Preparation of glyco-silica materials via thiol-ene click chemistry for adsorption and separation

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Experimental details

Materials and regents

Spherical silica (5 μm particle size, 100 Å pore size, 300 m^2 g^{-1} surface area) was purchased from Fuji Silysia Chemical (Kasugai,Japan). Toluene (99.0%) was obtained from Kermel (Tianjin, China). 3-Mercaptopropyl-trimethoxysilane (98%) was purchased from TCI (Tokyo, Japan). Mannose, galactose, glucose and maltose modified with alkenyl were obtained from Innotech (Dalian, China). Five polar compounds uracil, adenosine, inosine, cytidine, vernine were purchased from Acros (Fair Lawn, NJ, USA). Six peptides L-Leucy-Glycyl-Glycine, Glu-Glu, bivalirudin, Lys-Gly, Gly-Gly-His, exenatide were purchased from GL Biochem (Shanghai, China). Galactooligosaccharide and glucooligosaccharide were all kindly donated by the Natural Products and Glycobiotechnology group (Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, PR China). 2,2'-azobis[2-methylpropionamidine] dihydrochloride (AIBA,98%), ammonium bicarbonate (NH_4HCO_3), ammonium formate (97%) and formic acid (98%) were obtained from J&K Scientific (Beijing, China). Human IgG, concanavalin A (ConA), Bovine Serum Albumin (BSA), dithiothreitol (DTT), iodoacetamide, guanidine hydrochloride, monopotassium phosphate (KH_2PO_4, 99.0%), sodium dihydrogen phosphate (NaH_2PO_4, 99.0%) and disodium hydrogen phosphate (Na_2HPO_4, 99.0%) were purchased from Sigma Aldrich (St.Louis, MO, USA). Water was purified using a Milli-Q purification system (Billerica, MA, USA). Acetonitrile (ACN) and methanol (CH_3OH) of HPLC grade were obtained from Merck (Darmstadt, Germany).

Apparatus

The chromatographic system consisted of a 2695 HPLC pump, a 2489 ultraviolet-visible detector and a 2424 evaporative light scattering detection (ELSD) system. Data were collected and analyzed by Empower software version 3.0. These instruments and workstations were purchased from Waters (Milford, USA). The elemental analysis was performed on a Vario EL III elemental analysis system (Elementar, Hanau, Germany).

Synthesis of glyco-silica materials

The mercaptopropyl modified silica (SH-Silica) was prepared according to the following procedures. 50 g of silica gel (approximately 120.00 mmol silanol groups) was dispersed in 150 mL of toluene, 30 mL of 3-mercaptopropyltrimethoxysilane (160.09 mmol) and 10 mL (123.89 mmol) of pyridine were added. Then the mixture was refluxed for 24 h. The suspension was filtered and the solid was washed with toluene, methanol, water and methanol successively.

10 g of SH-Silica (approximately 10 mmol thiol groups) was added into the solution of AIBA (0.47 g, 2.16 mmol)

and alkenyl modified saccharide (For Man, Gal, Glu: 5 g, 22.70 mmol; For Mal: 6 g, 15.70 mmol) in 60 mL of water/methanol (v/v, 1:1) under nitrogen atmosphere. The reaction was continued for 24 h at 55-65 ℃. The resulting material was filtered, washed successively with water and methanol, then dried at 80 ℃ overnight. The resulting glyco-silica materials were obtained.

With 40 mL of methanol as slurry solvent and 80 mL of methanol as propulsion solvent under a pressure of 40 MPa, 2.4 g of resulting material was slurry-packed into a stainless steel column (150 mm × 4.6 mm I.D.).

