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Perovskite for the highly selective enrichment of phosphopeptides

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ABSTRACT

Selective and effective enrichment of phosphopeptides from complex samples is essential in phosphoproteome study by mass spectrometry (MS). In this work, we compared perovskites ($MgTiO_3$, $CaTiO_3$, $SrTiO_3$, $BaTiO_3$ and $CaZrO_3$) with metal oxides (ZrO_2 and TiO_2) in their capability for the selective enrichment of phosphopeptides. It was found here that perovskites exhibited higher selectivity towards phosphopeptides than commonly used ZrO_2 and TiO_2, even though they all have high affinity to phosphopeptides. As for perovskites, $CaTiO_3$ exhibited better selectivity for enrichment of phosphopeptides than $SrTiO_3$, $MgTiO_3$, $BaTiO_3$ and $CaZrO_3$, which might be ascribed to their crystal structures and electrophilic abilities. Moreover, to further confirm the performance of $CaTiO_3$, $CaTiO_3$ and TiO_2 were applied to the enrichment of phosphopeptides from tryptic digest of proteins of human Jurkat-T cell lysate, respectively. The results showed $CaTiO_3$ has much higher selectivity than TiO_2 in the enrichment of phosphopeptides from the complex biological sample. Taken together, here we show that $CaTiO_3$ is an excellent material for the highly selective enrichment of phosphopeptides and it could be potentially used in the large-scale phosphoproteome study.

1. Introduction

Protein phosphorylation, one of the most important post-translational modifications, is involved in many cellular processes, including proliferation, differentiation and apoptosis [1–3]. Abnormal phosphorylation is commonly associated with various diseases, such as cancer and metabolic disorders [4]. Therefore, the identification and quantification of protein phosphorylation are necessary to elucidate their functions and useful for diseases diagnostics and therapeutics. Mass spectrometry (MS) based techniques have become the foremost choice for phosphoproteomics analysis because of their high sensitivity and accuracy [5–9]. However, due to the low abundance, substoichiometry and low ionization efficiency of phosphopeptides, it remains challenging to detect and analyze phosphopeptides from complex biological samples. Therefore, isolation and enrichment of phosphopeptides are essential prior to MS analysis [10–12].

Up to date, various methods have been developed for phosphopeptide enrichment, and metal oxide affinity chromatography (MOAC) is one of the most powerful and widely used approaches because it is robust and highly tolerant towards buffers and detergents [12,13]. MOAC takes advantage of the affinity interaction of metal oxide and phosphate groups in the phosphopeptide. Based on this principle, different metal oxides have been used for phosphopeptide enrichment, including TiO_2, ZrO_2, Al_2O_3, Fe_2O_3 and SnO_2 [14–18]. Although these metal oxides play important roles in the development of phosphoproteome, MOAC approach remains some defects, which need to be improved. For example, most of metal oxides show preferential enrichment of mono-phosphopeptides [13,16]. Different metal oxides or the same one with different crystal structures or morphologies display distinct affinity for phosphopeptides, whereas the theoretical mechanism is ambiguous [19,20]. On the other hand, composite oxides containing at least two kinds of metal oxides or nonmetallic oxides possess more remarkable properties than single-component metal oxide and show great potential in the specific capture of phosphopeptides [21–23]. Therefore, it is very important to investigate the enrichment selectivity of composite oxides for phosphopeptides, and to get a better understanding of MOAC.

Several composite oxides have been used for phosphopeptide enrichment [21–29]. Titania-zirconia (TiO_2-ZrO_2) monodisperse microspheres were synthesized and applied to enrich phosphopeptides from tryptic digests of standard protein α-casein [21].

