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Application of open tubular capillary columns coated with zirconium phosphonate for enrichment of phosphopeptides

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ABSTRACT

A new approach utilizing open tubular capillary columns coated with zirconium phosphonate (ZrP-OTCC) for enrichment of phosphopeptides is described. The experimental conditions: interior diameter, length of capillary and flow rate was optimized using tryptic digest of α-casein (a phosphoprotein) as a model sample. The ZrP-OTCC was demonstrated to tolerate urea, sodium dodecyl sulphate (SDS), and NaCl. Further experimental results show that the ZrP-OTCC can trap the phosphopeptides even at the concentration of α-casein as low as 10^{-8} M. This column has also been successfully coupled online with nano-liquid chromatography for enrichment and then separation of phosphopeptides from a complex sample, and finally analyzed the phosphopeptides by mass spectrometry (MS).

1. Introduction

Reversible phosphorylation plays an important role in regulating many biological processes and functions such as cell proliferation, differentiation, metabolism, communication, and signal transduction pathways [1–3]. There are 25–30% of the proteins in eukaryotic cells that have been phosphorylated in their life cycle [4]. Therefore, the analysis of protein phosphorylation is very important for the understanding of cellular processes. Recently, mass spectrometry (MS), including matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI) mass spectrometry, has been widely used in proteomics research as a powerful tool for its high sensitivity, accuracy and resolution [5–7]. However, large-scale analysis of phosphoproteins by MS remains a great challenge because of the low abundance of phosphoprotein in cells and the suppressive effects from nonphosphopeptides in protein digestion products. Therefore, selective enrichment of phosphopeptides from the tryptic digest of a protein mixture is a critical step before MS analysis.

Several strategies have been developed to effectively enrich the phosphopeptides from complex samples, such as immobilized metal affinity chromatography (IMAC) [8–16], strong cation/anion exchange chromatography [17], metal oxide and zirconium phosphonate (ZrP)-based enrichment. The Fe(III) coated IMAC beads based on the affinity attraction between metal ion and phosphate groups are widely used. The limitation of this approach is that some acidic nonphosphopeptides may have similar affinity with Fe(III) as phosphopeptides do and results in non-specific enrichments. In recent years, metal oxides such as TiO_2 [18–21], ZrO_2 [22–25] and Al_2O_3 [26] have been used as substitutes for Fe^{3+} for the enrichment of phosphopeptides to reduce the non-specific binding of nonphosphopeptides [24]. A novel ZrP-based approach used for trapping phosphopeptides was reported recently and has been demonstrated to have higher selectivity and sensitivity for enriching phosphopeptides than the conventional Fe^{3+}-IMAC beads do [27–30]. The improved enrichment performance of the method is probably due to the coordination properties of phosphate group which lead to strong affinity interactions between phosphate group and ZrP [31].

Open tubular capillary columns (OTCC) have been used in proteome research recently [32,33]. The characteristics of high resolving power, good column-to-column reproducibility, and relatively high loading capacity of the OTCC facilitate the comprehensive proteomic research. But to our knowledge, the applications of the OTCC coated with ZrP in the study of phosphorylation modifications have not been reported. Our previous research [13]

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described a robust IMAC-capillary technology for the enrichment and identification of phosphopeptides, and such column-based affinity method is easy to fulfill an automated separation, enrichment and MS analysis. So in this study, we report our effort of preparation and application of the open tubular capillary columns coated with zirconium phosphonate (ZrP-OTCC) for enrichment of phosphopeptides. After the interior wall of silica capillary is derivatized by covalent coating with 3-aminopropyl-triethoxysilane, $POCl_3$ and Zr^{4+} in sequence to form the ZrP-OTCC, the tryptic digest of phosphoprotein could be directly applied to the column for the enrichment of phosphopeptides without any prior desalting process. Most importantly, our ZrP-OTCC has been successfully combined online with the nano-scale liquid chromatography (nanoLC)-MS to fulfill an automated separation, enrichment and MS analysis, making it very promising for future applications in large-scale phosphoproteome studies.

2. Experimental

2.1. Materials and methods

Bovine α-casein and bovine serum albumin (BSA) were obtained from Sigma (St. Louis, MO, USA). The standard phosphopeptide (FLpTEYVATR, m/z = 1179.55) was synthesized by SBS Genetech Co. Ltd. (Beijing, China). The bovine milk was purchased from Mengniu (Inner Mongolia, China).

