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Brush polymer modified and lectin immobilized core–shell microparticle for highly efficient glycoprotein/glycopeptide enrichment

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ABSTRACT

Protein glycosylation regulates numerous important biological processes and plays key roles in many diseases including cancer, diabetes and inflammation. The ability to efficiently profile variation of protein glycosylation in biological samples is very useful for identifying new diagnostic biomarkers or developing new therapeutic approaches. Due to the low availability of glycoprotein/glycopeptide from natural sources, enrichment before mass spectrometry (MS) analysis is usually a prerequisite. Affinity enrichment using lectins is currently one of the most widely adopted approaches. Conventionally, lectins are immobilized on solid supporting materials for sample recovery. However, the limited specific surface area, high steric hindrance and rigid nature of such supporting materials restricts lectin loading amount and results in low flexibility as well as accessibility of the immobilized lectins. Therefore, we proposed using core–shell microparticles composed of silica core and brush-like polymer chains shell for improved lectin immobilization. The surface bound brush-like polymer are synthesized by in situ growth of polymer chains from microparticle surface using surface initiated atom transfer radical polymerization (SI-ATRP). The flexible non-crosslinked polymer chains not only provide numerous binding sites, but also work as three-dimensional support for lectin immobilization, which leads to high loading amount and good accessibility of the immobilized lectin. Successful enrichment which facilitated glycoprotein/glycopeptide identification is demonstrated.

1. Introduction

As one of the most common post-translational modifications, protein glycosylation plays important roles in many biological processes, including protein trafficking and folding, cell–cell interactions and inter-cellular signaling [1–6]. Variation in the glycoforms of glycoproteins has long been considered correlating with cancer metastasis, differentiation and development, therefore can be used as a hallmark for cancer diagnosis [7–9]. Large scale profiling of such alternations is beneficial for identifying new diagnostic biomarkers [9–13] to monitor tumor progression as well as designing new drugs [14–16]. However, due to the extremely complex nature of protein samples extracted from blood, tissue or cells and the limited abundance of glycoproteins/glycopeptide, enrichment and separation of glycoproteins/glycopeptides from the highly complex mixture is widely adopted to facilitate identification [17–19]. A few enrichment approaches including hydrophilic interaction chromatography (HILIC), hydrazide chemistry and lectin based enrichment have been developed. HILIC, which relies on the hydrophilic interaction between glycopeptides and the stationary phase, provides the most broad spectrum enrichment regardless of the glycoform of the glycopeptides [20–28]. However, the enrichment selectivity is unsatisfactory due to the low physical affinity between the analyte and the hydrophilic stationary phase. Hydrophilic non-glycopeptides and other impurities often coelute with glycopeptides and interfere with the identification. On the contrary, hydrazide chemistry based enrichment in which the diol groups of glycans are oxidized to aldehyde groups and couple with hydrazide functionalized beads offers largely increased enrichment specificity [29–32]. However, the disadvantage of using this method is that the glycan structures are destroyed by oxidation, which makes glycoform analysis impossible. Compared with HILIC and hydrazide chemistry based enrichment, lectin enrichment [33–38] is currently the most powerful approach and has been widely adopted in glycoproteome research. However, the orientation and accessibility of the immobilized lectins is

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restricted by the conventional bulky solid substrate materials and results in impaired enrichment efficiency.

To improve the enrichment efficiency of lectin based approach, we developed a new type of immobilized lectin using core–shell microparticles composed of silica core and brush-like polymer chains shell prepared by surface initiated atom transfer radical polymerization (SI-ATRP). As one of the most successful "grafting from" strategies, SI-ATRP is particularly suitable for preparing core–shell particles with well controlled polymer shell thickness and structure, since the polymer grafting is highly controllable by in situ growth of polymer chains from the initiators immobilized surface via living/controlled polymerization. Compared with commonly used "grafting to" method, in which long polymer chains are directly attached on the substrate, SI-ATRP gives significantly increased surface grafting density. The densely packed polymer chains provide numerous binding sites and also work as three-dimensional support for lectin immobilization. As a result, high loading amount of lectin is achieved which leads to improved enrichment efficiency as demonstrated by the successful application in enrichment of glycoprotein/glycopeptides from complex mixture.

