Phosphopeptide Enrichment Using Titanium Dioxide & Zirconium Dioxide SPE Tips

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We report a rapid, highly selective enrichment procedure for phosphopeptides utilizing titanium dioxide (TiO_2) & zirconium dioxide (ZrO_2) SPE tips. β-casein digest samples purified via NuTip™ SPE tips, manufactured by Glygen Corporation (Columbia, MD), display exceptional signal to noise ratios for phosphopeptide analysis and eliminate many difficulties present in traditional IMAC methods.

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).

The following rapid enrichment procedure, developed by Glygen Corporation and New Objective, Inc. (Woburn, MA), maintains high enrichment selectivity without the complications and irreproducibility inherent in traditional IMAC methods (2).

Experimental

An overnight trypic β-casein digest was performed and the sample was then diluted with a 0.1% formic acid solution to generate a 1 pmol/µL solution. The enrichment procedure was as follows:

Product:	Titanium Dioxide & Zirconium Dioxide NuTip™ (part # NT1TIO & NT1ZRO)
Conditioning:	Tips conditioned with 5 aspiration/expulsion (A/E) cycles of HPLC grade water
Loading:	10 µL of sample loaded in 10 A/E cycles
Wash:	10 µL of HPLC grade water for 10 A/E cycles
Elution:	2 µL of 50/50 50mM NH_4HCO_3/50mM TEA in 5 A/E cycles
Post Elution:	Addition of 2 µL of a 50mM TEA in methanol solution followed by immediate mixing and centrifugation.
Detection:	All samples were analyzed via ESI-MS in negative-ion mode.

Figure 1 demonstrates performance of TiO_2 and ZrO_2 Trap'nTip™ (Trap'nTip™ is a miniaturized form of NuTip™, manufactured exclusively by Glygen Corporation for New Objective, Inc.). A phosphopeptide control set was used for tuning purposes and to confirm the identity of peaks in Figure 1. The results obtained on both TiO_2 and ZrO_2 compare favorably with traditional techniques, successfully enriching the phosphopeptides and thus greatly improving the signal-to-noise ratio for phosphopeptide analysis (2).

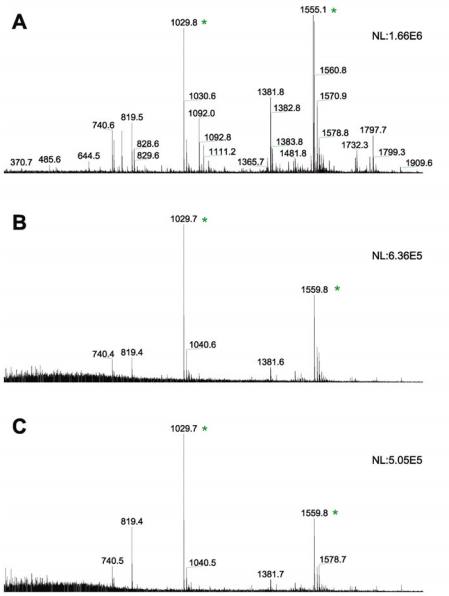

Figure 1: A) Spectrum of β-casein without enrichment B) Spectrum of β-casein after purification by TiO_2 Trap'nTip™ C) Spectrum of β-casein after purification by ZrO_2 Trap'nTip™ (2)

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 using TiO_2 and ZrO_2 SPE tips for phosphopeptide enrichment.

References

(1) Pinkse, M.W.H et al, *Analytical Chemistry*, **76**, 3935-3943 (2004).

(2) Toher, C.J., Perala, A.W., Shukla, A.K., Valaskovic, G.A., Shukla, M.M., Poster # TP13-215, ASMS 2006.

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