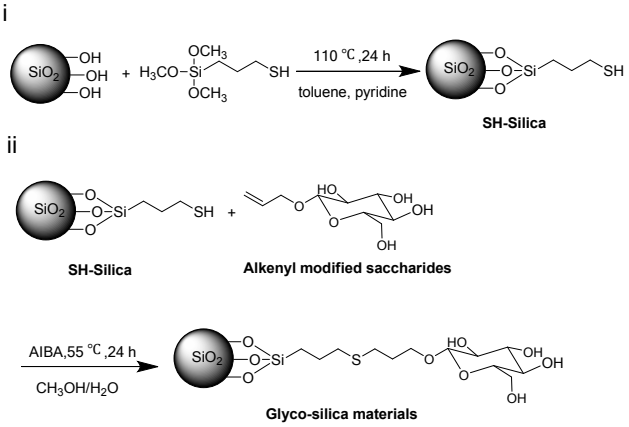

Fig. S1. The preparation of glyco-silica materials

SEM characterization of the materials

Characterization of the prepared materials was performed for morphology. SEM images (Fig. S2) revealed that the glyco-silica exhibited a similar morphology to that of the thiol-silica material, which guaranteed the application of the resulting glyco-silica particles. Besides, there was no aggregation between the particles in the SEM image, which illustrated that the thiol- thiol coupling of the particles had not occurred due to the steric hindrance between the silica particles.

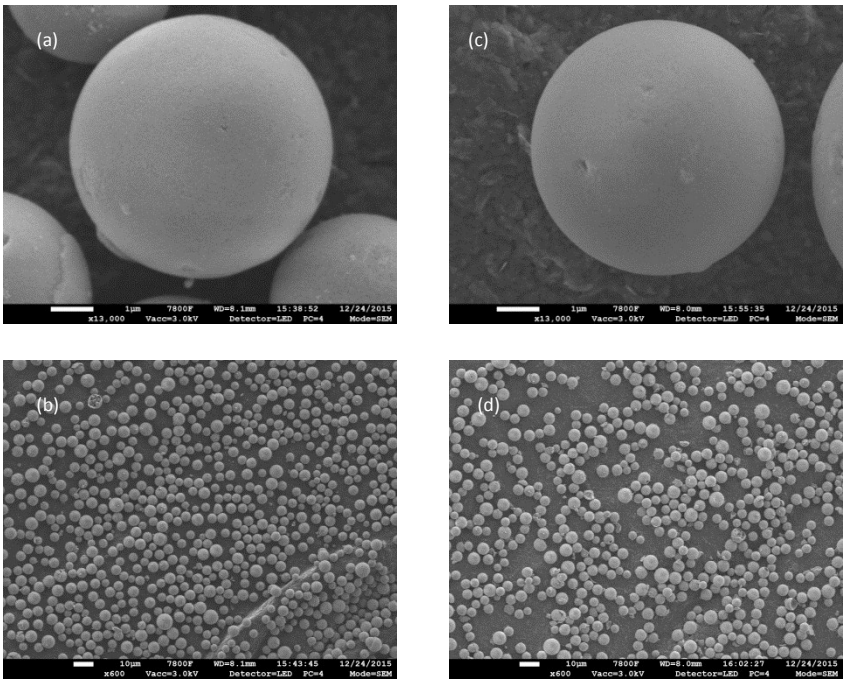

Fig. S2. SEM characterization of thiol-silica (a)(b) and maltose-silica (c)(d)

The adsorption properties of proteins on glyco-silica materials

20 mg of silica material was added into 0.5 mg/mL of protein solutions (1 mL, dissolved in 50 mM PBS, pH =7.0) and vibrated for 5 h (25 ◦C). The supernatant was obtained after centrifugation and injected into the chromatographic system. And the protein solutions before adsorption were also injected into the chromatographic system to serve as a reference. The chromatographic conditions were as follows: C4 column (150 mm×4.6 mm i.d.); mobile phase A, ACN/TFA (v/v, 100/0:1), mobile phase B, H_2O/TFA (v/v, 100/0:1), gradient: 20%-80% A, 6 min; injection volume 10 μL; flow rate 1.5 mL min^{-1}; column temperature 40 ◦C ; detection wavelength 280 nm. The obtained peak area was used to calculate the adsorption amount of proteins (△m, ng cm^{-2}) according to equation:

