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"Old" metal oxide affinity chromatography as "novel" strategy for specific capture of *cis*-diol-containing compounds

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ABSTRACT

The metal oxide affinity chromatography (MOAC) materials have been extensively used for extraction of phosphate compounds in the past decade. Actually, some of these materials also possess adsorption affinity towards *cis*-diol-containing compounds, which was seldom explored in separation field so far. Here we present the proof-of-concept study to evaluate the feasibility of expanding MOAC for specific capture of *cis*-diol biomolecules. Benefitting from the high commercialisation of the metal oxide materials, such MOAC strategy possesses several advantages, like synthesis-free, low cost and high expandability. Firstly, the recognition of adenosine against 2′-deoxyadenosine was performed using zirconium oxide and cerium oxide, two typical commercial MOAC materials. The results showed that efficient adsorption and elution could be achieved easily by pH switching from basic to acidic. The isotherm curves demonstrated the adsorption process fitted well with Freundlich isotherm model and was spontaneous at room temperature ($\Delta G^0 < 0$) with an exothermic nature ($\Delta H^0 < 0$). Afterwards, the highly efficient and selective enrichment of various model *cis*-diol biomolecules, including ribonucleosides, glycopeptides and glycoproteins, was achieved using this MOAC strategy. Finally, the endogenous ribonucleosides and modified ribonucleosides were successfully purified from human urine sample, which demonstrated the potential application of MOAC materials in the enrichment of target compounds from complex biological samples. Besides the excellent performance of extraction for *cis*-diol-containing compounds, equally important is that these materials are commercially available with low cost, which makes the MOAC a promising strategy for the study of *cis*-diol biomolecules in metabolomics and proteomics.

1. Introduction

The metal oxides, such as titanium dioxide (TiO_2) and zirconium dioxide (ZrO_2), have long been utilised in separation science for their good mechanical/chemical stability and unique amphoteric nature. Recently, benefiting from the strong affinity towards phosphate group, these materials have been extensively applied to extract phosphopeptides/phosphoproteins from high abundant unmodified peptides/proteins before mass spectrometry (MS) analysis [1–4]. During extraction, phosphate is proposed to bind with metal oxide in a bridging bidentate form under neutral or acidic condition and then dissociate upon changing to basic condition, which was presented as metal oxide affinity chromatography (MOAC) in the first place [5–8]. Since then, many related studies of MOAC have been carried out, including synthesis of novel MOAC materials (individual metal oxides and composites), developing different applicable forms (particles, monoliths and fibres) and useing MOAC in various biosystems for proteomic studies [9–13]. Up to date, many MOAC-based products have been commercialised, like Phos-trap™ Phosphopeptide Enrichment Kit (PerkinElmer Life and Analytical Sciences, Inc.), Agilent HPLC phosphochip (Agilent Technologies).

On the other side, *cis*-diol-containing biomolecules are of great significance in life science and attract increasing attention these years. Nucleosides in urines have been considered as potential biomarkers for early cancer diagnostics [14]. And protein glycosylation is one of the essential issues for cell development, cell division and prion diseases [15]. However, these *cis*-diol-containing biomolecules are normally present in complex matrix with sub-stoichiometric amounts so that proper enrichment is indispensable before detection. So far, lectin affinity chromatography [16], hydrazide chemistry [17], hydrophilic interaction

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chromatography [18] and boronate affinity chromatography (BAC) have been developed for such purpose. Thereinto, BAC, which relies on the reversible generation of cyclic esters between boronic acids and *cis*-diol groups, has been widely used due to the covalent-binding mechanism, good specificity and controllability [19,20]. Whereas, the high cost of boronate ligands, multiple synthesis steps, harsh conditions for functionalisation (non-aqueous solvent, high temperature, etc.), and lack of commercialisation make the BAC materials still hard to be universally applied [21,22].

Actually, in physical chemistry, surface adsorption of acyclic polyols on metal oxides has been extensively studied as it is the prerequisite step for many important catalytic processes [23]. Al_2O_3, ZrO_2, CeO_2 and TiO_2, which are commonly used in MOAC, are all proved possessing special interaction with ethylene glycol and glycerol [24–27]. These phenomena encourage us to explore the potential applicability of MOAC strategy towards enrichment of *cis*-diol-containing biomolecules. Although several previous reports utilised TiO_2 or ZrO_2 to extract glycopeptides, the interaction mechanism was explained as hydrophilic interaction or coordination effect through acid residues [28–31]. The definite concept and explicit application prospect of MOAC materials for the enrichment of *cis*-diol-containing compounds were unfortunately still elusive yet [32–34]. Here we present this proof-of-concept study to not only clarify the MOAC mechanism in the related extracting process but also put out MOAC strategy for specific capture of *cis*-diol-containing biomolecules, which is synthesis-free and low cost. To this end, by investigating the recognition ability towards adenosine (a typical *cis*-diol molecule), two proper commercial MOAC materials, ZrO_2 and CeO_2, were selected from a series of candidates, including Al_2O_3, ZrO_2, CeO_2, TiO_2, Co_2O_3, Cr_2O_3 and GaO_2. Then, through chromatographic study, we found that basic condition was necessary for the recognition of adenosine for both materials, while acidic condition could induce the effective dissociation of the captured target compounds. In addition, adsorption isotherms revealed a better fitting of Freundlich isotherm model than Langmuir isotherm model for the adsorption process of adenosine onto the two MOAC materials. And the thermodynamic parameters showed a spontaneous and exothermic nature of such adsorption process at room temperature. Finally, the two MOAC materials were used to capture various *cis*-diol-containing biomolecules, including nucleosides, glycopeptides and glycoproteins. Excellent efficiency and selectivity were achieved even in complex matrix. Along with the high commercialisation, the MOAC strategy presents a novel option with promising application for enriching *cis*-diol biomolecules.

