Phosphopeptide Enrichment using Zirconium Dioxide and Titanium Dioxide

Literature Review

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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. IMAC methods can vary widely in effectiveness depending on the type of metal ion and loading/elution procedure. The technique also uses valuable research time for the required metal ion loading and washing steps and is difficult to incorporate into an on-line application (6). 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). Recently, several papers and posters have been published demonstrating the unique ability of titanium dioxide and zirconium dioxide to selectively retain phosphopeptides contained in complex biological mixtures (1, 2).

Titania Pre-Columns

For example, Pinkse et al. report and innovative approach to automate the method for the enrichment of phosphopeptides using a 2D technique with titanium dioxide particles as the first dimension and a reversed phase silica C18 column as the second dimension (1). The complex proteolytic digests were loaded onto the titanium dioxide pre-column using acidic conditions; retaining the phosphorylated peptides and allowing the rest of the digest to concentrate on the reversed phase column. After the analysis of the digest peptides on the reversed phase column is complete the phosphopeptides are eluted, under alkaline conditions, from the titanium dioxide for analysis. The authors report the method has a recovery above 90% and allows for the identification of previously uncharacterized phosphorylation sites (1). Additionally, they report the titanium dioxide pre-columns could be used for over 200 runs without reduced performance (1). However, using this method, the titanium dioxide does appear to have non-specific binding issues especially of nonphosphorylated peptides with acidic residues.

Larsen et al. took the Pinkse research one step further and dramatically improved the selectivity of the Pinkse method by loading the peptide samples onto the titanium dioxide in 2,5-dihydrobenzoic acid (DHB) (2). In a direct comparison of the titanium dioxide and IMAC methods for semi-complex samples the titanium dioxide pre-columns had a greater yield of phosphorylated peptides and fewer contaminating non-phosphorylated peptides (2). This effect was enhanced as the complexity of the samples increased (2).

Zirconia Microtips

At the recent ASMS 2005 conference Kweon et al. report the successful use of a zirconium dioxide microtip for the enrichment of phosphopeptides (3). Phosphopeptides from proteolytic peptide mixtures were selectively isolated and enriched by binding to zirconia microtips. For this application, the zirconia phosphopeptide enrichment proved superior to titanium dioxide and IMAC methods.

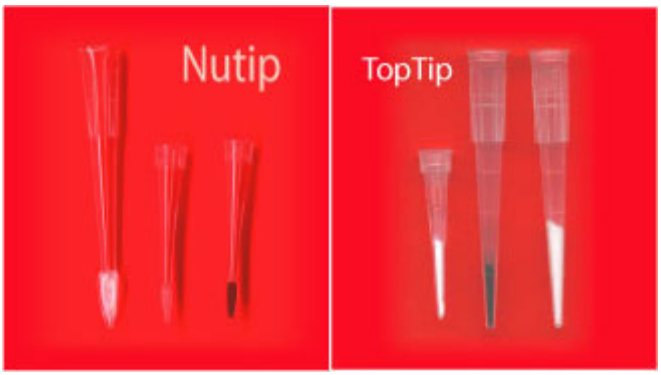

Figure 1. Glygen's Lab-in-a-tip™ SPE pipette tips

Sachtopore-NP (titanium dioxide) and ZirChrom-PHASE (zirconium dioxide) are available as bulk particles or packed analytical, semi-prep or prep sized HPLC columns. In addition, both materials are available as packed or embedded particle SPE pipette tips (Glygen's Lab-in-a-tip™ SPE pipette tips, Figure 1). More information is available on our website at www.zirchrom.com or by contacting a ZirChrom technical specialist by phone at 1-866-STABLE-1 or by e-mail at support@zirchrom.com.

References

(1) Pinkse, M.W.H.; Uitto, P.M.; Hilhorst, M.J.; Ooms, B.; Heck, A.J.R., Analytical Chemistry 2004, 76, 3935-3943.

(2) Larsen, M.R.; Thingholm, T. E.; Jensen, O.N.; Roepstorff, P.; Jorgensen, T.J.D., Mol Cell Proteomics, 2005, 4, 873-886.

(3) Kweon, H.K; Hakansson, K.; ASMS 2005 Poster "Characterization of Phosphopeptides by EDD in FT-ICR Mass Spectrometry"

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