Size-Selective Enrichment of N-Linked Glycans Using Highly Ordered Mesoporous Carbon Material and Detection by MALDI-TOF MS

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Ⓢ *Supporting Information*

ABSTRACT: Many diseases are characterized by the changes of either glycan structure or glycosylation site of glycoproteins. The glycan profiling can provide the overview of glycosylation in despite of the absence of the glycosylation sites, which in turn simplifies the complexity of disease diagnosis. Herein, we describe a simple method to profile the N-linked glycans by MALDI-TOF MS with the enrichment using oxidized ordered mesoporous carbon, taking advantages of the size-exclusive effect of mesopore against proteins as well as the interaction between glycans and carbon. Twenty four N-linked glycans derived from ovalbumin could be efficiently detected with high signal-to-noise (S/N) ratios and sufficient peak intensities. In the analysis of complex serum samples, 32 N-linked glycans could be profiled, and 5 (4 core-fucosylated glycans) of them were distinguished from liver cancer and healthy samples.
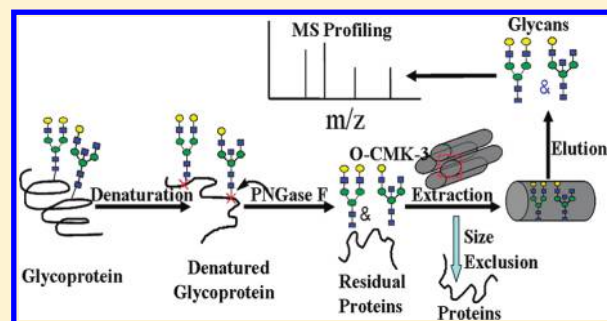
The enzymatic process of linking carbohydrate moieties to a protein molecule is referred to as protein glycosylation, which is one of the most important protein post-translational modifications for proteins involved in biological systems. The linked oligosaccharide moieties (glycans) have been found to be associated with many essential cell biological processes, including cell adhesion, molecular trafficking and clearance, receptor activation, signal transduction, and endocytosis.[1,2] The tumor progression, cancer metastasis, and other immunodeficiency diseases have been considered to be related to the changes of glycans on proteins.[3-5] Generally, protein glycosylation can be classified into two main types: the N-glycosylation (glycans link to Asn residues presenting in a tripeptide consensus sequence of Asn-X-Ser/Thr, X could be any amino acid except proline) and O-glycosylation (glycans link to a Ser or Thr residue). The characterization of N-linked glycans has been widely applied in diseases diagnoses.[3,6,7] Due to the complexity of multienzymatic processes in protein glycosylation, the glycans linked to proteins are highly heterogeneous in structure and component.[8] Therefore, the comprehensive determination of glycans is a thus crucial step in the understanding of these biological processes.

Up to date, various analytical technologies such as mass spectrometry (MS) have been attempted to accomplish the glycan mapping assignment.[9,10] Due to the interference of residual proteins during MS detection, selective enrichment of glycans from a protein matrix is needed. Organic solvent precipitation has long been used in protein removal.[10] However, the incomplete precipitation of residual proteins by organic solvents and the likely sample loss arising from solvent dilution are the major disadvantages. Lectin can be applied in the enrichment of glycans by taking the advantage of affinity interactions between lectin and glycans. Unfortunately, lectin, such as ConA, shows low affinity to diantennary N-glycans and no affinity to tri- and tetra-antennary complex-type glycans in spite of its good affinity to high-mannose and hybrid N-glycans.[11] Moreover, each lectin can sometimes only be applied to enrich its corresponding glycans, which thus limits the overall glycans enrichment and subsequent comprehensive glycan profiling.[12] It is reported that active carbon material is a promising material in enrichment of glycans based on the hydrophobic and polar interactions between carbon and glycans.[13,14] However, a complex protein matrix would still be adsorbed by active carbon due to the existence of a macroporous structure.[13,15]

Mesoporous materials have attracted intensive attention in the fields of catalyst, adsorbent, sensor, and nanodevice due to the large surface area, well-defined mesoporous structure, and narrow pore size distribution.[16-20] The application of mesoporous materials in biological sample preparation, such as selective capture of endogenous peptides[21,22] and phosphopeptides[23-25] as well as the size-selective enzymatic process,[26] has been carried out, where the unique mesoporous structures were superior in capturing objective peptides and repelling against interfering large molecular weight proteins due to the size-exclusive effect.

