Analytica Chimica Acta 942 (2016) 146–154

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Titanium dioxide nanoparticle coating of polymethacrylate-based chromatographic monoliths for phosphopetides enrichment

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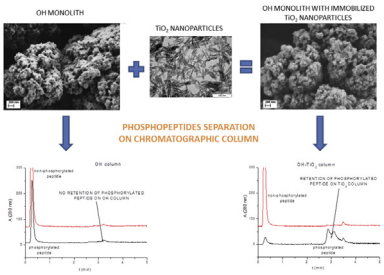
HIGHLIGHT

- Simple immobilisation of rutile TiO_2 nanoparticles onto polymethacrylate monolith.
- Stable and homogeneous monolayer of TiO_2 nanoparticles was proven.
- Application of MOAC in combination with chromatographic monolith.
- Chromatographic separation of phosphorylated peptides from nonphosporylated.
- Enrichment of phosphorylated peptides from digested model protein.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 7 June 2016
Received in revised form
28 August 2016
Accepted 29 August 2016
Available online 19 September 2016

Keywords:
Proteomics
Monolith
Phosphopeptide enrichment
Chromatography

ABSTRACT

Metal oxide affinity chromatography has been one of the approaches for specific enrichment of phosphopeptides from complex samples, based on specific phosphopeptide adsorption forming bidentate chelates between phosphate anions and the surface of a metal oxide, such as TiO_2, ZrO_2, Fe_2O_3, and Al_2O_3. Due to convective mass transfer, flow-independent resolution and high dynamic binding capacity, monolith chromatographic supports have become important in studies where high resolution and selectivity are required. Here, we report the first synthesis and characterization of immobilisation of rutile TiO_2 nanoparticles onto organic monolithic chromatographic support (CIM-OH-TiO_2). We demonstrate the specificity of CIM-OH-TiO_2 column for enrichment of phosphopeptides by studying chromatographic separation of model phosphorylated and nonphosphorylated peptides as well as proving the phosphopeptide enrichment of digested bovine α-casein. The work described here opens the

Abbreviation: ACN, acetonitrile; ADHP, ammoniumdihydrogen phosphate; ATR, attenuated total reflection; BET, Brunauer–Emmett–Teller; BSA, bovine serum albumin; CID, collision induced dissociation; CIM, convective interaction media; CIM-OH, CIMac™ hydroxyl-based analytical column; CIM-OH-TiO_2, CIMac™ hydroxyl-based analytical column with immobilized TiO_2 nanoparticles; CV, column volume; DBC, dynamic binding capacity; ESI, electrospray ionization; FA, formic acid; FDR, false discovery rate; FTIR, Fourier transformation infrared spectroscopy; HPLC, high performance liquid chromatography; IMAC, immobilized metal affinity chromatography; LC, liquid chromatography; MeOH, methanol; mgf, mascot generic file; MPA, binding mobile phase – mobile phase A; MPB, elution buffer - mobile phase B; MOAC, metal oxide affinity chromatography; MS, mass spectrometry; MS/MS, tandem mass spectrometry; NaOH, sodium hydroxide; nPh, non-phosphopeptides; OH, hydroxyl group; Ph, phosphopeptides; RPLC, reverse phase chromatography; SEM, scanning electron microscopy; TEAB, triethylammonium bicarbonate; TEM, transmission electron microscopy; TFA, trifluoroacetic acid; TFE, trifluroethanol; XRD, x-ray diffraction.

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http://dx.doi.org/10.1016/j.aca.2016.08.044

Metal oxide affinity chromatography
TiO_2 nanoparticles

possibility for a faster, more selective enrichment of phosphopeptides from biological samples that will enable future advances in studying protein phosphorylation.

1. Introduction

Protein phosphorylation is an essential ubiquitous post translational modification and plays important roles in regulation of vast array of cellular and molecular processes [1]. Due to the significance of protein phosphorylation in biological processes, many efforts have been made to investigate protein phosphorylation [2]. The complexity of phosphorylation (low stoichiometry, wide dynamic range, heterogeneity of phosphoprotein isoforms present in biological samples) requires highly sophisticated methods for in-depth investigations of protein phosphorylation, such as mass-spectrometry-based phosphoproteomics [3]. For biological significance, the latter requires high enrichment of phosphorylated molecules upstream of MS analyses [4]. The most common enrichment strategies are based on immobilized metal affinity chromatography (IMAC) and metal oxide affinity chromatography (MOAC). Due to its higher tolerance for low pH treatment of samples, required for protonation of carboxyl groups while keeping the negative charges on phosphorylated residues, MOAC leads to higher selectivity for phosphorylated molecules compared to IMAC [4]. Different metal oxides form complexes with phosphates in peptides and proteins, such as TiO_2, ZrO_2, Fe_2O_3, or Al_2O_3 [5–8]. If used in parallel, these MOAC materials have shown differences in affinity and specificity for phosphate groups and could therefore provide complementary information in phosphoproteome analysis [9]. Taking advantages of selectivity, recovery, and relatively high salt tolerance [10] TiO_2-based MOAC materials have been regarded as the most powerful and promising materials for phosphopeptide sample preparation [11].