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Compared with commercial TiO_2, TiO_2-ZrO_2 mixed microsphere is more specific for phosphopeptides. Zhang et al. reported the preparation of Ti-hexagonal mesoporous silica (Ti-HMS) for selective enrichment of phosphopeptides [22]. Because of the lower Lewis acidity as compared to TiO_2, Ti-HMS displayed less non-specific adsorption of acidic non-phosphopeptides. Our group has also developed ceria-zirconia composite (CeO_2-ZrO_2), silica-zirconia composite (SiO_2-ZrO_2), and titanium-containing magnetic mesoporous silica sphere for the effective enrichment of phosphopeptides [23–25]. And very recently, SnO_2-$ZnSn(OH)_6$ and $NiZnFe_2O_4$ were proven to display predominant selectivity towards multi-phosphopeptides [28,29]. Nevertheless, the application of composite oxides for the enrichment of phosphopeptides is still at an early stage, and how the change of the structure affects their selectivity towards phosphopeptides remains to be elucidated.

The broad conception of perovskite is a class of minerals which have the same type of crystal structure. The general formula of perovskite is ABO_3, where A can be Na^+, K^+, Ca^{2+}, Sr^{2+}, Pb^{2+}, Ba^{2+}, etc., and B can be Ti^{4+}, Zr^{4+}, Mn^{4+}, Fe^{3+}, Nb^{5+}, Ni^{3+}, Ta^{5+}, Th^{4+}, etc. [30–33]. In a narrow sense, perovskite means calcium titanate ($CaTiO_3$), which can be synthesized by sintering of CaO and TiO_2 at temperatures above 1300 °C [34]. Although $CaTiO_3$ can be seen as the combination of CaO and TiO_2, the properties of $CaTiO_3$ and TiO_2 are largely different. The ideal crystal structure of perovskites is cubic system. However, the change of A or B atoms will result in various types of structural distortions, ranging from cubic, orthorhombic to tetragonal crystals etc., which is likely to cause different properties of them [35].

In this work, we demonstrated the high selectivity of $CaTiO_3$ towards phosphopeptides. First, different perovskites including $MgTiO_3$, $CaTiO_3$, $SrTiO_3$, $BaTiO_3$ and $CaZrO_3$ were compared with ZrO_2 or TiO_2 for the enrichment of phosphopeptides from tryptic digest mixture of β-casein and BSA. And then, the selectivity of different perovskites was examined. The relationship between the selectivity and the structures of various materials was further discussed. Finally, the performance of $CaTiO_3$ and TiO_2 were compared in the enrichment of phosphopeptides from tryptic digest of proteins of Jurkat-T cell lysate.

2. Materials and methods

2.1. Chemicals and materials

Calcium titanium oxide ($CaTiO_3$, powder, <2 μm) and strontium titanium oxide ($SrTiO_3$, powder, <2 μm) were purchased from Dibai Chemical Reagent Co. (Shanghai, China). Magnesium titanium oxide ($MgTiO_3$, powder, <2 μm) and barium titanium oxide ($BaTiO_3$, powder, <2 μm), calcium zirconium oxide ($CaZrO_3$, ~325 mesh powder) were purchased from Aladdin Chemical Reagent Co. (Shanghai, China). HPLC grade acetonitrile (ACN) was purchased from Fisher Scientific (Pittsburgh, PA, USA). Titanium(IV) oxide (TiO_2, anatase), zirconium(IV) oxide (ZrO_2, powder), phosphoric acid (H_3PO_4), trifluoroacetic acid (TFA), 2,5-dihydroxybenzoic acid (2,5-DHB), bovine β-casein, bovine serum albumin (BSA), dithiothreitol (DTT), iodoacetamide (IAA) and trypsin were purchased from Sigma–Aldrich (St. Louis, MO, USA). Tris(Hydroxymethyl)aminomethane (Tris), urea, acetone, ethanol, acetic acid and calcium chloride ($CaCl_2$) were all of analytical reagent grade and supplied by Shanghai General Chemical Reagent Factory (Shanghai, China). Purified water was obtained with a Milli-Q apparatus (Millipore, Bedford, MA, USA).

2.2. Sample pretreatment

Bovine β-casein was originally made up into stock solutions at 1 mg/mL with Milli-Q water. Proteins were digested with trypsin by using an enzyme to substrate ratio of 1:50 (w/w) in 100 mM Tris-HCl (pH 8.5) at 37 °C for 16 h.