2. Experiments

2.1. Materials and reagents

Concanavalin A (Con A), wheat germ agglutinin (WGA), ricinus-communis agglutinin (RCA_{120}) were received from Vector Laboratories (Burlingame, CA, USA). peptide-N-glycosidase F (PNGase F) was obtained from New England Biolabs (Beverly, MA, USA). Bovine serumalbumin (BSA), bovine pancreatic ribonuclease B (RNase B), Albumin from chicken egg white (OVA), bovine fetuin, bovine asialofetuin, glycidylmethacrylate (GMA), 2-bromoisobutyrate bromide, copper(II) chloride, cuprous chloride, D-Glucosamine Hydrochloride, methyl-a-D-mannopyranoside, N-Acetyl-b-D-glucosamine, lactose and α-Cyano-4-hydroxycinnamic acid (CHCA) were obtained from Sigma (St. Louis, MO,USA). Porous silica microparticles (10 μm, 150 Å) were obtained from Agela Technologies (Tianjin, China). 3-aminopropyl-triethoxysilane, and N, N, N′, N″, N″-pentamethyldiethylenetriamine were purchased from Acros (Belgium). Sequencing grade porcine trypsin was received from Promega (Madison, WI, USA). commercial agarose based WGA was purchased from GE Healthcare (USA). Deionized water used in all experiments (with resistance >18 MΩ/cm) was prepared by a Millipore purification system (Billerica, MA, USA).

2.2. Preparation of 2-methyl-acrylic acid 3-(2,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-3-ylamino)-propylester (GMA-G) monomer for SI-ATRP reaction

GMA (1 mL) was treated with sulfuric acid (0.1 M) for 4 h to hydrolyze the epoxy groups to form vicinal diols. Next, 0.01 M freshly prepared sodium periodate was used to oxidize the vicinal diols to aldehyde groups. The obtained aldehyde–GMA was thoroughly mixed with glucosamine (0.5 M) and 1% $NaBH_3CN$ in methanol and reacted for 2 h to form GMA-G monomer. The monomer was washed with methanol and separated by crystallization at 4 °C.

2.3. SI-ATRP of GMA-G on silica microparticle surface to form core–shell structure

The SI-ATRP reaction initiator, 3-(2-bromoisobutyramido) propyl (triethoxy)-silane (BIBAPTES) was synthesized using procedures reported in our previous work [39]. After treating the silica microparticles with BIBAPTES (0.002 M) for 12 h for initiator immobilization on the surface of the microparticles, excess initiators were removed by repeated washing with methanol. SI-ATRP grafting was carried out by mixing the initiator immobilized silica microparticles with GMA-G (2 M), 0.01 M CuCl, 0.001 M $CuCl_2$, and 0.015 M N,N,N′, N″,N″-pentamethyldiethylenetriamine. The reaction mixture was sonicated to form homogeneous solution, bubbled with nitrogen for 10 min to remove oxygen, sealed and put in a 37 °C water bath. After reaching the preset reaction time, excess reagents were removed by repeated washing with methanol and 1% FA. The obtained polymer-brush shell hybrid silica microparticles (PSHSM) were dried under ultra pure nitrogen flow.

2.4. Characterization of PSHSM

The surface morphology of bare silica microparticles without modification and PSHSM was characterized by scanning electron microscope (SEM) using a XL30 ESEM-TMP scan electron microscope (Philips-FEI Corporation, Holland) operated with acceleration voltage of 120 keV under high-vacuum conditions. Thermo gravimetric analysis (TGA) performed on a SDT Q600 instrument (TA instrument, U.S.A.) was used to demonstrate the core–shell structure of PSHSM and determine content of the polymer brush shell. 10 to 15 mg samples (initial weight) were heated from 20 to 800 °C at a heating rate of 10 °C/min under nitrogen flow.

2.5. Preparation of PSHSM–lectin and conventional single layer modified silica lectin particles

PSHSM was treated with 10 mM sodium periodate for 1 h to convert the vicinal diols of the glucosamine on the side chains of GMA-G polymer brushes to aldehyde groups. For lectin immobilization, 2 mg lectin and 1% $NaBH_3CN$ were mixed with 10 mg PSHSM and agitated at 4 °C for 12 h. Finally, PSHSM–lectin was washed with PBS (pH = 8) three times to remove excess reagents and kept in 4 °C for further usage. For preparation of conventional single layer modified silica lectin particles, amine modified silica was mixed with 40.0% glutaraldehyde at room temperature overnight for 4 h to convert the amine groups to aldehyde groups for lectin immobilization. The loading amount of lectin on PSHSM was determined by comparing the UV absorption value (at the wavelength of 280 nm) of the supernatant solution before and after immobilization reaction using nanodrop2000c Spectrophotometer (Thermo Scientific).