TiO_2 naturally appears in three different crystalline forms and can be prepared as nano-sized particles or macroscopic crystals [12]. The main industrial application area is using it as a pigment in white paints, plastics, paper, textiles, cosmetics and food. Besides its use as a white pigment, TiO_2 has been studied for applications in photocatalysis [13], and nanocoating with different applications, such as super-hydrophilic surfaces and anti-reflective coatings [14]. Moreover, due to its inertness and high biocompatibility [15], TiO_2 presents a very suitable support as a biochromatographic stationary phase. Its usefulness as a chromatographic support has thus far been limited by the challenges presented by introduction of chromatographically active ligands onto solid TiO_2 chromatographic supports. To date, organic molecules could only be immobilized onto titania surface by exploiting the strong chemical coordinative adsorption mechanism of carboxyl groups in dicarboxylic or tetracarboxylic molecules (binding constant $K \approx 10^4$–10^5 M^{-1} and 10^5–10^6 M^{-1}, respectively) [16,17]. A similar coordinative adsorption mechanism was proposed for phosphate groups ($K \approx 10^4$–10^5 M^{-1}) [18], which has been used to rationalise the affinity interaction between phosphorylated proteins and peptides and TiO_2 surface [19].

Traditionally, phosphoprotein enrichment has been achieved using macroscopic TiO_2 particles [8,11], though nano-sized TiO_2 would be advantageous due to higher contact area with phosphopeptides and phosphoproteins. This approach suffers from difficult handling, aggregation of particles and possible toxicity of nanoparticle [20–22]. An elegant solution combining the advantages of macroscopic and nano TiO_2 is to prepare a pure TiO_2 monolithic column from titanium precursors, but due to the complexity of such synthesis and problems with leaking there are just few successful reports of TiO_2 capillary monoliths [23]. We reasoned, therefore, that the immobilisation of nano-sized TiO_2 onto a surface of a macroporous chromatographic support would harness the benefits both of nano-particulate TiO_2 as ligands for selective phosphopeptide enrichment, and unique chromatographic properties of monolith supports, while minimising the issues with handling and toxicity of TiO_2. Polymethacrylate monolithic supports have been reported as innovative chromatographic matrices with high porosity and convective mass transport [24,25], therefore integration of nanoparticles onto such material appeared as an alternative solution [26]. Different types of nanoparticles (gold, iron oxides, latex, carbon nanotubes, and hydroxyapatite) have been integrated onto monolithic supports either via encapsulation during polymerization [27–29] or by immobilisation of nanoparticles (but not TiO_2) directly onto monolith pore surface [30,31]. The latter approach results in a significant increase in the effective surface area due to the high surface-to-volume ratio of nanomaterials. To our knowledge there are no existing reports in the literature, describing the immobilisation of TiO_2 nanoparticles onto monolithic support. TiO_2 reacts coordinatively with aromatic diol groups (bidentate mechanism), such as in catechol [32], strong interactions of aliphatic diols with TiO_2 surface (molecularly adsorbed) have also predicted [33], which have not yet been exploited to immobilize TiO_2 nanoparticles to a polymeric support. In this article we describe a successful coating of rutile TiO_2 nanoparticles onto hydroxyl-based (OH) Convective Interaction Media™ (CIM™) chromatographic support (CIM-OH-TiO_2 column). Composite material characterization data on CIM-OH-TiO_2 columns are evaluated and a selectivity of the column for model peptides is demonstrated.

2. Materials and methods

2.1. Materials

Pure rutile TiO_2 nanoparticles sol in aqueous system at pH 3.6 (40 mg mL^{-1}) were obtained from University of Debrecen, Hungary. Phosphorylated and nonphosporylated peptides were designed at the Medical University of Vienna and synthesized at University of Debrecen (Table 1). Raw phosphopeptides were purified using reversed phase chromatography on a PepMap C18 column (300 μm ID × 15 cm length, 100 Å pore size, 3 μm particle size, Thermo Scientific, Germering, Germany). Sodium hydroxide (NaOH, 98%), 96% H_2SO_4, acetic acid (AA, 99.8%), 37% HCl (99.8% purity), sodium chloride (NaCl, 99.8%), trisodium phosphate (99%), formic acid (FA, 98%), HPLC grade acetonitrile (ACN), triethylammonium bicarbonate (TEAB, 1 M, pH 8.5 ± 0.1), 2,5-dihydroxybenzoic acid (DHB, >99.0%) and proteins (ovalbumin, phosvitin, α-casein, cytochrome C, lysozyme and human serum albumin; all of them with purity higher than 95%) were purchased from Sigma-Aldrich (St. Louis, MO, USA); ammonium hydroxide, trifluoroacetic acid (TFA, 98%), heptaflurobutyric acid (HFBA, 99.5%), and sodium 1-octanesulfonate monohydrate (1-OSA,~98%) were purchased from Fluka (St. Gallen, Switzerland); disodium hydrogen phosphate (99%) was purchased from Honeywell (Morristown, NJ, USA). HPLC grade methanol (MeOH) and ammonium dihydrogen phosphate (ADHP, PhEur) were