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COMMUNICATION

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

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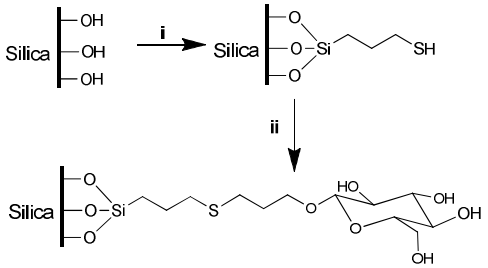
Glyco-silica materials were successfully developed based on thiol-ene click chemistry between alkene-saccharides and mercapto-silica, which behaved well in HILIC separation and exhibited high affinity to specific proteins.

Saccharides are a class of biomolecules and have essential functions in the biological systems. They can recognize and interact with specific proteins, which involved in intercellular communication in physiological processes[1]. The highly hydrophilic character and unique chemical structure of saccharides make them be employed as functional molecules, and they could apply in materials design and development[2].

Immobilization of saccharides on the solid support surface to fabricate glycol-materials is one of the most fascinating techniques with applications of many fields, including bioactive screening[3], biosensor development[4] and hydrophilic interaction chromatographic separation in HPLC[5]. However, covalently bonding of saccharides with strict structures is a very challenging work due to the multiple hydroxyl groups and side reactions. Click chemistry showed great potential in the saccharides immobilization due to its high selectivity and efficiency[6]. Copper catalyzed azide-alkyne cycloaddition (CuAAC) is one of the primary click chemistry and had been intensively used in the immobilization of saccharides[3, 6b, 7]. For example, Wong et al immobilized the oligosaccharides on the microtiter plates for high-throughput screening[3, 6b] and Santoyo-Gonzalez et al covalently bonded the mannose on the silica surface as affinity chromatography materials[7b]. However, the copper ion used in the CuAAC click chemistry would remain in the resulting materials and affect the properties of the materials. In addition, the azide used in the CuAAC reaction is dangerous due to its high-explosive property. Therefore, the development of alternatives to CuAAC click chemistry for efficient and selective bonding saccharides on solid supports is required.

In recent years, thiol-ene click chemistry, which is metal-free, has attracted great interest. It has been extensively used in polymer science, surface modification and bioorganic chemistry[8]. There are many reports on preparing glycoconjugates via thiol-ene click chemistry[9], some of which successfully immobilized the saccharides onto solid support surface, including silica wafer[9d] and polymers[10]. However, the immobilization of saccharides onto porous silica surface has not been reported. The porous glyco-silica has wide applications in chromatographic separation[5a, 11], and due to its affinity to specific protein, it could be used in the enrichment and purification of proteins[7b]. In this work, thiol-ene click chemistry was employed to immobilize saccharides onto porous silica surface. Monosaccharide and disaccharide bonded silica materials were synthesized. Then the specific binding ability to proteins and the chromatographic performance of the resulting glyco-silica materials were evaluated, demonstrating great potentials in separation sciences.

The synthetic route of this new strategy is shown in Scheme 1. The mercapto-silica was prepared via the silylation reaction of 3-marcaptopropyltrimethoxysilane on porous silica. A thiol-ene click reaction between mercapto-silica and alkenyl saccharides was carried out in the presence of 2,2'-Azobis(2-methylpropionamidine) dihydrochloride (AIBA) to obtain the glyco-silica materials.



Scheme 1 Reagents and conditions: (i) 3-marcaptopropyltrimethoxy-silane, toluene, 110 °C; (ii) alkenyl saccharides, AIBA, H_2O, 55 °C, nitrogen atmosphere

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Table 1 Illustration of glyco-silica materials and the surface coverage of the saccharides

Entry	glycol-silica	stationary phases	Carbon content %[a]	Surface coverage (μmol/m^2)[b]
1	TE-Click Man		7.79	1.75
2	TE-Click Gal		7.21	1.52
3	TE-Click Glu		7.60	1.68
4	TE-Click Mal		7.17	0.91

a: The carbon content was determined by elemental analysis.
b: Thesurface coverage was calculated according to the literature[12].

