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Efficient enrichment of glycopeptides with sulfonic acid-functionalized mesoporous silica

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ABSTRACT

This work presents an efficient and selective enrichment method for glycoprotein/glycopeptides with sulfonic acid-functionalized mesoporous silica (SBA-15-SO_3H), which is synthesized via simple oxidation of -SH groups with H_2O_2. The functionalized SBA-15 shows large surface area and accessible pores, and can selectively adsorb glycopeptides via hydrogen bond and hydrophilic interaction. Upon the selective enrichment prior to the mass spectrometric (MS) analysis, the signals of glycopeptides are significantly enhanced, which leads to the identifiable signals of 21 glycopeptides from the digest of HRP, 16 glycopeptides from the digest of human IgG, and 16 glycopeptides from the digest of chicken avidin. The SBA-15-SO_3H gives significant selectivity for glycopeptides even at a low molar ratio of glycopeptides to nonglycopeptides with an enrichment time of 15 min. Therefore, this work provides a powerful material for selective enrichment and identification of low abundant glycopeptides in glycoproteomic analysis.

1. Introduction

Protein glycosylation is one of the most vital post-translational modifications and commonly links with regulation of cell division, tumor immunology, inflammation, and protein-protein interactions [1–8]. Therefore, glycoprotein research has important biological significance. The detection of the glycosylated sites of proteins plays crucial roles in diverse biological processes and discovery of new targets for drugs at molecular level [9,10]. Currently mass spectrometry (MS) coupled with matrix-assisted laser desorption/ionization (MALDI) or electrospray ionization (ESI) has become benchmark analytical techniques for the determination of glycoprotein. However, these techniques are generally lack of the sensitivity demanded for low abundant glycopeptides, and the ion suppression effect caused by the co-presence of non-glycosylated peptides often decreases the ionization efficiency [11,12]. Thus, an enrichment step prior to MS detection is important for the analysis of glycoproteins to improve the detection sensitivity. As a result, it is necessary to develop efficient enrichment methods for detailed glycoproteomic studies [13–15].

Several methods or materials have been reported to enrich glycopeptides, including the application of lectin [16], boronic acid [11,17], hydrazine beads [18], magnetic beads [19,20] and gold nanoparticles [21], immobilized metal affinity chromatography [22], size-exclusion chromatography [23], and hydrophilic interaction chromatography with solid phase extraction (HIC-SPE) [24]. The HIC adsorbents have also been applied for isolation of glycopeptides from digests of purified glycoproteins and more complex protein mixtures using sepharose, cellulose, saccharides, zwitterionic beads and metal–organic frameworks [25–29]. These methods are sensitive but have limitations in selectivity. To solve this problem, boronic acid modified nanomaterials have been reported for selective enrichment [11,17,32] and solid-phase extraction [30–33] of glycopeptides. The boronic-based chemically selective strategy shows much improved selectivity for enrichment and isolation of glycopeptides and enhanced sensitivity for MS analysis of glycopeptides.

Here, we use sulfonic acid-functionalized mesoporous silica (SBA-15-SO_3H) to develop a method for selective enrichment of glycopeptides via hydrogen bond and hydrophilic interaction, and then convenient elution to perform following MS analysis (Scheme 1). The functionalization of SBA-15 can conveniently be performed by immobilizing 3-mercaptopropyl trimethoxysilane (MPTMS) on SBA-15 and then oxidizing the -SH groups with H_2O_2 to form active sulfonic acid groups [34] (Fig. 1). The porous structure of SBA-15 with abundant sulfonic groups assures the adsorption selectivity and capacity of glycopeptides. Thus the proposed strategy shows high sensitivity for detection of glycopeptides from the digests of different glycoproteins. The SBA-15-SO_3H provides promising application in selective enrichment and identification of glycopeptides for glycoproteomic research.

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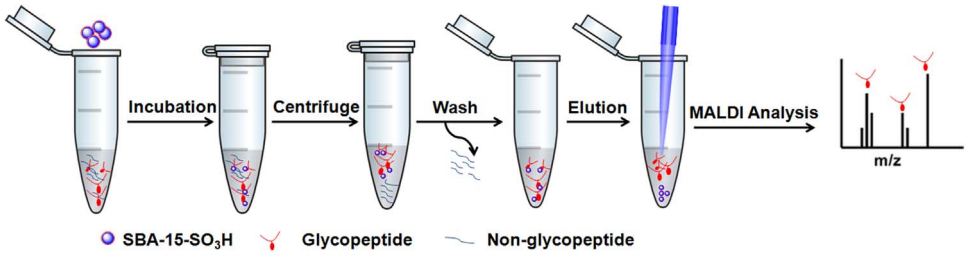

Scheme 1. Workflow of specific enrichment with SBA-15-SO_3H and MALDI-MS analysis of glycopeptides.