2.6. Glycoprotein enrichment by PSHSM–lectin and SDS-PAGE characterization

Three types of immobilized lectin PSHSM–ConA, PSHSM–WGA and PSHSM–RCA_{120} were prepared using the above mentioned method. Model glycoproteins with different glycoforms including bovine pancreatic ribonucleaseB (RNase B), bovine fetuin and bovine asialofetuin were used to evaluate the enrichment efficiency of the prepared PSHSM–lectin. Briefly, each type of glycoprotein (20 μg) was mixed with corresponding type of PSHSM–lectin, i.e. PSHSM–ConA for RNase B, PSHSM–WGA for fetuin and PSHSM–RCA_{120} for asialofetuin in 100 μL binding buffer (20 mM Tris–HCl pH = 7.4, 500 mM NaCl, 1 mM $MnCl_2$, 1 mM $CaCl_2$) in a spin column. After 2 h incubation with gentle agitation at 4 °C, the PSHSM–lectin with captured glycoprotein was washed three times with 400 μL binding buffer. Next, the captured glycoprotein was eluted by 100 μL elution buffer with appropriate sugar in binding buffer (ConA: 200 mM methyl-a-D-mannopyranoside, WGA: 300 mM N-Acetyl-b-D-glucosamine, RCA_{120}: 500 mM lactose) for 30 min at room temperature with gently spinning for three times and The eluate was concentrated to 50 μL by deionized water using a 3-kDa filter (Merck Millipore), then characterized by SDS-PAGE to assess the enrichment efficiency. For enrichment

selectivity evaluation, protein mixture composed of RNase B and BSA (1:10, w/w) was prepared and enriched by PSHSM–ConA. After repeated wash with 20 mM Tris–HCl (pH=7.4), 500 mM NaCl,1 mM $MnCl_2$ and 1 mM $CaCl_2$, the captured RNase B was eluted and characterized by SDS-PAGE. SDS-PAGE separation was carried out using GE Amersham™ ECL™ Gel system with 8–16% gradient gel, 1 × Running Buffer (25 mM Tris–HCl, 192 mM glycine, 0.1% SDS), 160 V constant voltage for 1 h under room temperature. The gel was stained with Coomassie Bright Blue G-250 and imaged by UMAX PowerLook 2100xl.

2.7. Protein digestion and glycopeptide enrichment by PSHSM–lectin

1 μg fetuin was mixed with 10 μg BSA and dissolved in 50 mM NH_4HCO_3 buffer (pH=7.8) followed by DTT reduction and IAA alkylation. Next, trypsin was added to the solution at trypsin to protein substrate ratios of 1:50 and incubated at 37 °C for 12 h. After that, 2 μL of formic acid was added to the solution to deactivate trypsin and terminate the digestion reaction.

100 μL commercial agarose based WGA or PSHSM–WGA was added to a spin column and washed with 400 μL binding buffer (20 mM Tris–HCl pH=7.4, 500 mM NaCl, 1 mM $MnCl_2$ and 1 mM $CaCl_2$) for three times. The proteolytic peptides mixture was lyophilized and redisposed in 100 μL binding buffer and loaded to the spin columns either containing the commercial agarose based WGA or PSHSM–WGA and incubated at 4 °C for 4 h with gentle agitation. After that, the two kinds of lectin beads were washed with 400 μL binding buffer for three times, the captured glycopeptides were eluted by 100 μL elution buffer (binding buffer plus 300 mM N-Acetyl-b-D-glucosamine). Next, the eluted glycopeptides were subjected to PNGase F (1 Unit) treatment overnight at 37 °C.

2.8. MALDI-TOF-MS analysis

The obtained de-glycosylated peptides were desalted by C_{18} zip–tip and lyophilized. After re-dispersed the de-glycosylated peptides in 10 μL CHCA solution (5 mg/mL, 50% ACN, 0.1% TFA), 1 μL sample was spotted on the MALDI-TOF-MS target plate and air dried. MALDI-TOF-MS analysis was carried out using a 4800 MALDI-TOF-TOF analyzer (AB Sciex, MA, USA) equipped with a Nd: YAG laser at excitation wavelength 355 nm. All the mass spectra (1000 laser shots for every spectrum) were acquired in positive reflection mode after being calibrated using peptides originated from myoglobin and analyzed by Data Explorer (Version 4.5).

3. Results and discussion

3.1. Preparation and characterization of polymer-brush shell Hybrid silica microparticles (PSHSM)

The core–shell PSHSM consisting of silica core and brush-like polymer shell are synthesized using SI-ATRP technique. As a versatile tool for synthesis of hybrid materials, SI-ATRP is particularly powerful for surface confined growth of densely packed polymer brushes which can be heavy conjugated with large quantity of recognition molecules for highly efficient enrichment. The preparation procedure of PSHSM is illustrated in Fig. 1. Briefly, the surface of 10 μm silica microparticles was immobilized with 3-(2-bromoisobutyramido)propyl(triethoxy)-silane (the initiator for SI-ATRP) via Si–O bond and SI-ATRP reaction was carried out using 2-Methyl-acrylicacid 3-(2,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-3-ylamino)-propyl ester as the monomer which results in highly controlled growth of brush-like polymer chains on the microparticles surface. After reaching the preset grafting time, SI-ATRP reaction was stopped by repeated washing to remove excess reagents. Next, the vicinal diols on the side chains of the obtained GMA-G polymer chains were converted to aldehyde groups by sodium periodate oxidation for lectin immobilization. Surface morphology of the prepared PSHSM was characterized by scanning electron microscopy (SEM). Fig. 2 is the SEM image of PSHSM with 12 h SI-ATRP grafting and unmodified porous silica microparticles. After SI-ATRP grafting, the surface of silica microparticlesis entirely covered by a smooth and uniformed polymer