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Highly ordered mesoporous carbon material is a new type of mesoporous material, possessing similar mesoporous structure and large surface area but a difference in the nature of chemistry to silica mesoporous materials.[27] Taking advantages of carbon interacting with glycans and mesoporous structure excluding large proteins, in this work, the oxidized mesoporous carbon material (O-CMK-3) was applied in the selective enrichment of N-linked glycans from a complex biological matrix by the size-exclusion mechanism. This high throughput approach presented herein offers higher efficiency in enrichment of glycans than the previous reported methods.

■ EXPERIMENTAL SECTION

Materials and Reagents. Pluronic P123, tetraethyl orthosilicate (TEOS), 2,5-dihydroxybenzoic acid (2,5-DHB), sinapinic acid (SA), formic acid (FA), trifluoroacetic acid (TFA), hen ovalbumin and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO); peptide N-glycosidase (PNGase F) was obtained from New England Biolabs (Ipswich, MA, USA); cytochrome C was acquired from Aladdin regents (Shanghai, China); centrifugal filter with MWCO of 10 kDa was purchased from Millipore (Bedford, MA); human serum from healthy volunteers and liver cancer patients were provided by Dalian Medical University and stored at −80 °C before analysis; acetonitrile (ACN) was chromatographic grade from Merck (Darmstadt, Germany). Deionized water was purified by a Milli-Q water system (Millipore, Milford, MA). All other chemicals including hydrochloric acid (HCl), ethanol, nitric acid (HNO₃), hydrofluoric acid (HF), and sucrose were of analytical grade.

Preparation of O-CMK-3. SBA-15 used for the preparation of CMK-3 was synthesized with the procedure reported elsewhere.[18] Briefly, 4.5 mL of TEOS was added to 75 mL of 1.6 M HCl solution containing 2.0 g of P123. The resulting mixture was magnetically stirred at 35 °C for 0.5 h and placed in an oven at 35 °C for 24 h and then at 100 °C for 6 h. The as-synthesized SBA-15 product was collected by filtration, dried at 100 °C under vacuum overnight, and finally calcinated at 550 °C for 6 h.

The synthesis of CMK-3 was carried out with the reference procedure.[27] Briefly, a mixture consisting of 1.25 g of sucrose, 1 g of above prepared SBA-15, 75 μL of H₂SO₄, and 5 mL of H₂O was first placed in an oven at 100 °C for 6 h and then at 158 °C for 6 h. The sample, containing partially polymerized and carbonized sucrose, was further treated as above after the addition of 0.8 g of sucrose, 50 μL of H₂SO₄, and 5 mL of H₂O. The above sample was carbonized at 900 °C under nitrogen gas for 3 h. Afterward, the CMK-3 was obtained by removing the silica template of SBA-15 from the resulting carbon−silica composite with 5 wt % HF at room temperature for 12 h, filtered, washed with ethanol, and dried at 120 °C for further use.

To further prepare the oxidized CMK-3 (O-CMK-3), 0.3 g of resulting CMK-3 was refluxed with 50 mL of HNO₃ (26 wt %) at 90 °C for 4 h, which was filtered, washed to neutral, and dried under vacuum at 120 °C overnight.[28]

Characterization of O-CMK-3. The O-CMK-3 was characterized by infrared spectrometry (IR), transmission electron microscopy (TEM), and nitrogen sorption, respectively. The IR spectra of raw CMK-3 and O-CMK-3 materials, including before and after oxidization, were collected by a Bruker Tensor 27 FT-IR spectrometer. The TEM images of O-CMK-3 were taken by a JEOL JEM-2000 EX transmission electron microscope (JEOL, Tokyo, Japan) operated with the acceleration voltage of 120 keV.

The nitrogen sorption measurements of CMK-3 and O-CMK-3 degassed under 200 °C for 12 h were performed at 77 K (liquid nitrogen temperature) using a static-volumetric method on ASAP 2010 (Micromeritics). Pore diameter and the distribution curves were calculated by the BJH (Barrett−Joyner−Halenda) method from the desorption branch.

