PHOSPHOPEPTIDE ENRICHMENT ON A MONOLITHIC COLUMN CONTAINING SUPERFICIALLY IMMOBILIZED NANO TIO_2

Fichtenbaum, Andreas 1; Pungor, András 2; Nagy, Zoltán 2; Černigoj, Urh 3; Vidič, Jana 3;Barut, Miloš3 ; Mitulović, Goran 1
1Medical University of Vienna; 2University of Debrecen, Debrecen, Hungary;3BIA Separations d.o.o. , Ajdovščina, Slovenia

INTRODUCTION

Enrichment of phosphopeptides prior to LC-MS analysis is a crucial sample preparation step because of their low stoichiometry in biological sample, longer retention on reversed phase columns, and lower ionization efficiency compared to non-phosphorylated peptides [1].The use of metal oxides, most prominently of TiO_2 enabled efficient and relatively simple phosphopeptide-enrichment. In this study a new monolithic column from BIA Separations containing immobilized TiO_2-nanoparticles was tested for its ability to enrich phosphopeptides. The TiO_2-column was also tested for possible carryover originating from biological samples. In conclusion, tested monolithic TiO_2 columns show significant binding ability for phosphopeptides and are considered as suitable for phosphopeptide enrichment.

MATERIAL AND METHODS

PROTEIN DIGEST

Bovine α – casein, Biological sample (50mM TEAB)

Trypsin 1:50 (w/w)

37°C: Incubation 17h

+ 3µl TFA → Aliquotation and Storage -20°C

PHOSPHOPEPTIDE ENRICHMENT

Loading
- Column equilibration and sample loading with Loading Solvent (LS):
- Collection of Flow Through → (FT)

Wash 1
- Solvent = LS → (W1)

Wash 2
- 80% ACN/0.1%TFA → (W2)

Elution
- Peptide Elution with Elution Solvent (ES)→ (E1,E2)
- → Acidification with TFA

Fig.1: Workflow for phosphopeptide enrichment on the monolithic nano-TiO_2 column.

LS=Loading solvent:
20%acetic acid, 420mM 1-octane-sulfonic acid, 50mg/ml Dihydroxybencoic acid, 0.1% Heptaflurobutyric acid
ES=Elution Solvent:
50%ACN/50%ammonium dihydrogenphosphate, pH10.5
ACN=Acetonitrile

SAMPLE PREPARATION for NANO-LC-MS

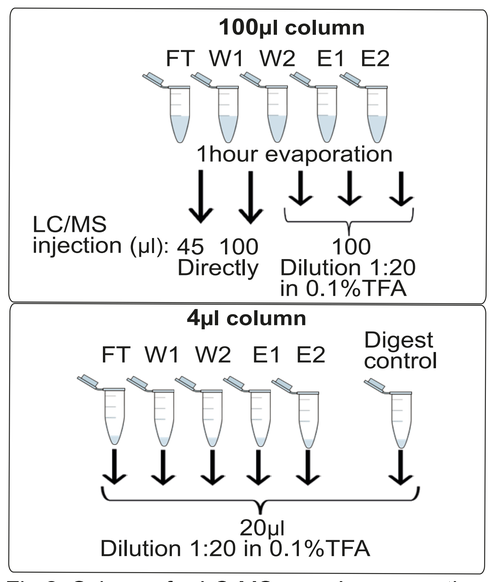

Fig.2: Scheme for LC-MS sample preparation of fractions obtrained from phosphopeptide enrichment of α-casein on the monolithic nano-TiO2 column.

NANO-LC-MS ANALYSIS

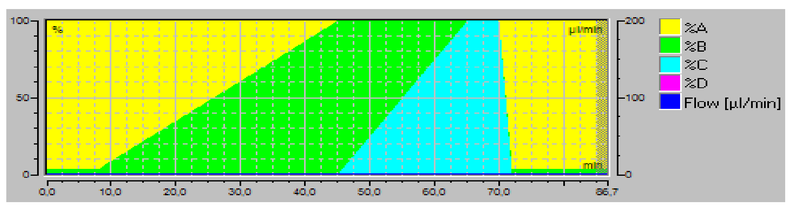

Fig.3: Gradient of the NANO-RP separation system. Mobile Phases:
A: 5%ACN, 0.1% FA
B: 15%ACN 15%MeOH, 0.1%FA
C: 60%ACN 30%MeOH 10%TFE. 0.08%FA

DATA ANALYSIS

MS database search was performed using “Mascot Daemon” and the most current SwissProt database. Further analysis were performed using the software “Scaffold Q+S”. Peptide threshold was set to 90% and the minimum number of peptides was set to “2”

RESULTS - Phosphopeptides retained on TiO_2 column versus Blank column

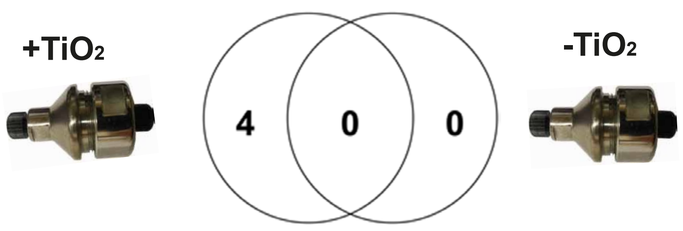

Fig.4: Comparison of identified phosphopeptides of tryptical digested bovine α-casein in the elution fraction “E1” collected from phosphopeptide enrichment on a monolithic column with and without immobilized nano-TiO_2 particles.