$$\triangle m = (A_0 - A_1)\, C_0 V_0 \times 10^5 / (A_0 m_1)$$

Where A_0 and A_1 is the peak area of protein solutions before and after adsorption respectively; C_0 is the concentration of protein solutions before adsorption, which is 0.5 mg mL^{-1}; V_0 is the volume of protein solutions, which is 1 mL; m_1 is the amount of resulting silica material, which is 20 mg; S is the surface area of the silica material, which is 300 m^2 g^{-1}.

After adsorption of ConA, each kind of silica materials was washed by 1 mL of 50 mM PBS (pH =7.0). Then 1 mL of 50 mM PBS (pH =7.0) + 1 M mannose solutions were mixed with silica materials and vibrated for 1 h (25 ◦C) for the elution of ConA. The elution rates of ConA on different silica materials were shown in Table S1.

Table S1. The adsorption amounts and elution rate of proteins on different silica materials

Glyco-silica materials	ConA		BSA
	△m / ng▪cm^{-2}	Elution rate	△m / ng▪cm^{-2}
TE-Click Gal	0.17	0%	0
TE-Click Mal	1.39	69.0%	0
TE-Click Man	1.96	70.1%	0
TE-Click Glu	3.04	80.4%	0
Silica	7.48	6.41%	5.21

The enrichment of glycopeptides on glyco-silica materials

Glycoprotein digestion: Human IgG (1 mg) were dissolved in guanidine hydrochloride (4 M) in NH_4HCO_3 buffer (50 mM, 100 μL). The denatured protein samples were reduced with DTT (200 mM, 5 μL) for 45 min at 56 °C. Then, IAA (200 mM, 20 μL) was added for alkylation and the resulting solution was incubated in dark for 30 min at ambient temperature. After being diluted tenfold with NH_4HCO_3 buffer (50 mM, 875 μL), the solution was mixed with trypsin at an enzyme/substrate ratio of 1:40 (w/w) and incubated overnight at 37.5 °C. At last, formic acid (5 μL) was added to deactivate the enzyme.

Desalting: C18 material (about 1 mg) was slurried in ACN (40 μL) and packed into the GELoader tip. The microcolumn was activated in turn with ACN/H_2O/FA (50:50:0.1 (v/v), 40 μL) and H_2O/FA (100:0.1 (v/v), 40 μL). Then tryptic digest of human IgG (10 μL) was loaded onto the column. After rinsing with H_2O/FA (100:0.1 (v/v), 40 μL), the peptide fraction was eluted with ACN/H_2O/FA (85:15:1 (v/v), 40 μL), collected.

Enrichment of glycopeptides: An inert sieve was placed in the end of the GELoader tip prior to packing. For microcolumn packing, acetonitrile slurry (45 μL) containing TE-Click Mal (about 1 mg) was slurry-packed into the

GELoadertip. Then the microcolumn was activated with ACN/H_2O/formic acid (FA) (50:50:0.1(v/v), 40 μL) and equilibrated with ACN/H_2O/FA (85:15:1 (v/v), 40 μL). Then tryptic digest IgG was loaded onto the column and it was rinsed with ACN/H_2O/FA (80:20:1 (v/v), 40μL) and ACN/H_2O (70:30 (v/v), 10 mM NH_4FA, 40 μL) to remove non-glycopeptides. The glycopeptide fraction was eluted with ACN/H_2O (60:40 (v/v), 10 mM NH_4FA, 20 μL) and directly infused to MS.

MS experiments were performed on an X'TremeSimple nano-LC system (Micro-Tech Scientific, Vista, CA) coupled to a quadrupole time-of-flight (Q-TOF) mass spectrometer (Waters, Manchester, UK). The nano-ESI source was operated under positive ion mode with nanospray voltage at 2.0 kV. MS data was acquired at m/z 500–2000.