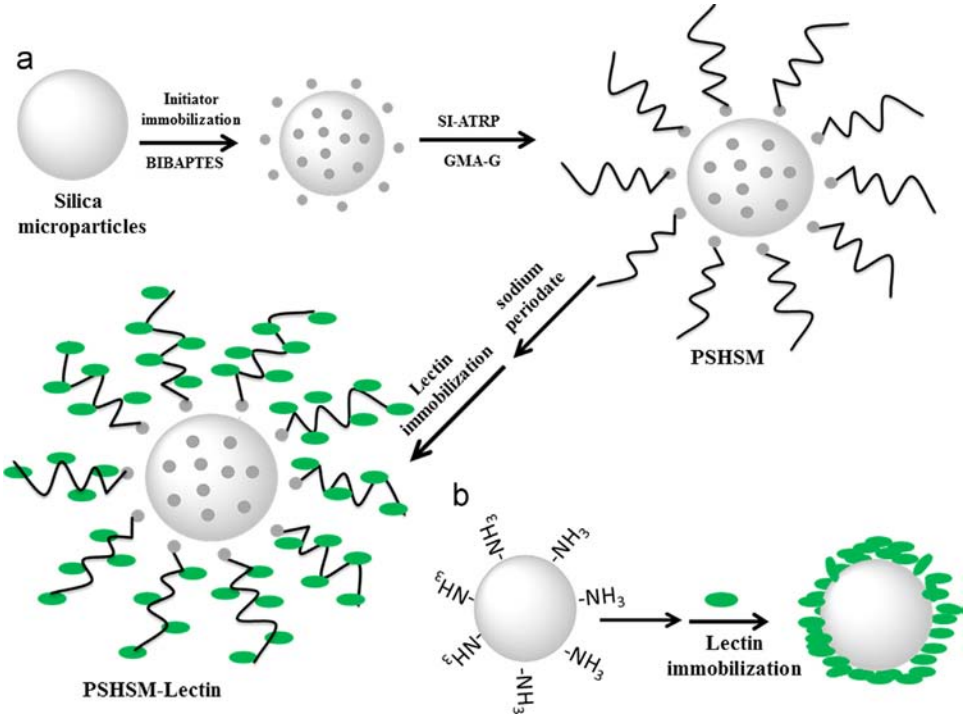

Fig. 1. Schematic diagram of preparation of the PSHSM and PSHSM–lectin (a), and single layer of lectin (b).

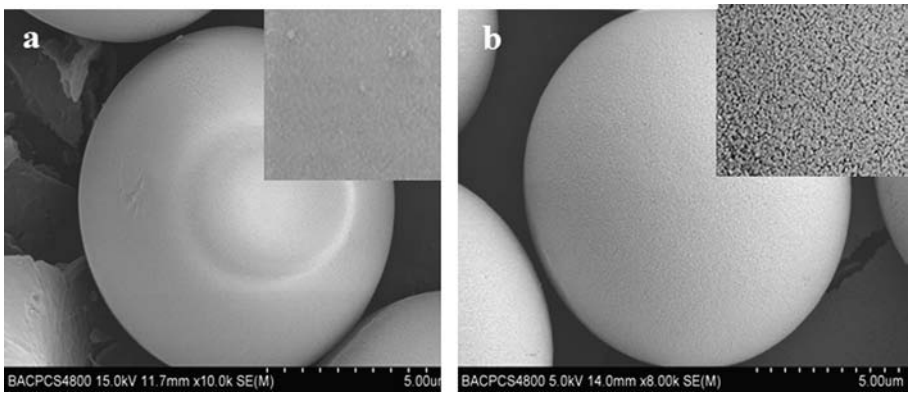

Fig. 2. SEM images of PSHSM (a) and unmodified bare silica microparticles (b).

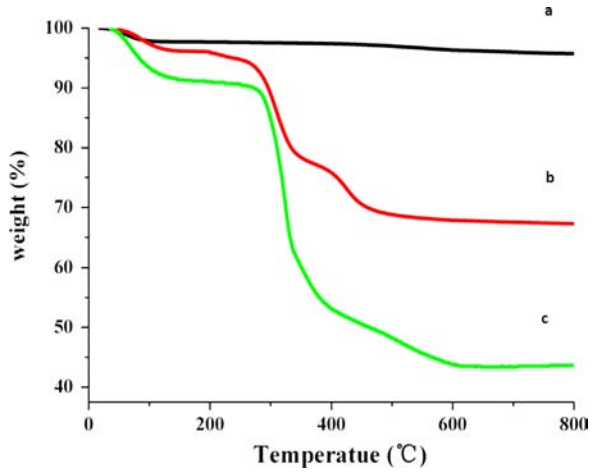

Fig. 3. TGA curves of bare silica microparticles (a), PSHSM with 6 h polymerization (b) and 12 h polymerization (c).