BSA (1 mg) was dissolved in 100 μL of denaturing buffer solution (8 M urea in 100 mM Tris-HCl pH 8.5). The dissolved BSA was reduced by 10 mM DTT for 30 min at 37 °C and alkylated by 20 mM IAA for 30 min at room temperature in the dark. The reduced and alkylated protein mixture was diluted with 300 μL 100 mM Tris-HCl (pH 8.5), and then digested with trypsin at an enzyme to substrate ratio of 1:50 (w/w) by incubating at 37 °C for 16 h. All the tryptic digests were lyophilized to dryness and stored at −80 °C before use.

Human Jurkat-T cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. The cells were harvested and lysed in RIPA lysis buffer (1% NP-40, 0.25% deoxycholate) with protease and phosphatase inhibitors on ice for 30 min. The cell lysate was centrifuged at 16,000 *g* under 4 °C for 30 min and the protein concentration of the supernatant was determined by Bicinchoninic Acid Assay. Proteins were precipitated with 1.5 mL of 50% acetone/50% ethanol/0.1% acetic acid on ice for 1 h, and then centrifuged at 1,800 *g* under 4 °C for 30 min. The protein pellet was re-suspended in 2 mL of buffer containing 8 M urea, 0.2 M Tris (pH 8.0), and 4 mM $CaCl_2$. The following digestion procedure was the same as that of BSA, and the digested products were desalted by C18 cartridge and stored at −20 °C before use.

2.3. Phosphopeptide enrichment

For phosphopeptide enrichment from tryptic digests of standard proteins, commercial metal oxides or perovskites (~300 μg) were added to 20 μL of peptides mixture (containing 2 pmol of tryptic digest of β-casein) and incubated at 37 °C for 30 min. After washing with 30 μL of 1% TFA/50% ACN twice, the trapped peptides were eluted with 20 μL 2.5% ammonium hydroxide. The eluted solution was then lyophilized to dryness. Two microliter of matrix solution (mixture of 20 mg/mL 2,5-DHB in 50% (v/v) ACN, 1% (v/v) phosphoric acid) was introduced into the eluate and 1 μL of the mixture was used for MALDI-TOF MS analysis. Each experiment was performed in twice.

For phosphopeptide enrichment from tryptic digests of proteins of Jurkat-T cell lysate, 50 mg $CaTiO_3$ or TiO_2 were mixed with 1 mg tryptic digests in 1 mL 1% TFA/50% ACN and incubated at 37 °C for 45 min. After washing with 1 mL 1% TFA/50% ACN twice, the trapped peptides were eluted with 1 mL 2.5% ammonium hydroxide. The eluted solution was then lyophilized to dryness, desalted with Ziptip C18 and used for RPLC-ESI-MS/MS analysis.

2.4. Mass spectrometry analysis

All MALDI-TOF MS spectra were recorded with an Axima TOF^2 mass spectrometry (Shimadzu, Kyoto, Japan). The instrument was equipped with a 337 nm nitrogen laser with a 3 ns pulse width. The detection was performed in positive ion reflector mode with an accelerating voltage of 20 kV. Typically, 500 laser shots were averaged to generate each spectrum.

RPLC-ESI-MS/MS was used to analyze the sample from Jurkat-T cells. The analysis was carried out on a hybrid quadrupole-TOF LC-MS/MS mass spectrometer (TripleTOF 5600+, ABSciex) equipped with a nanospray source. Peptides were first loaded onto a C18 trap column (5 mm × 0.3 mm i.d., 5 μm, Agilent Technologies) and then eluted into a C18 analytical column (150 mm × 75 μm i.d., 3 μm, 100 Å, Eksigent). Mobile phase A (3% DMSO, 97% H_2O, 0.1% formic acid) and mobile phase B (3% DMSO, 97% ACN, 0.1% formic acid) were used to establish a 100 min gradient, which was comprised of 0 min in 5% B, 65 min of 5-23% B, 20 min of 23–52% B, 1 min of