Adsorption of the Standard Glycan. γ-CD was selected as the standard carbohydrate to evaluate the adsorption of CMK-3 and O-CMK-3, respectively. All the materials were first activated with ethanol, and then, the materials were dispersed in a 0.4 mg/mL γ-CD solution to give with the final concentration of materials at 2 mg/mL. Each mixture was shaken at room temperature. Kinetic experiment was conducted to determine the amount adsorbed as a function of incubation time. At a given time, 0.1 mL of emulsion was taken from the bulk solution and centrifuged at 20 000g for 3 min. The amount of γ-CD left in supernatant was calculated by the peak intensity detected using MALDI-TOF MS with a given dilution. The peak intensity was the average of six times. Additionally, the γ-CD left in supernatant was analyzed by HPLC with an evaporative light scattering detector (ELSD, Varian 380, PL-ELSD2100) under evaporation temperature at 50 °C and drift tube temperature at 90 °C. The peak area was adopted for the quantification of γ-CD left in supernatant.

Releasing of N-Linked Glycans from Proteins. Standard glycoproteins with the concentrations of 1 mg/mL were prepared in 10 mM ammonium bicarbonate solution (pH 7.5). The release of the N-linked glycans from the proteins was carried out as follows: the protein solutions (100 μL) were boiled for 5 min, cooled to room temperature, and incubated at 37 °C for 24 h after adding 10 U of PNGase F. To release the N-linked glycans from human sera, the serum samples from healthy volunteers and liver cancer patients were thawed at 4 °C and centrifuged at 12 000g for 10 min. The collected supernatants (50 μL) were diluted to 500 μL by 10 mM ammonium bicarbonate (pH 7.5), and the samples were ultrafiltrated through a membrane with MWCO of 10 kDa at 14 000g for 20 min. The collected proteins on the membrane were rinsed by with 500 μL of 10 mM ammonium bicarbonate (500 μL, pH 7.5) for three times and dissolved in 500 μL of 10 mM ammonium bicarbonate (10 mM, pH 7.5). The other procedures of releasing glycans from the collected human serum proteins were the same as for the above standard proteins.

Enrichment of N-Linked Glycans. A 10 μL of protein digestion was mixed with 10 μL of O-CMK-3 (30 mg/mL) aqueous solution and 30 μL of deionized water. After being incubated at room temperature for 0.5 h and followed by the removal of supernatant by centrifuging at 20 000g for 2 min, the precipitate was washed with 30 μL of deionized water for three times. Finally, the adsorbed glycans on O-CMK-3 materials were eluted using 10 μL of 50% ACN solution.

Residual Protein Detection. The same process as the glycans enrichment was applied for enrichment of proteins and peptides in order to test the adsorption properties of the active carbon and O-CMK-3. Additionally, the eluted fraction was concentrated, and the residual proteins were detected by MALDI using SA as matrix. The LOD of BSA and cytochrome C using MALDI-TOF MS was determined. Additionally, the residual proteins of BSA and cytochrome C in the elution were also quantified by the BCA assay.

To estimate the MWCO of O-CMK-3, a mixture of protein and peptides was prepared by adding 10 μg of cytochrome C (12 kDa) into a peptide sample (MW < 10 kDa, approximately) extracted from 20 μL of human serum, and the mixture of
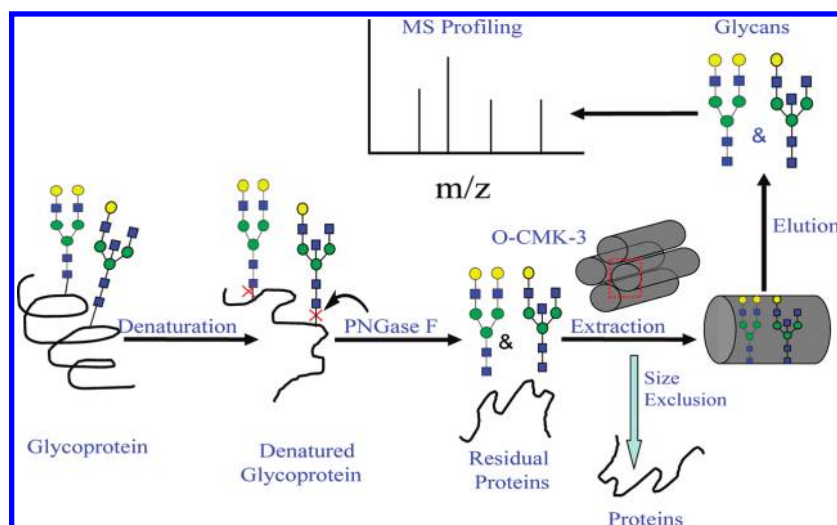
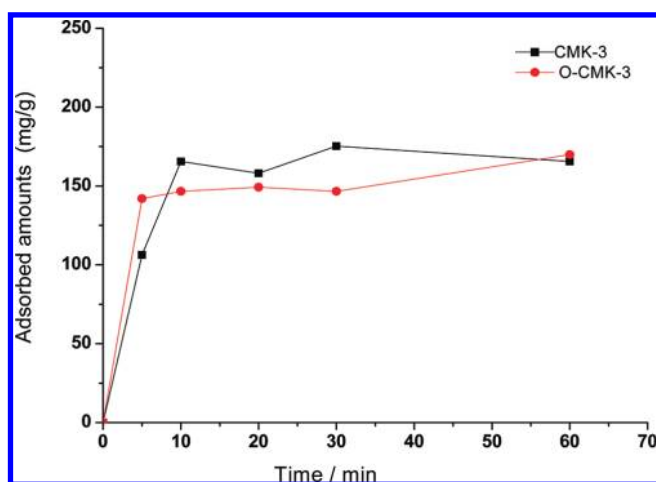**Figure 1.** Scheme for size-selective enrichment of glycans by O-CMK-3.