Elemental analysis results (shown in Table 1) indicated that the alkene-saccharides were successfully bonded to the mercapto-silica.

First of all, the specific protein binding capacities of glyco-silica materials were studied. Concanavalin A (ConA), a widely used lectin which can specifically interact with glucose and mannose, was employed as model protein[7b, 9d]. Bovine serum albumin (BSA) and bare silica were used as contrasts. As shown in Fig.1, BSA was adsorbed on the bare silica but not on the glyco-silica materials, while ConA was effectively adsorbed on the glyco-silica materials except for the TE-Click Gal. This means that the glyco-silica materials have specific ConA binding capacities due to the specific interaction of ConA to glucose and mannose but not galactose. After the adsorption procedure, the glyco-silica materials were washed by 1 M mannose solutions. Most of ConA was eluted from the glyco-silica (eluted rate ≥ 69.0%, shown in Table S2). Although the bare silica had strong protein binding capacity, the adsorbed ConA was difficult to be eluted (eluted rate, 6.4%), thus it was non-specific adsorption on the bare silica. The results of specific protein recognition of the glyco-silica materials demonstrated that thiol-ene chemistry was a promising approach for bonding saccharides on silica particles and the resulting materials had great application potential in the recognition or purification of specific proteins.

Saccharides modified silica materials possess abundant hydroxyl groups, and they have been successfully employed in hydrophilic interaction chromatography (HILIC)[5a]. In this paper, the chromatographic performance of glyco-silica materials under HILIC mode was evaluated. As shown in Fig.2, five nucleosides have good retention on TE-Click Mal column under hydrophilic interaction chromatography (HILIC) mode. Moreover, the separations of oligosaccharides, including galactooligosaccharides and gluco-oligosaccharides were performed on TE-Click Man column, both of them were well separated (Fig.3). In addition, peptides with different hydrophilicity were also separated with good resolution and peak shapes (Fig.4).

To further demonstrate the applicability of the glyco-silica in

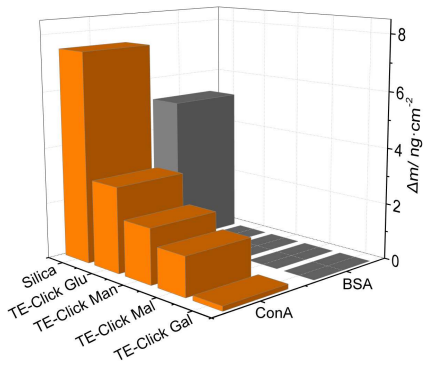

Fig. 1 The specific binding capacity of glycol-silica materials. The concentration of proteins was 0.5 mg/mL in 50 mM PBS solutions, pH=7.0; adsorption: vibration for 5 h, 25 °C.

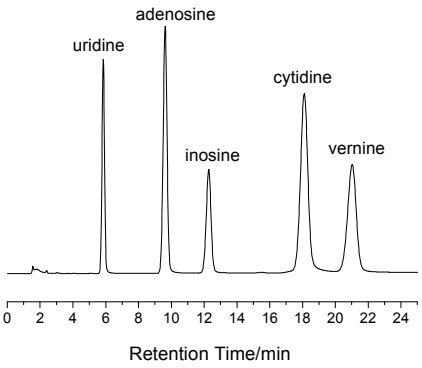

Fig. 2 The separation of nucleosides on the TE-Click Mal column (150 mm×4.6 mm i.d.). Conditions: flow rate: 1.0 mL min^{-1}; 30 °C; mobile phase: 10 mM $HCOONH_4$ in ACN/H_2O (85:15), pH 3.2; UV detection: 270 nm.