Sequencing grade porcine trypsin was obtained from Promega (Madison, WI, USA). Trifluoroacetic acid (TFA) and 2,5-dihydroxybenzoic acid (2,5-DHB) were purchased from Aldrich (Steinheim, Germany). 1,4-Dithio-DL-threitol (DTT) and iodoacetamide (IAA) were purchased from Pierce (Rochford, IL, USA). Fused silica capillaries with different specifications were obtained from Yongnian (Hebei, China). Zirconyl chloride octahydrate ($ZrOCl_2{\cdot}8H_2O$), 3-aminopropyl-triethoxysilane and 2,4,6-collidine were obtained from Acros (Morris plains, NJ, USA). Phosphorus oxychloride ($POCl_3$), hydrochloric acid, sodium hydroxide, urea, sodium dodecyl sulphate (SDS), sodium chloride and methanol were of analytical grade reagent. Deionized water (R > 18.2 MΩ) was prepared by using Millipore purification system (Billerica, MA, USA) and used throughout this work. Matrix (20 mg/mL) was prepared by dissolving 2,5-DHB in acetonitrile/water (50/50, v/v) solution containing 1% H_3PO_4 [34].

2.2. Preparation of the ZrP-OTCC

The ZrP-OTCCs of different lengths or inner diameters were prepared for the examination of their performance on the enrichment of phosphopeptides. The preparation process of the ZrP-OTCC was shown in Fig. 1. First, the capillary was manually infused with 0.1 M HCl at a low flow rate for 0.5 h using a syringe. After rinsing with deionized water, the capillary was injected with 0.1 M NaOH for 2 h. Subsequently, the capillary was washed by methanol and dried under N_2, and then 3-aminopropyl-triethoxysilane was added for reaction at 70 °C for 12 h with the capillary ends sealed. After that, the capillary was washed by methanol and dried under N_2 again. Next, $POCl_3$ (40 mM, diluted in 40 mM 2,4,6-collidine in anhydrous acetonitrile) was injected into it for reaction at room temperature for 12 h. After the same washing and drying process as

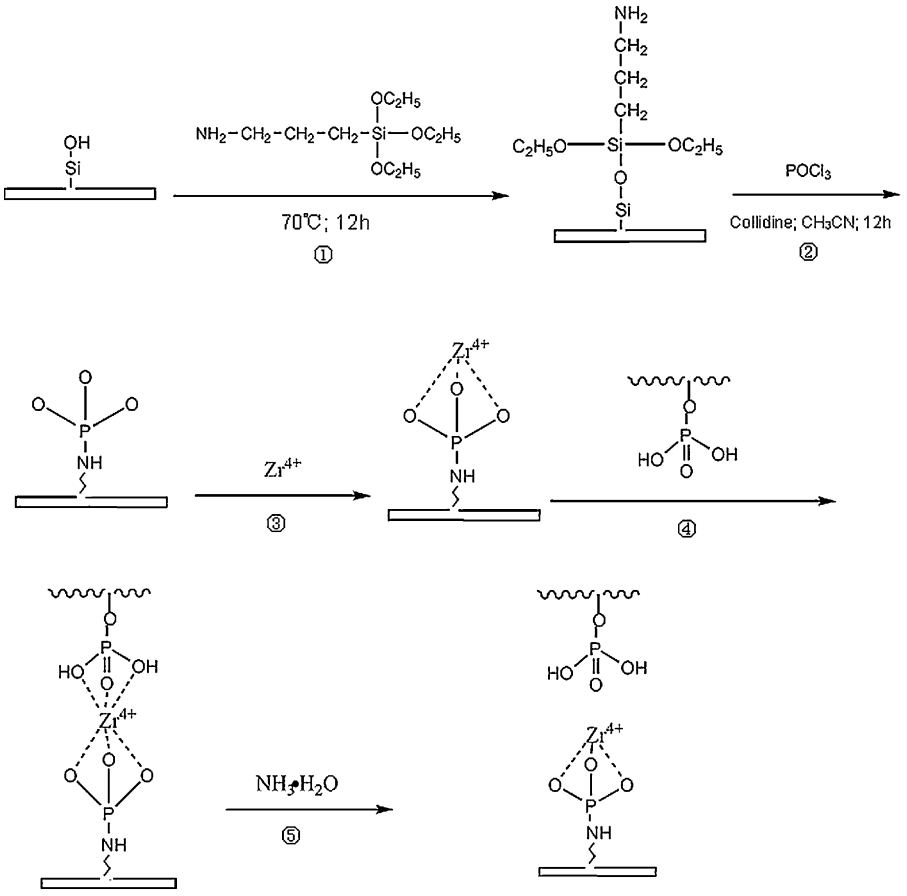

Fig. 1. Model for preparation of the ZrP-OTCC and the procedure of enrichment of phosphopeptides. Detailed description for each step: ① coating a thin layer of aminopropyl onto the interior surfaces of the OTCC; ② transforming the aminopropyl-terminated OTCC into the phosphate-terminated OTCC; ③ loading zirconium ions into the phosphate-terminated OTCC to form the ZrP-OTCC; ④ capturing the phosphopeptides from the tryptic digest; ⑤ eluting the captured phosphopeptides from the ZrP-OTCC.