Figure 2. Adsorption of γ-CD on CMK-3(■) and O-CMK-3(red ●) materials detected by MALDI-TOF MS.

cytochrome C and the extract human serum peptides were then treated by O-CMK-3. The supernatant and eluted fractions obtained by O-CMK-3 were detected by MALDI-TOF MS to estimate the MWCO of O-CMK-3 to proteins and peptides.

Mass Spectrometric Analysis. All MALDI-TOF mass spectrometric experiments were carried out on a BRUKER Autoflex time-of-flight mass spectrometer (Bruker Daltoniccs Autoflex, Germany) equipped with a delayed ion-extraction device and a pulsed nitrogen laser (UV, 337 nm). The MALDI-TOF mass spectrometer used a ground-steel sample target with 384 spots. The range of laser energy was adjusted to slightly above the threshold for obtaining the good resolution and signal-to-noise ratio. All measurements were carried out in linear positive-ion mode with delayed ion extraction. The delay time for ion extraction and the extraction voltage were set at 90 ns and 20 kV, respectively. Each TOF-MS spectrum was acquired by the accumulation of 30 laser shots. 2,5-Dihydroxy-benzoic acid (DHB) (10 mg/mL, in 50% ACN/H_2O solution with 10 mM of NaCl) was used as the matrix for the analysis of N-linked glycans by MALDI-TOF MS. Sample aliquots (0.5 μL) were placed on MALDI plate and dried at room temperature. After

adding DHB matrix (0.5 μL), the sample spots were dried under vacuum prior to TOF-MS analysis. Residual proteins were mixed with saturated SA with the ratio of 1:1, and then, a 0.5 μL solution was dipped onto the plate and dried at room temperature before the MS analysis.

■ RESULTS AND DISCUSSION

Glycan attached proteins and lipids are often involved in interaction, recognition and defense performances of biological systems. The determination of the diverse glycan moieties of such proteins is considered to be of crucial importance for the understanding of biological processes. Yet, the residual proteins disturb the determination of glycans, and extraction of glycans from the mixtures is the essential step. Due to the high surface area and the interactions between carbon material and oligosaccharides, as well as the size exclusion to proteins caused by the mesoporous structures, O-CMK-3 has been used in the selective enrichment of N-linked glycans from complex biological samples by the size-exclusion mechanism (Figure 1).

