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

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The following compares and contrasts the selectivity of zirconium dioxide (ZrO_2) and titanium dioxide (TiO_2) SPE tips with varying concentrations of α-casein for rapid enrichment of phosphopeptides. The results demonstrate that phosphopeptide selectivity for α-casein was not compromised when sample amount was decreased to 25 pmol. Below 25 pmol, a smaller (25 μg) SPE tip is required to obtain acceptable signal to noise ratios.

Introduction

Immobilized metal affinity chromatography (IMAC), the most widely utilized technique for phosphopeptide enrichment, can vary widely in effectiveness, uses valuable research time for the required metal ion loading/washing steps and is difficult to incorporate into on-line applications (1). As non-specific binding further hampers the technique, researchers need 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 various sized ZrO_2 and the TiO_2 NuTip™ SPE tips, manufactured by Glygen Corporation (Columbia, MD), to analyze the sensitivity of the enrichment of phosphopeptides from several concentrations of 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 trypic α-casein digest was performed and the sample was then diluted with 3.3% formic acid (pH 2) to generate a 100 pmol solution. Samples of decreasing concentration were created from this solution, and the sensitivity of the enrichment protocol was tested. The enrichment procedure was as follows:

Product:	50 & 25 μg ZrO_2NuTip™ (part # NT1ZRO) 50 & 25 μg TiO_2 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.

Table 1 demonstrates clearly that phosphopeptide selectivity is not compromised when sample amount is decreased from 100 pmol to 50 and then finally to 25 pmol. As the MS analysis system was not optimized for high sensitivity, poor signal to noise values were obtained with samples lower than 25 pmol in concentration. Halving the size of the SPE tips (refer to Figure 1) dramatically improves the signal to noise ratios for a 1 pmol sample however selectivity is still compromised when compared to higher concentration samples.

Table 1. Selectivity[a] (%) of 50-ug ZrO_2 and TiO_2 Microtips for Phosphopeptide Enrichment of a Tryptic Digest of a-casein as a Function of Sample Amount

	100 pmol	50 pmol	25 pmol
without enrichment	27	29	29
ZrO_2 Enrichment	67	85	83
TiO_2 Enrichment	62	77	74

[a] *Defined as relative phosphopeptide signal*

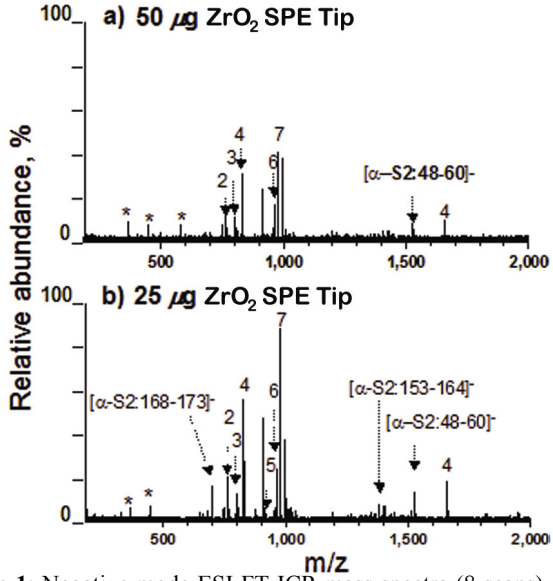

Figure 1: Negative mode ESI FT-ICR mass spectra (8 scans) from 1pmol of a trypsin digest of α-casein obtained following phosphopeptide enrichment with a 50-ug ZrO_2 (a) and a 25-ug ZrO_2 NuTip™ SPE tip (b). Identified nonphosphorylated peptides are labeled with their corresponding amino acid residue numbers and α-casein isoform.

NuTip™ is a trademark of Glygen Corporation.

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).

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