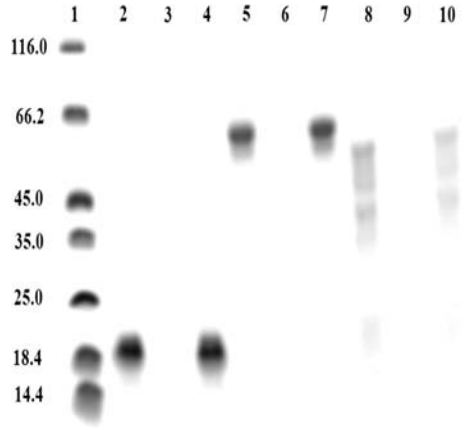

Fig. 4. SDS-PAGE gel picture of RNase B, fetuin and asialofetuin before and after PSHSM–lectin enrichment. **Lane 1:** The molecular weight standards (M_r in kDa), **lane 2, 5 and 8:** RNase B, fetuin and asialofetuin before enrichment. **Lane 3, 6 and 9:** supernatant of RNase B, fetuin and asialofetuin after enrichment. **Lane 4, 7 and 10:** eluted RNase B, fetuin and asialofetuin after enrichment.

Table 1
Loading amount of ConA, WGA and RCA_{120} on PSHSM ($n=3$).

No.	ConA (μg/mg)	WGA (μg/mg)	RCA_{120} (μg/mg)
1	78.5	79.3	55.6
2	79.0	81.0	55.9
3	78.1	79.7	56.1
Average loading	78.5	80.0	55.9
Standard deviation (%)	0.5	0.9	0.3

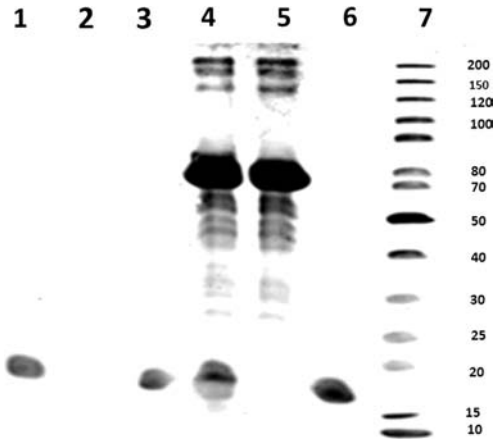

Fig. 5. SDS-PAGE gel picture of mixture of RNase B & BSA before and after PSHSM–ConA enrichment. **Lane 1 and 4:** mixture of RNase B & BSA (1:0 and 1:10) before enrichment. **Lane 2 and 5:** supernatant of RNase B & BSA (1:0 and 1:10) after enrichment. **Lane 3 and 6**: eluted RNase B (mixed with BSA in 1:0 and 1:10) after enrichment. **Lane 7:** The molecular weight standards (*Mr* in kDa).

layer (Fig. 2a), which is clearly different from the porous surface of the unmodified ones (Fig. 2b). This difference in surface morphology is more clearly shown in the enlarged images inserted in Fig. 2a and b. As revealed by Fig. 2a, the polymer layer is solely grown from the microparticles surface with no bulk polymer structure formation. Furthermore, the modified microparticles remain separated and no crosslinked microparticles can be found after SI-ATRP grafting, indicating highly efficient and controlled surface growth of polymer brushes is achieved. This surface confined polymer grafting is hard to achieve using conventional free radical polymerization (CFRP), since in CFRP the initiators are dissolved in the polymerization solution and formation of bulk polymer or crosslinked microparticles is inevitable.

Next, thermogravimetric analysis (TGA) was adopted to further confirm the successful preparation of GMA-G polymer-brush hybrid silica core–shell microparticles as well as determine the content of the surface grafted polymer brushes. Fig. 3 is the TGA analysis of bare silica microparticles, PSHSM with 6 and 12 h grafting. Unmodified bare silica microparticles (Curve a) show less than 5.0% of total weight loss which might be attributed to the loss of water residue. In contrast, PSHSM which consists of thermally stable silica core and thermo decomposable polymer brushes showed noticeable weight loss after thermo treatment. The weight

loss is 34.0% for PSHSM with 6 h polymerization (Curve b) and increases to 57.5% after 12 h polymerization (Curve c) indicating highly efficient surface grafting of polymer chains is achieved. Such high content of polymer grafting is difficult to be realized by conventional "graft to" strategy, due to the large steric hindrance induced by direct attachment of large polymer chains to silica microparticles. Furthermore, the roughly proportional increase of polymer content of PSHSM with increasing polymerization time is a typical characteristic of controlled/"living" polymerization, in which side reactions such as chain transfer and termination is minimized and therefore leads to surface confined and thickness controlled polymer grafting.