Characteristics of O-CMK-3. The highly ordered mesoporous carbon material of CMK-3 was prepared and then oxidized to O-CMK-3 by nitric acid for providing better aqueous dispersion in sample preparation.[27,28] In a comparison, the raw CMK-3 material is poorly soluble in aqueous solution, with severe aggregation and adhesion on the tube (right tube in Figure S1A, Supporting Information), while the O-CMK-3 could be well dispersed in aqueous solution, eliminating the disrelished aggregation and adhesion on the tube (left tube in Figure S1A, Supporting Information). As characterized by FT-IR (Figure S1B, Supporting Information), a band at 1730 cm^{-1} ascribed to the carbonyl bond indicates the oxidization of CMK-3. The structure and morphology of the materials before and after oxidization were also charatcterized by XRD and TEM, respectively. The strong peak intensity with XRD measurment indicated the reservation of the highly ordered mesoporous structure (Figure S2A, Supporting Information), and the highly ordered structure of materials before and after the oxidization was also observed by TEM (Figure S2C and D, Supporting Information). Obviously, the mesoporous structure was not seriously influenced by the oxidation process. Furthermore, the nitrogen sorption
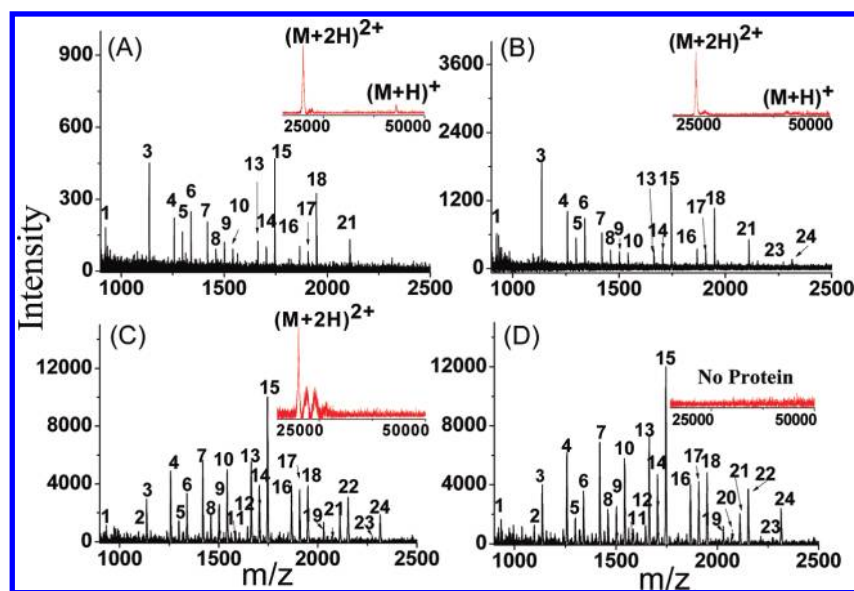
Figure 3. MALDI-TOF MS spectra of N-linked glycans of ovalbumin digest: (A) by direct analysis (1 mg/mL); (B) after precipitation with 75% cold ethanol (1 mg/mL); (C) after extraction by active carbon (10 mg/mL) and (D) by O-CMK-3 (10 mg/mL). The up-right insets were mass spectra of detected residual proteins. (The structures of these 24 glycans are presented in Figure S6B in the Supporting Information.)
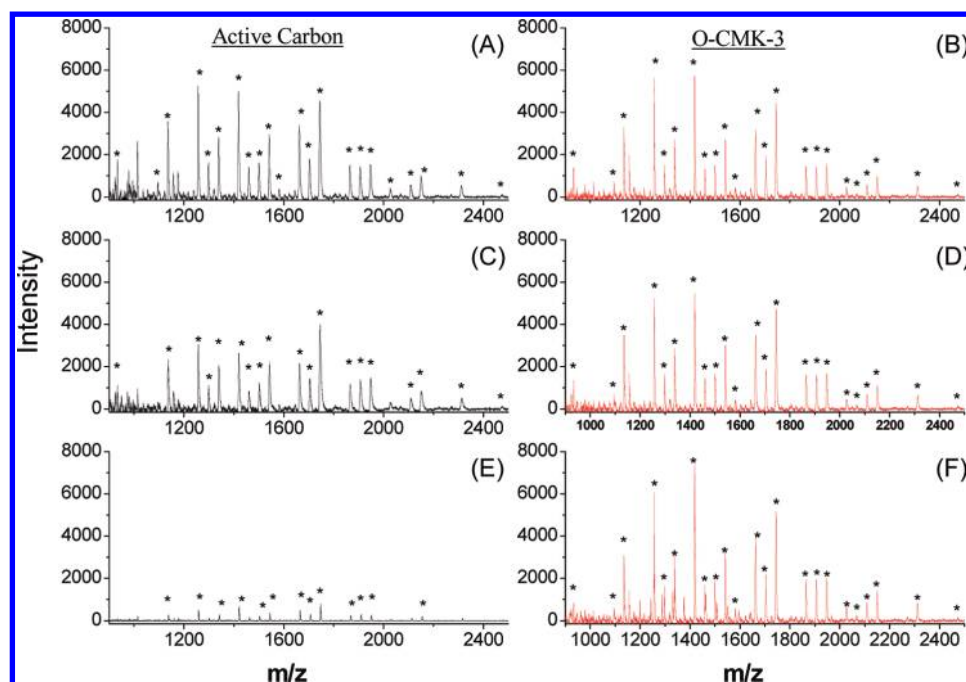

