The reversible phosphorylation of serine, threonine, and tyrosine is one of the most abundant and significant posttranslational modifications involved in a variety of cellular functions. Identification of phosphorylated sites by mass spectrometry is challenging due to the low abundance of phosphopeptides and their low ionization efficiency. Therefore, it is critical to select the phosphopeptides prior to MS analysis.

One of the most commonly used enrichment techniques is immobilized metal affinity chromatography (IMAC). With this approach, the phosphorylated peptides are enriched by the selective elution with metals, such as Fe³⁺. With these methods, optimal enrichment and recovery depend largely on the type of metal used, loading matrix, and the elution/backwash procedures. Recently, Threonine-selective TiO₂ has been used as an alternative to IMAC. [1]

IMAC sorbents, along with TiO₂ sorbent, were evaluated for phosphopeptide affinity extraction in terms of selectivity and recovery, optimizing various binding and elution solutions using a standard sample containing synthetic phosphopeptides. All three types of phosphorylated amino acids, such as phosphoserine, phosphothreonine and phosphotyrosine are represented in the sample. The synthetic phosphopeptides are spiked into an enolase tryptic digest in various ratios. All sorbents in this study were packed in a 96-well micro ELUTION plate. Selectivity was evaluated using LC/MS.

Processed using TiO₂ (w/ Enhancer)

TiO₂: After Extraction

78% 65%

Figure 2: A mixture of enolase tryptic peptides with a synthetic phosphopeptide in 50:1 molar ratio solubilized in 0.1% TFA with 80% MeCN. Significant loss of T43_1P was observed for TiO₂ (with 0.1% TFA loading condition and the IMAC (NTA-Fe III)).

CONCLUSION

TiO₂ is tolerable towards contaminants such as detergents

Selection Comparison

TiO₂: After Extraction

Control: Enolase Digest spiked with 4 Phosphopeptides in a 1:1 Molar ratio

T43_1P

Control: Enolase Digest spiked with 1 Phosphopeptide

T19_1p HLADL(pS)K

72% 65%

800 1000 1200 1400 1600 1800 2000 2200 2400 2600 2800 3000 3200 3400 3600 3800

Figure 3. The left panel is the LC/MS data of 200 µl of TiO₂ digestion solution. The right panel shows the MALDI-TOF analysis of T15-16 before and after affinity enrichment using TiO₂ and IMAC. The right panel shows that the phosphopeptide selectivity between TiO₂ and IMAC. LC/MS analysis of the elution from TiO₂ and IMAC is shown. The last two chromatograms are the two IMAC materials with Poros MC and NTA both chelated with Fe (III). TiO₂ with 0.1% TFA loading condition and the IMAC (NTA-Fe III). Significant loss of T43_1P was observed for TiO₂ (with 0.1% TFA loading condition and the IMAC (NTA-Fe III)).

SPE protocol for TiO₂ involves less steps than IMAC since metal chelation is not required.

IMAC sorbent amount used: 2.5 mg Poros MC, 50 µl NTA-Ni agarose gel

Elution: 50 µl of 0.3N Ammonium Hydroxide

Metal chelation: Fe (III)

Elution condition: 0.3N Ammonium Hydroxide

Washing condition: 200 µl of 25% MeCN in 1% Acetic Acid

Phosphopeptide Enrichment Protocols

IMAC protocol

Sample loading conditions: 10 ml of 1% TFA, 5% Acetic Acid

Elution: 50 µl of 3% Ammonium Hydroxide

SPE protocol

Sample loading conditions: 100 µl of 0.1% TFA on 50 µl of 1% Acetic Acid

Metal chelation: 1% Fe³⁺

TiO₂ protocol

Sample loading conditions: 100 µl of 0.1% TFA on 50 µl of 1% Acetic Acid

Elution: 50 µl of 3% Ammonium Hydroxide

Instrumentation

Waters Separation Module 2795 and Waters Micromass ZQ

Waters Micromass Q-TOF

Waters Q-TOF API Q-TOF

Waters MALDI Protein MS

References


TO DOWNLOAD A COPY OF THIS POSTER VISIT WWW.WATERS.COM/POSTERS