Table 2
Sequence and m/z of the de-glycosylated peptides of fetuin.

No.	Peptide sequence	m/z
1	LCPDCPLLAPLN * DSR	1741
2	VVHAVEVALATFNAESN * GSY LQLVEISR	3017
3	RPTGEVYDIEIDTLETTCHVLDPTPLAN * CSVR	3673

3.2. Lectin loading amount on PSHSM

Three types of the most widely used lectins including ConA, WGA and RCA_{120} which have specific affinity towards high mannose, N-acetylglucosamine or sialic acids, and galactose glycoforms were used to prepare PSHSM–lectin. The amount of lectin immobilized on PSHSM was determined by measuring the difference of UV absorbance at 280 nm of lectin solution before and

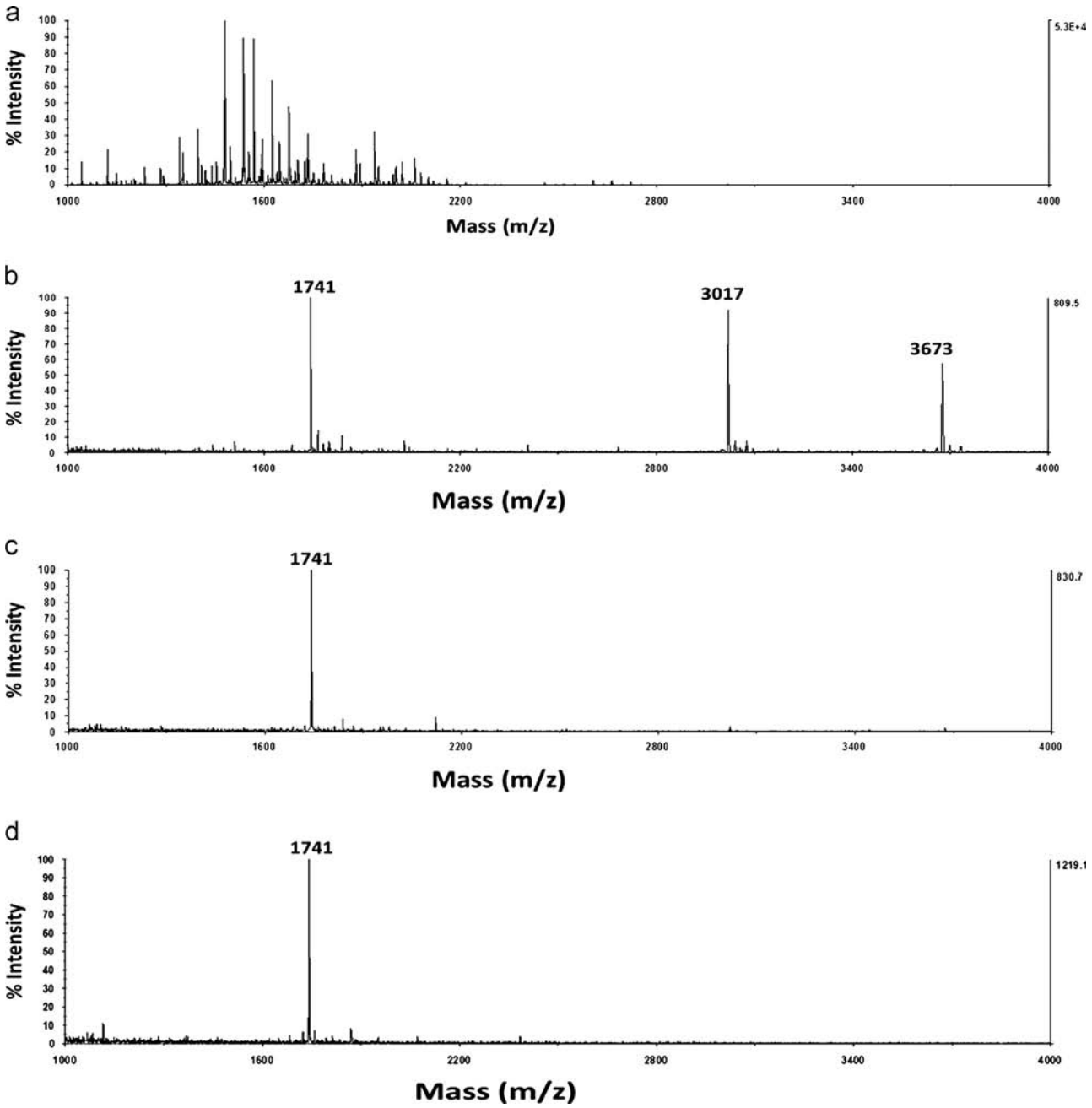

Fig. 6. MALDI-TOF-MS spectra of de-glycopeptides before enrichment of fetuin and BSA (1:10, w/w) (a), after enrichment by PSHSM–WGA from mixture of fetuin and BSA (1:10, w/w) (b), after enrichment by commercial agarose based WGA from mixture of fetuin and BSA (1:10, w/w) (c) and after enrichment by PSHSM–WGA from mixture of fetuin and BSA (1:50, w/w) (d).