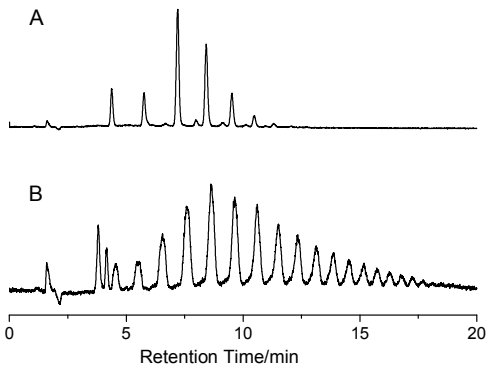

Fig. 3 The separation of galactooligosaccharide (A) and glucooligosaccharide (B) on the TE-Click Man column (150 mm×4.6 mm i.d.). Conditions: flow rate: 1.0 mL min^{-1}; 30 °C; ELSD: gas pressure 30 psi, tube temperature 70 °C, gain 100; mobile phase A, H_2O; mobile phase B, ACN; galactooligosaccharide: 0-10 min, 30% A→50% A; 10-20 min, 50% A; glucooligosaccharide: 0-20 min, 30% A→50% A.

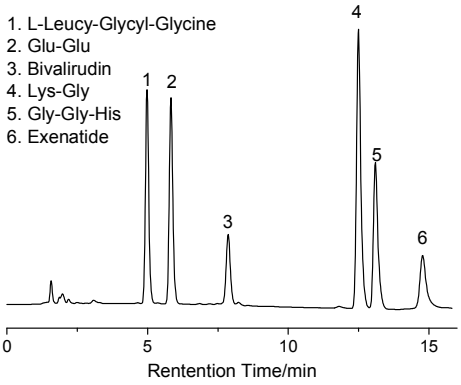

Fig. 4 The separation of six peptides on the TE-Click Mal column (150 mm×4.6 mm i.d.). Conditions: flow rate: 1.0 mL min-1; 30 °C; mobile phase A, 100 mM KH_2PO_4, pH 2.4; mobile phase B, ACN; mobile phase C, H_2O; gradient: 0-15 min,20% A, 75% B,5% C→20%A, 50% B,30% C; UV detection: 210 nm.

HILIC mode, the enrichment of glycopepitdes from the digest of IgG was performed. The TE-Click Mal material (about 1.5 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)) and loaded onto the column and it was rinsed to remove nonglycopeptides. Then the glycopeptides fraction was eluted, collected and directly infused to MS. As shown in Fig.5, much more glycopeptides in IgG digest could be detected after the enrichment by TE-Click Mal material. TE-Click Mal exhibited good selectivity, indicating that it was a promising material for the enrichment of glycosylated peptides.

In summary, thiol-ene click chemistry, which is simple and metal-free, has been successfully employed for the immobilization of

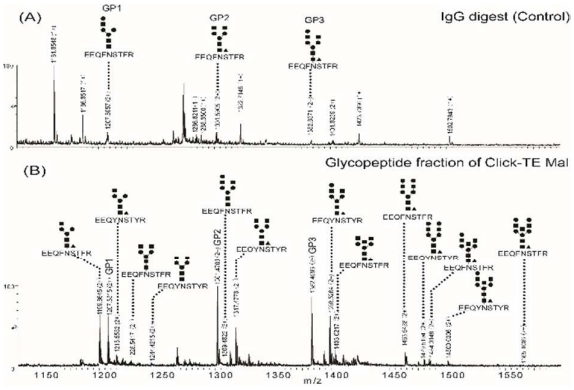

Fig. 5 Nano-ESI-MS of the human IgG digest before and after the enrichment on the TE-Click Mal materials. (A) the direct analysis of IgG digest (B) the analysis of IgG digest after enrichment by Click-TE Mal material. Loading conditions: ACN/H_2O/FA (85:15:1 (v/v), 40 μL); rinsing condition: ACN/H_2O/FA (80:20:1 (v/v), 40μL); ACN/H_2O (70:30 (v/v), 10 mM NH_4FA, 40 μL); eluting conditions: ACN/H_2O (60:40 (v/v), 10 mM NH_4FA, 20 μL).

saccharides on porous silica surface. The obtained glyco-silica materials exhibited high affinity to the specific proteins and behaved well in the separation of nucleosides, oligosaccharides and peptides. In addition, the selective enrichment of glycopetides was successfully achieved on the glyco-silica, showing a great potential in glycoproteomics.

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Graphical Abstract

Saccharides bonding method based on thiol-ene chemistry was developed and the resulting glyco-silica materials demonstrated great potentials in separation science.