Comparison of Titanium Dioxide & Zirconium Dioxide SPE Tips for Phosphopeptide Enrichment

ZirChrom Separations, Inc.

Technical Bulletin # 325

The following compares and contrasts zirconium dioxide (ZrO_2) and titanium dioxide (TiO_2) SPE tips for rapid enrichment of phosphopeptides. Although, for the α-casein digest samples tested, either technique proves more effective than traditional methods, interestingly, the ZrO_2 tips enriched singly phosphorylated peptides in greater abundance.

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). Immobilized metal affinity chromatography (IMAC) techniques, the most widely utilized technique for phosphopeptide enrichment, 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 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 Kweon and Hakansson at the University of Michigan (Ann Arbor, MI), was applied to both the ZrO_2 and the TiO_2 NuTip™ SPE tips, manufactured by Glygen Corporation (Columbia, MD), to compare and contrast the enrichment of phosphopeptides from a trypic α-casein digest (2). The ZrO_2 and TiO_2 materials used in this study were manufactured by ZirChrom Separations and Sachtleben Chemie GmbH, (Duisburg, Germany), respectively.

Experimental

An overnight tryptic α-casein digest was performed and the sample was then diluted with 3.3% formic acid (pH 2) to generate a 100 pmol solution. The enrichment procedure was as follows:

Product:	50 µg Zirconium Dioxide NuTip™ (part # NT1ZRO) & 50 µg Titanium Dioxide NuTip™ (part # NT1TIO)
Conditioning:	Tips conditioned with 10 µL 3.3% formic acid (pH 2) for 3 aspiration/expulsion (A/E) cycles.
Loading:	10 µL of sample loaded in 10-20 A/E cycles
Wash:	10 µL of HPLC grade water for 2 A/E cycles
Elution:	10 µL of 0.5% piperidine (pH 11.5) for 2 A/E cycles
Post Elution:	Eluted samples were dried and reconstituted in 2-propanol/ACN/water (1:1:2) with 0.25% piperdine.
Detection:	All samples were analyzed via ESI FT-ICR in negative-ion mode.

Figure 1 compares three mass spectra; (a) before enrichment , (b) after enrichment using ZrO_2 SPE tips, and (c) after enrichment using TiO_2 SPE tips . Phosphopeptides are numbered and non-phophorylated peptides are labeled with their corresponding amino acid residue numbers.

The most abundant species following enrichment with the ZrO_2 material is the singly phosphorylated peptide 7. Whereas the most abundant signal following TiO_2 enrichment is the doubly phosphorylated peptide 6. This phenomenon is not hypothesized to be due to irreversible binding of the multiply phosphorylated peptides to ZrO_2 as these peptides were present in the solution left-over after enrichment.

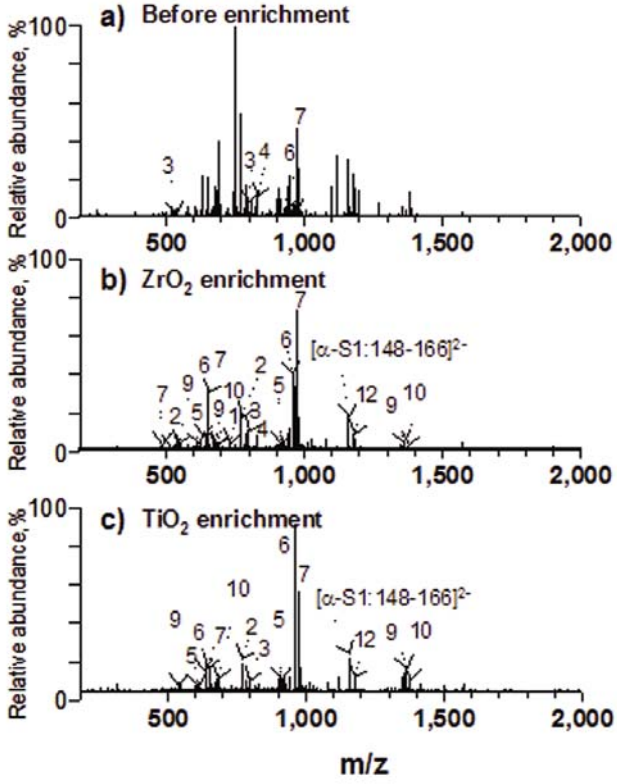

Figure 1: Negative mode ESI FT-ICR mass spectra (8 scans) of a tryptic digest of α-casein obtained; (a) prior to enrichment, (b) after ZrO_2 enrichment, and (c) after TiO_2 enrichment.

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.

References

(1) Pinkse, M.W.H et al, *Analytical Chemistry*, **76**, 3935-3943 (2004).
(2) Kweon, H.K; Hakansson, K.; *Analytical Chemistry*, **78**, 1743-1749 (2006).

ZirChrom Separations, Inc.
617 Pierce Street, Anoka, MN 55303
1-866-STABLE-1
support@zirchrom.com