Figure 4. Enrichment of N-linked glycans by active carbon and O-CMK-3 from ovalbumin digests containing BSA with different ratios (W/W). (A), (C), and (E) are MALDI-TOF spectra for samples using AC with the ratio of ovalbumin/BSA at 1:0, 1:10, and 1:50, respectively. (B), (D), and (F) are MALDI-TOF spectra for samples using O-CMK-3 with ratio of ovalbumin/BSA at 1:0, 1:10, and 1:50, respectively. The loading amount of ovalbumin digest is 0.5 μg/μL (∗, glycans released from ovalbumin).

curves of CMK-3 and O-CMK-3 were measured (Figure S2B, Supporting Information); they exhibited the typical IV isotherm with hysteresis loop as defined by IUPAC for mesoporous materials. The abrupt increase of P/P^0 from 0.40 to 0.80, suggesting the uniform pore size distribution for both materials, is clearly observed, which indicated again the preservation of mesoporous structure for O-CMK-3. The O-CMK-3 retains the

highly ordered mesoporous structure with pore size of ca. 3.4 nm (Figure S2D, Supporting Information), which is a suitable pore size allowing the intrapore capture of glycans (about 0.9—3 kDa) but avoiding the interference of the released protein residuals (e.g., ovalbumin, 43 kDa) due to the size-exclusion mechanism of mesopore to proteins.[22] The BET surface areas of CMK-3 and O-CMK-3 are 1217.0 and 1576.0 m^2/g, respectively. The
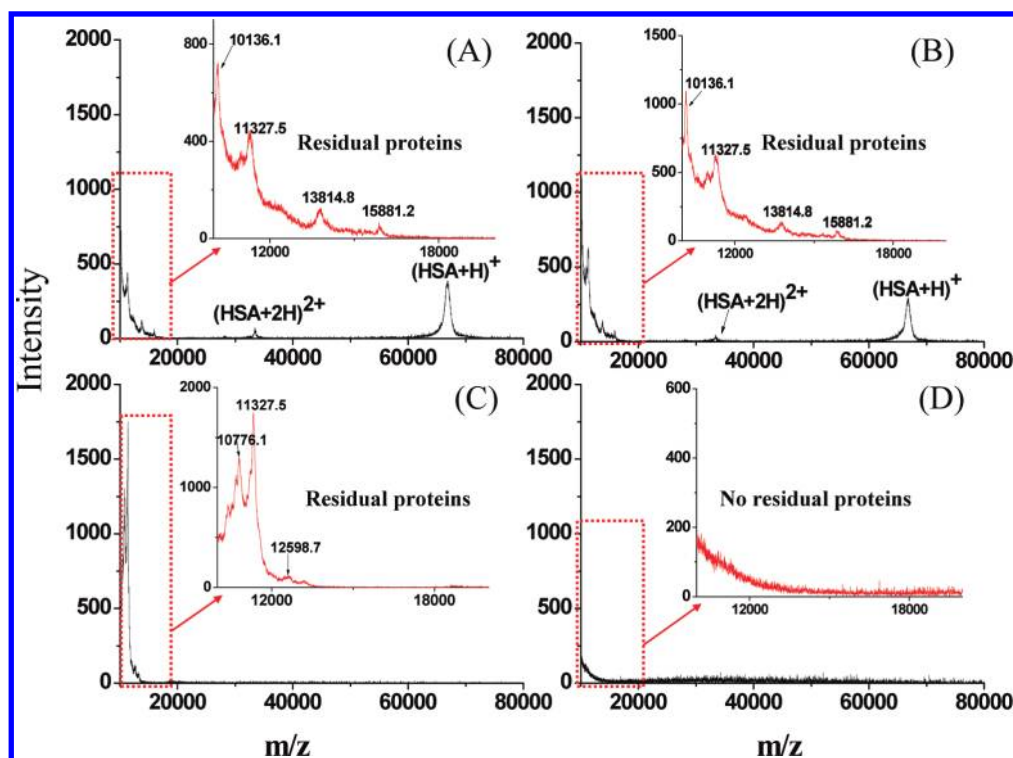
Figure 5. Serum residual proteins detected by MALDI-TOF MS: (A) without any treatment; (B) with solvent precipitation using 75% cold ethanol; (C) with extraction by active carbon; (D) with extraction by O-CMK-3.