after immobilization. The loading amount of ConA, WGA and RCA_{120} on PSHSM was measured three times and the average loading and standard deviations are shown in Table 1. Compared 16.2 (ConA), 18.9 (WGA) and 11.6 (RCA_{120}) μg/mg loading amount obtained from conventional single layer lectin modified silica microparticles, PSHSM–lectin has obviously higher lectin loading amount. We attribute this increased lectin loading amount to the particular shell structure of PSHSM. The polymer shell is composed of large amount of non-cross-linked poly-GMA-G brushes that provide numerous of binding sites and also work as scaffolds to support 3D lectin immobilization (Fig. 2a). In contrast, since only a single layer of lectin can be immobilized on the surface of conventional microparticles, the lectin loading amount is limited by the surface area of the microparticles (Fig. 2b). Furthermore, no cross-linkage is applied between the surface attached GMA-G polymer brushes, therefore glycoproteins maybe allowed to penetrate into the internal spaces between these polymer brushes and results in improved accessibility towards the immobilized lectins.

Next, the enrichment efficiency and recovery of PSHSM–lectin was evaluated using three kinds of glycoproteins including RNase B (high mannose glycoform), fetuin (sialic acids glycoform) and asialofetuin (galactose glycoform). Fig. 4 is the SDS-PAGE gel picture of RNase B, fetuin and asialofetuin before and after PSHSM–lectin enrichment. As shown in the picture, lanes 2–4 are RNase B before enrichment, supernatant and elution after enrichment. There is no glycoprotein left in the supernatant after enrichment by PSHSM–ConA, therefore highly efficient glycoprotein capturing is achieved. Furthermore, the amount of eluted RNase B by Commassie Blue Staining in lane 4 is almost equal to the loading amount before enrichment (lane 2) indicating little irreversible adsorption of glycoproteins on PSHSM–lectin and therefore leads to minimized sample loss. Similar results are also obtained for both PSHSM–WGA and PSHSM–RCA_{120} as shown in Fig. 4 lanes 5–7 and 8–10 demonstrating that efficient enrichment with high recovery is achieved for glycoproteins with various of glycoforms. The ability to specifically enrich glycoproteins in the presence of a large amount of non-glycoproteins is a key issue for glycoproteome analysis. To further evaluate the enrichment specificity of PSHSM–lectin, a more complex sample with a substantial fraction of non-glycoproteins (BSA) was used. Protein mixtures composed of RNase B and BSA (1:0) and (1:10) were prepared and enriched by PSHSM–ConA. SDS-PAGE gel picture of these protein mixtures before and after enrichment is shown in Fig. 5. Lane 1 and 4 are RNase B and BSA (1:0) and (1:10) before enrichment. Two bands corresponding to RNase B and BSA are shown in the lanes. However, only the band representing RNase B is appeared and almost no residue of BSA can be found in lane 6, even though a large fraction of BSA is used, suggesting that only glycoproteins can be captured from the mixture by PSHSM–lectin with little nonspecific adsorption of non-glycoproteins. Besides the increased loading amount of lectin, another possible explanation for the high enrichment specificity of PSHSM–lectin is the strong hydrophilic nature of the GMA-G polymer brushes on the microparticle surface. Carrying numerous of hydroxyl groups, the GMA-G polymer brushes are excellent anti-folding material that resist nonspecific adsorption of non-glycoproteins.

Due to its higher compatibility with mass spectrometry analysis, bottom-up based glycoproteome research strategy is widely adopted. Therefore, we further evaluated the enrichment efficiency and specificity of PSHSM–lectin towards glycopeptides using mass spectrometry. Fetuin which has three N-linked glycosites (Asn_{99}, Asn_{156}, and Asn_{176}, m/z of the de-glycosylated peptides in Table 2) was digested into peptides and enriched by PSHSM–WGA followed by PNGase F treatment for de-glycosylation which facilitates MS analysis. We compared the enrichment efficiency of our PSHSM–lectin and commercially lectin beads towards glycopeptides using mixture of