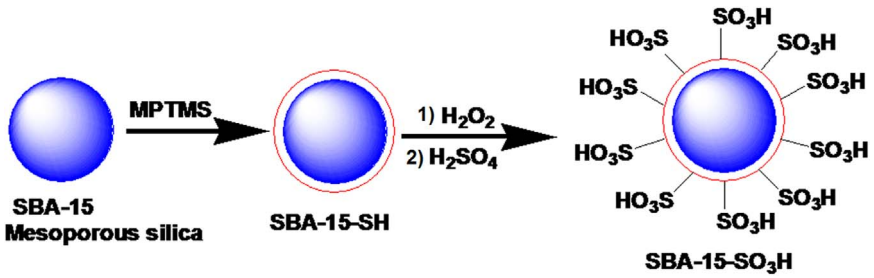

Fig. 1. Synthesis of SBA-15-SO_3H.

2. Experimental

2.1. Materials and reagents

Horseradish peroxidase (HRP, MW ~44 kDa), bovine serum album (BSA, MW ~66 kDa), human serum immunoglobulin G (human IgG), chicken avidin, trypsin (from bovine pancreas, ≥ 10,000 BAEE units mg^{-1}), ammoniumbicarbonate (NH_4HCO_3), DL-dithiothreitol (DTT), iodoacetamide (IAA), trifluoroacetic acid (TFA, ≥ 90%), α-cyano-4-hydroxycinnamic acid (CHCA) and MPTMS ≥ 90% were purchased from Sigma-Aldrich Inc. (USA). Sulfuric acid, anhydrous toluene, dichloromethane, ethyl acetate, hydrogen peroxide and methanol were obtained from Shanghai Reagent Company (Shanghai, China). All these reagents were used without further purification. Ultrapure water obtained from a Millipore water purification system (≥ 18 MΩ, Milli-Q, Millipore) was used in all experiments.

2.2. Apparatus

Fourier-transform infrared (FT-IR) spectra were recorded on Thermo Nicolet 380 spectrometer using KBr pellets (Thermo Nicolet, Wisconsin, USA). Zeta potential analysis was performed on a Zetasizer instrument (Nano-Z, Malvern, UK). X-ray powder diffraction data was recorded using an X-Ray Powder Diffractometer (Bruker AXS Ltd, Germany). Nitrogen absorption/desorption measurement was performed on a porosimeter (ASAP 2020, Micromeritics, USA).

2.3. Preparation of SBA-15-SO_3H

Mesoporous silica (SBA-15) was firstly synthesized according to the procedure reported previously [35]. The obtained SBA-15 was dried in oven at 150 °C, overnight under vacuum to remove absorbed water. The functionalization of SBA-15 with -SH groups was carried out by mixing 4 g of dried SBA-15 and 1.45 g of MPTMS in 460 ml of anhydrous toluene, which was vigorously stirred and refluxed for 24 h [34]. The solid product was recovered by filtration and added a mixture containing 151 ml dichloromethane and 223 ml ethyl acetate. The mixture was stirred and refluxed for more 24 h. After filtration, the thiol modified SBA-15 was obtained.

The thiol modified SBA-15 (4 g) was then dispersed in a mixture containing 25 ml H_2O_2, 108 ml CH_3OH and 286 ml H_2O. The resulting solution was stirred at room temperature for 12 h and filtered. The desired solid product was dispersed in 200 ml of 0.5 M H_2SO_4 and stirred at room temperature for 12 h. The solid product was then filtered, washed thrice with ethanol and water, and dried overnight under vacuum oven at 100 °C to obtain SBA-15-SO_3H.

2.4. Digestion of proteins

HRP as a model glycoprotein was firstly dissolved in NH_4HCO_3 buffer (50 mM, pH 8.3) to a final concentration of 1 mg ml^{-1} and denatured at 100 °C for 5 min. The solution was incubated with trypsin at an enzyme-to-protein ratio of 1:40 (w/w) at 37 °C for 16 h. The digestion of human IgG, chicken avidin and BSA was performed by incubating the mixture of 50 μL of 200 mM DTT and 1 ml of 1 mg ml^{-1} protein in 50 mM NH_4HCO_3 buffer (50 mM, pH 8.3) at 100 °C for 5 min. After the mixture was cooled down to room temperature, 40 μL of 1 M IAA was added in dark for 45 min, and the excessive IAA was consumed with 200 μL of 200 mM DTT at room temperature for 1 h. Then, trypsin was added to the protein solution at an enzyme-to-protein ratio of 1:40 (w/w) and incubated at 37 °C for 16 h. Finally, 0.5% FA was used to stop the digest reaction. The obtained samples were stored at −20 °C prior to enrichment.

2.5. Enrichment of glycopeptides

SBA-15-SO_3H (1 mg) was dispersed in 100 μL ACN/H_2O/TFA (88:11.9:0.1, v/v/v), and mixed with 100 μL sample of digested glycoprotein to incubate with shaking at room temperature for 15 min. The glycopeptide-loaded SBA-15-SO_3H was collected by centrifugation at 14,000 rpm for 3 min, and washed three times with 100 μL ACN/H_2O/TFA (88:11.9:0.1, v/v/v) to remove the non-specifically adsorbed peptides. Then, 20 μL of ACN/H_2O/TFA (50:49:1, v/v/v) was added to release the captured glycopeptides from SBA-15-SO_3H over a period of 30 min.

2.6. MALDI-TOF MS analysis

Equivalent amounts (0.5 μL) of the elute and saturated CHCA solution in ACN/H_2O/TFA (60:39.9:0.1, v/v/v) as the matrix were