RESULTS - Carryover of the monolithic nano-TiO_2 column

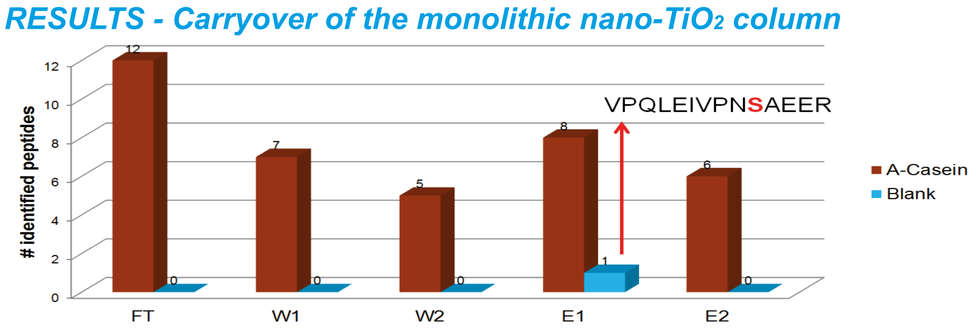

Fig.7: Testing carryover of the nano-TiO_2 monolithic column after phosphopeptide enrichment with α-casein (brown) and a blank sample (blue).

RESULTS - Phosphopeptides enriched on the monolithic nano-TiO_2 column

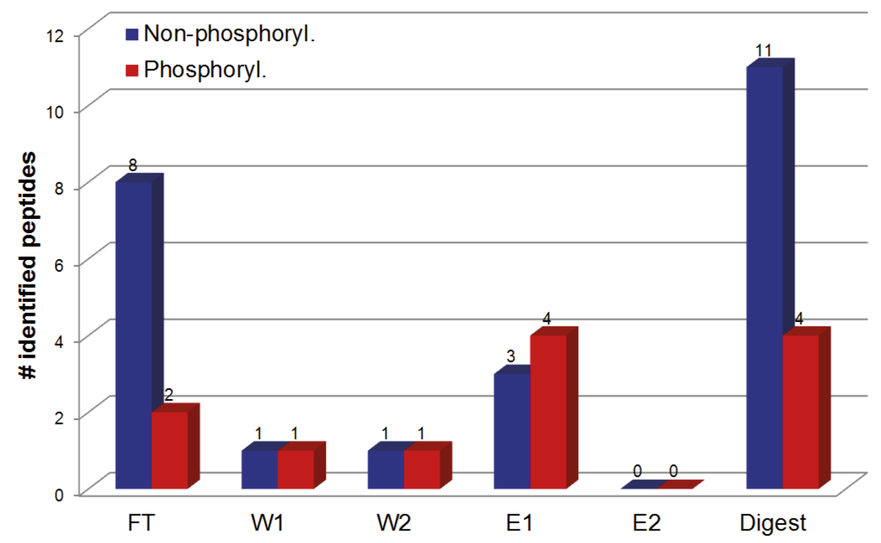

Fig.5: Number of phospho- and non-phosphorylated peptides from tryptically digested bovine α-casein. Fractions (FT,W1,W2,E1,E2) were collected upon phosphopeptide enrichment on a monolithic column containing immobilized nano-TiO_2 particles. α-casein digest (Digest) directly injected to LC-MS served as control sample.

DIGSESTEDQAMEDIK
DIGSESTEDQAMEDIK
DIGSESTEDQAMEDIK
EQLS/TSEENSK
EQLSTSEENSK
EQLSTSEENSKK
EQLSTSEENSKK
KTVDMES/TEVFTK
TVD[oM]ES/TEVFTK
TVDMES/TEVFTK
TVDMESTEVFTK
VPQLEIVPNSAEER
YKVPQLEIVPNSAEER

Fig.6: Phosphopeptides from α-casein identified in the elution fraction “E1” after phosphopeptide enrichment on the monolithic nano-TiO_2 column.

DISCUSSION

A new monolithic column containing superficially immobilized TiO_2 nano-particles was tested for its ability to enrich phosphopeptides from a tryptical digest of bovine α- casein . Binding ability of phosphopeptides to the particles was confirmed compared to a blank column (Fig.4).
As expected the majority of non-phosphorylated peptides didn’t bind to the nano-TiO_2 support. These were detected in the flow-through (FT) fraction. Elution fraction E1 contained most unique phosphorylated peptides (Fig.5).
Results of this evaluation study indicate the suitability of the tested nano-TiO_2 coated monolithic column for enrichment of phosphopeptides.
Carryover on the monolithic nano-TiO_2 column was not observed except in elution fraction “E1” the phosphopeptide VPQLEIVPNpSAEER was identified (Fig.7). Since this phosphopeptide was found constantly in all fractions (FT,W1,W2,E1,E2) it indicates a strong binding affinity to the column. Therfore, further protocol optimization in terms of column cleaning is suggested.

CONCLUSION

Mesoporous nano-TiO_2 particles immobilized on a monolithic support may provide higher capacity than common TiO_2 material and lower backpressure in HPLC which makes it capable also being applied to a nano-sized approach. Evaluating the binding ability and carryover of the column was a first step in this study but protocol optimization has to be evaluated especially on tryptical digests derived from a complex biological sample. Moreover, since a study of Bodenmiller et al [3], that compares several phosphopeptide enrichment methods revealed only 30% of overlapping phosphopeptides, another outlook to further improve MS identification rates on phosphorylations is the combination of the nano-TiO_2 support with a second enrichment technique such as e.g. Electrostatic Repulsion Chromatography.

REFERENCES

1. L. Beltran et al, Amino acids 43, 1009 (Sep, 2012).
2. M. Mazanek et al., Journal of chromatography. B, Analytical technologies in the biomedical and life sciences 878, 515 (Feb 15, 2010).
3. B. Bodenmiller et al, Nature methods 4, 231 (Mar, 2007).

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