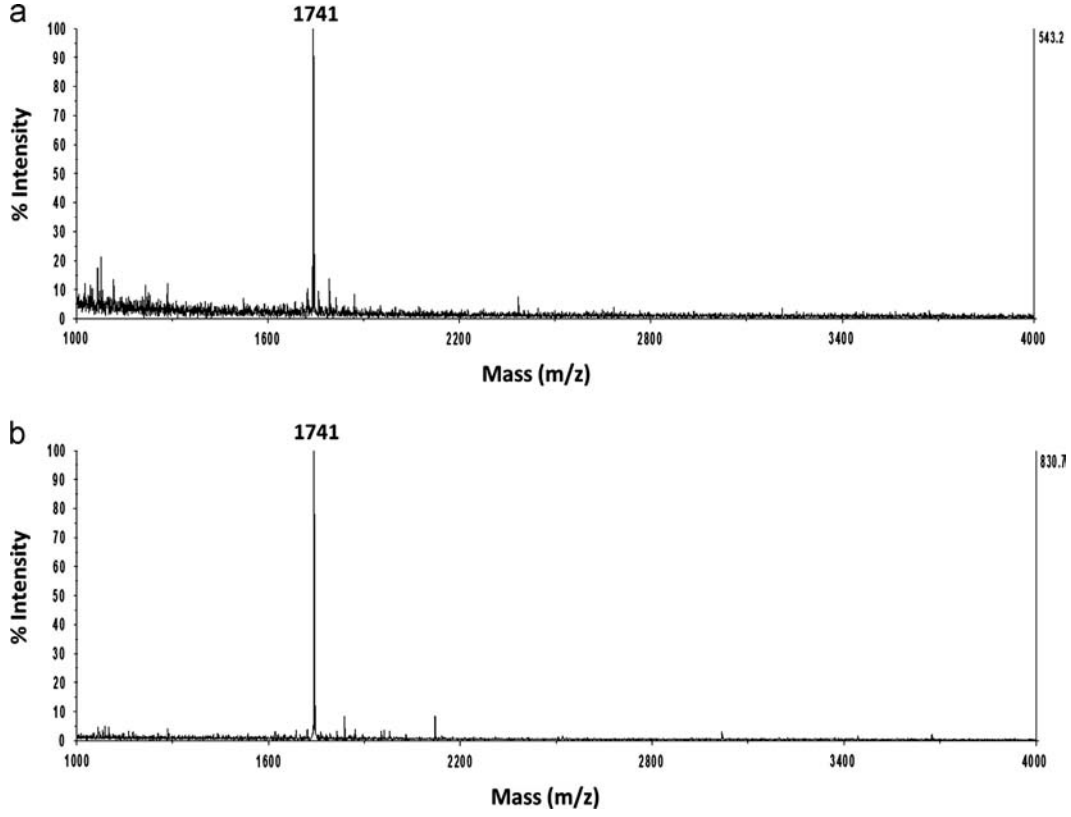

Fig. 7. MALDI-TOF-MS spectra of de-glycopeptide enriched from mixtures of 0.2 μg fetuin and 2 μg BSA by PSHSM–WGA (a) and 1 μg fetuin and 10 μg BSA by commercial based WGA (b).

fetuin and BSA (1 μg:10 μg) as the model sample. The MALDI-TOF-MS spectra of the de-glycosylated peptides before and after enrichment by PSHSM–WGA and commercial WGA beads were shown in Fig. 6. Without enrichment, the signals of the de-glycopeptides are completely overwhelmed by the intensive signals of non-glycopeptides (Fig. 6a). Both PSHSM–lectin and commercial lectin beads efficiently removes the highly abundant non-glycopeptides and results in a clean background. However, only one de-glycopeptide (m/z=1741) was detected after commercial WGA beads enrichment (Fig. 6c), but PSHSM–WGA enrichment leads to the successful identification of all three glycopeptides of fetuin (m/z=1741, 3017, 3673) with high S/N ratio indicating its higher enrichment efficiency (Fig. 6b). To further challenge the PSHSM–lectin, we increased the ratio between fetuin and BSA to 1:50. As shown in Fig. 6d, one de-glycopeptide (m/z=1741) can still be detected after PSHSM–WGA enrichment. Finally, the detection limit of PSHSM–lectin enrichment was measured using varying amount of fetuin and BSA mixture (1:10, w/w) and compared with that of commercial lectin beads. We taked the identification of at least one de-glycopeptide as the successful detection of the glycoprotein. The detection limit of PSHSM–WGA and commercial WGA beads enrichment is 0.2 μg and 1 μg fetuin, respectively (Fig. 7) indicating a 5-fold enhancement in enrichment efficiency is achieved.

Compared with conventional lectin beads in which lectins are immobilized on solid surface, PSHSM–lectin has the advantage of attaching large amount of lectins on highly flexible non-crosslinked polymer chains, therefore reduced steric hindrance and improved accessibility may be expected.

4. Conclusion

In summary, a new type of lectin immobilized microparticle composed of silica core and brush-like hydrophilic polymer chains shell is developed in this work. The core–shell microparticles prepared by SI-ATRP lead to increased lectin loading amount and accessibility, therefore highly efficient and selective glycoprotein/glycopeptide enrichment is achieved. Successful enrichment of glycoprotein/glycopeptide from complex mixture by PSHSM–lectin indicates its promising application in glycoproteome research.

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