2. Experimental

2.1. Chemicals and reagents

ZrO_2 (Z104401 – 100 g), CeO_2 (C103980 – 25 g), Al_2O_3 (A102005 – 25 g), Co_2O_3 (C105670 – 100 g), Cr_2O_3 (C118496 – 25 g) and GaO_2 (G110981 – 5 g) were all obtained from Aladdin Industrial Inc. (Shanghai, China). TiO_2 powders were synthesised according to our previous report [33]. Briefly, acetic acid (0.1 g) and water (1 g) was dissolved in ethanol (25 g). After cooling down to 0 °C, tetrabutyl titanate (10 g) was added into the solution in an ice bath. The mixture was then kept in a closed system at 40 °C for 10 h to complete the sol–gel reaction and ageing process. After washing with ethanol and water (100 mL each) and drying at 30 °C, the material was ground and sifted to obtain particles ranging from 20 to 40 μm. Finally, these powders were rinsed with water and dried again before use.

2′-Deoxycytidine (dC), 2′-deoxyguanosine (dG), 2′-deoxyadenosine (dA), thymidine (T), cytidine (rC), guanosine (rG), adenosine (rA), uridine (rU) and 5-methylcytidine (5-mrC) were purchased from Sigma–Aldrich (Beijing, China). N^6-Methyladenosine (m^6A) was purchased from Hanhong Chemical Co., Ltd. (Shanghai, China).

Horseradish peroxidase (HRP) was purchased from Shanghai Kayon Biological Technology (Shanghai, China). Trifluoroacetic acid (TFA), ammonia hydroxide (NH_3H_2O, 25%), trypsin, RNase A, RNase B, bovine serum albumin (BSA), 2,5-dihydroxybenzoic acid (2,5-DHB) and a-cyano-4-hydroxycinnamic acid (CHCA) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Other chemicals were purchased from Sinopharm Chemical Reagent (Shanghai, China). All the chemicals used in the experiments were analytical grade. Purified water was obtained with a Milli-Q apparatus (Millipore, Bedford, MA, USA).

2.2. Sample preparation

HRP (1 mg) was dissolved in NH_4HCO_3 solution (50 mM, 1 mL) and denatured at 95 °C for 5 min. To the above solution, trypsin (1 mg/mL, 40 μL) was added and the mixture was incubated at 37 °C for 16 h. The obtained HRP digestion mixture was diluted 100 times with sampling solution before use.

The urine sample was collected from a tumour patient and obtained from the Hospital of Wuhan University according to the standard clinical procedures. The utilisation was complied with guidelines of Ethics Committee of the Institute, and the participant gave his informed consent. The urine was diluted 20 times by 1% ammonia solution and then centrifuged at 10,000 × *g* for 5 min before use.

2.3. Instruments

The microscopic morphology of ZrO_2 and CeO_2 was examined by a Quanta 200 scanning electron microscopy (SEM) (FEI, Holand). The energy-dispersive spectrometer data were determined by Shimadzu EDX-720 energy-dispersive X-ray analysis (EDX, Kyoto, Japan) using Mg Kα radiation as the excitation source. Nitrogen sorption experiments were carried out at 77 K using JW-BK surface area and pore size analyser (JWGB Sci. & Tech., Beijing, China). The specific surface areas were calculated according to the Brunauer–Emmett–Teller (BET) equation at P/P_o between 0.05 and 0.3.

All the liquid chromatography experiments were carried out with an Agilent 1100 system (Agilent Technologies, Waldbronn, Germany). The LC–MS/MS experiments were performed on the LC–ESI-MS/MS system consisting of an AB 3200 QTRAP LC–MS/MS (Applied Biosystems, Foster City, CA) with an electrospray ionisation source (Turbo Ionspray) and a Shimadzu LC-20AD HPLC (Tokyo, Japan) with two LC-20AD pumps, a SIL-20A autosampler, a CTO-20AC thermostatted column compartment, and a DGU-20A3 degasser. Data acquisition and processing were performed using AB SCIEX Analyst 1.5 Software (Applied Biosystems, Foster City, CA).

All MALDI-TOF-MS spectra were recorded with an Axima TOF^2 mass spectrometry equipped with a 337 nm nitrogen laser with a 3 ns pulse width (Shimadzu, Kyoto, Japan). The detection was performed in positive ion reflector mode with an accelerating voltage of 20 kV. Typically, 200 laser shots were averaged to generate each spectrum.

2.4. Experimental details

2.4.1. pH effect on the retention behaviour of MOAC materials

ZrO_2 or CeO_2 (300 mg) was respectively packed as stationary phase into a short column (50 mm × 2.1 mm i.d.). The flow rate was set at 0.3 mL/min with the mobile phase of 10 mM sodium bicarbonate at certain pH values (pH 10.0, 11.0, 11.8 for ZrO_2 and pH