysis method for phosphopeptides because isolation of the molecules from non-phosphorylated peptides is frequently required before examination of the complex samples can proceed (1). Previously, immobilized metal affinity chromatography (IMAC) was the most widely utilized technique for phosphopeptide enrichment by mass spectroscopy. However, IMAC methods can vary widely in effectiveness, use valuable research time for the required metal ion loading/washing steps and are difficult to incorporate into on-line applications (1). As non-specific binding of non-phosphorylated peptides further hampers the technique, researchers using mass spectroscopy needed a more specific on-line technique for isolating the phosphopeptides in order to fully realize the time saving benefits of mass spectroscopy (1, 2).

The following rapid (less than 5 min/sample) highly selective enrichment procedure, developed by the Department of Biochemistry and Molecular Biology, University of Southern Denmark (Odense, Denmark), dramatically increases the selectivity of enrichment in comparison to traditional IMAC methods.

Experimental

A complex mixture of phosphorylated and non-phosphorylated peptides, diluted 1:5 in loading buffer, was enriched using titanium dioxide bulk particles packed into a 3mm long micro-column. The enrichment procedure was as follows:

Column:	Sachtopore®-NP titanium dioxide micro-column [Part# TI02-0310-5(100)]
Loading Buffer:	10uL-300 mg/mL DHB in 80/20 ACN/1% TFA, pH 1.9
Wash Buffer:	1. 10uL-300 mg/ml DHB in 80/20 ACN/0.1% TFA pH 1.9 2. 20uL-80/20 ACN/0.1% TFA, pH 1.9
Elution Buffer:	20uL - NH4OH, pH 10.5

Figure 1 demonstrates performance of the material with a relatively simple mixture (1:1 ratio) of non-phosphorylated and phosphorylated peptides. At this level of complexity the titania based method compares favorably with traditional techniques, enabling detection of equal numbers of phosphopeptides and reducing the number of non- phosphorylated peptides retained. As sample complexity increases so does the selectivity of the binding for the phosphorylated versus the non-phosphorylated peptides (see reference 2 for additional data).

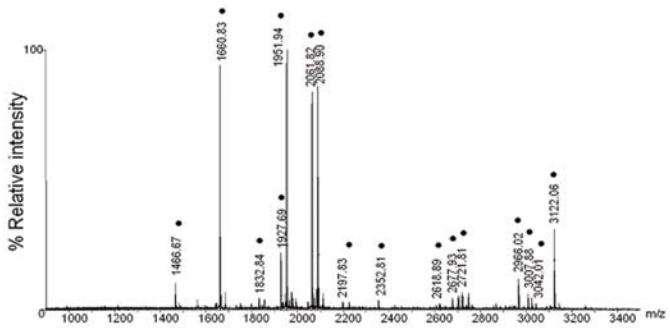

Figure 1: This MALDI mass spectra demonstrates the performance of TiO2 micro-columns for the selective enrichment of phosphorylated peptides (marked with dots) from a complex mixture (a tryptic digestion of 0.5 pmol of the phosphorylated proteins [β-casein, α-casein and ovalbumin] and 0.5 pmol of the non-phosphorylated peptides [serum albumin, β-lactoglobulin and carbonic anhydrase]).

This method can be tailored to your specific application needs. ZirChrom technical support can help to optimize and transfer this method to your site. Please contact ZirChrom technical support at 1-866-STABLE-1 or support@zirchrom.com for details about Sachtopore®-NP bulk or Sachtopore®-NP guard inserts.

Acknowledgements

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References

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(2) Larsen, M.R.; et al., Mol Cell Proteomics, **4**, 873-886 (2005).

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