Effect of Loading Buffer on Phosphopeptide Enrichment using Zirconium Dioxide SPE Tips

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The following text investigates the effect of sample loading buffer on a rapid, highly selective enrichment procedure for phosphopeptides utilizing zirconium dioxide (ZrO_2) SPE tips. For α-casein digest samples enriched using ZrO_2 NuTip™ SPE tips, manufactured by Glygen Corporation (Columbia, MD), a low pH formic acid loading buffer enabled the most effective and specific enrichment of phosphopeptides.

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).

This rapid enrichment procedure, developed by Kweon and Hakansson at the University of Michigan (Ann Arbor, MI), optimizes sample loading conditions for the enrichment of phosphopeptides using zirconium dioxide SPE tips (2).

Experimental

An overnight tryptic α-casein digest was performed and the sample was then diluted with loading solution (see figure 1) to generate a 100 pmol solution. The enrichment procedure was as follows:

Product:	50 µg Zirconium Dioxide NuTip™ (part # NT1ZRO)
Conditioning:	Tips conditioned with 10 µL loading solution (see figure 1) 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 solution for 2 A/E cycles
Post Elution:	Eluted samples were dried and reconstituted in 2-propanol/ACN/water (1:1:2) with 0.25% piperidine.
Detection:	All samples were analyzed via ESI FT-ICR in negative-ion mode.

Figure one compares the spectra of five different sample loading buffer conditions. The superior loading buffer is the 2.4% formic acid buffer (pH 2.0). Even raising the pH to 3.0 makes a large difference in the number of contaminating non-phosphopeptides (2). To achieve maximum recovery of the bound analytes the washing and elution solutions were also optimized. The highest phosphopeptide recovery was achieved with water washing solution and a 0.5% piperidine (pH 11.5) elution solution (2).

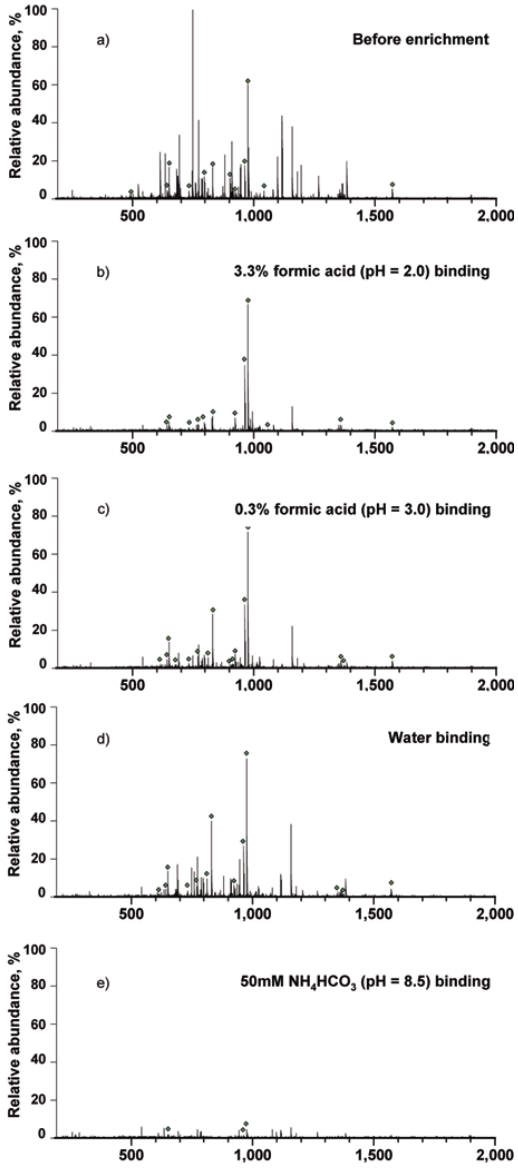

Figure 1: Negative mode ESI FT-ICR mass spectra (8 scans) of a tryptic digest of α-casein obtained prior to enrichment (a) and following enrichment using various loading solutions (b-e). Phosphopeptides indicated by green diamonds.

This method can be tailored to your specific application needs. 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).