remaining high surface area of O-CMK-3 makes it a promising enrichment adsorbent to capture glycans.

To examine the adsorption of polysaccharide on CMK-3 material, γ-cyclodextrin (γ-CD, 8 sugar ring cyclic oligosaccharide) was used as the test compound to evaluate the interaction between carbon materials and polysaccharide. Both CMK-3 and O-CMK-3 materials demonstrated good adsorption to γ-CD with maximum adsorption capabilities of 170 and 150 mg/g of γ-CD on materials, respectively, within 10 min (Figure 2), and the similar results were also obtained using HPLC-ELSD determination (Figure S3, Supporting Information). The amount of γ-CD adsorbed by O-CMK-3 and CMK-3 was pretty close, indicating that O-CMK-3, which would be well dispersed in aqueous solution, could enrich glycans as well as CMK-3.

Additionally, the MWCO of O-CMK-3 material to peptides and proteins was estimated. The obtained result indicated that the MWCO of O-CMK-3 to protein and peptides was ca. 9–12 kDa (Figure S4, Supporting Information), indicating that proteins with molecular weight greater than this mass range would not enter into the pore inside of O-CMK-3 due to the size-exclusion effect. For the analysis of glycans from serum, the endogenous peptides and small proteins with molecular-weight less than 10 kDa were removed by UF (10 kDa) in advance.

Enrichment of N-Linked Glycans by O-CMK-3. With the highly ordered mesoporous structure of O-CMK-3 (pore size, ca. 3.4 nm), the size-exclusion interaction of mesopores of O-CMK-3 to proteins is thus anticipated during the glycan enrichment of the PNGase F digested glycoproteins. To verify the clearance performance of O-CMK-3 to residual proteins, ovalbumin (44.6 kDa, 4.0 × 5.0 × 7.0 nm) was selected as the standard glycoprotein and digested by PNGase F enzyme to

release the N-glycans. For comparison, the organic solvent precipitation with 75% ethanol as well as the solid phase extraction of the use of active carbon material to remove the residual proteins were carried out. Though there is a lower ionization efficiency of proteins than that of peptides, there is still obvious protein signal with as low as 25 ng BSA and even much lower for cytochrome C (0.5 ng in 0.5 μL of H_2O) (Figure S5, Supporting Information), and the residual proteins were detected by MALDI-TOF MS using SA as the matrix. As illustrated at the right-upper inset in Figure 3A, there are two peaks of [M + 2H]$^{2+}$ and [M + H]$^+$ detected by MALDI-TOF MS, corresponding to mono- and discharge states of the residual ovalbumin after the release of N-glycans by PNGase F enzyme. Using 75% cold ethanol, the residual ovalbumin is still apparent (right-upper inset, Figure 3B), indicating the low efficiency in removal of residual protein by organic solvent precipitation. When ovalbumin digest was extracted by active carbon material, the residual protein peak of (M + 2H)$^{2+}$ could still be detected (right-upper inset, Figure 3C). The adsorption of residual protein on active carbon material is likely attributed to the protein trapping inside macropores of active carbon material. Interestingly, when the ovalbumin digest was extracted by O-CMK-3, the peaks corresponding to residual ovalbumin are absent in MALDI-TOF mass spectrum (right-upper inset, Figure 3D), suggesting the extensive removal of residual protein by the size-exclusion interaction of the mesoporous O-CMK-3 material.

To demonstrate the effect of residual protein removal on the analysis of N-glycans released from a glycoprotein, the N-glycans released from the ovalbumin by PNGase F enzyme were thus analyzed by MALDI-TOF MS. Figure 3A represents the direct analysis of N-glycans by MALDI-TOF MS without any
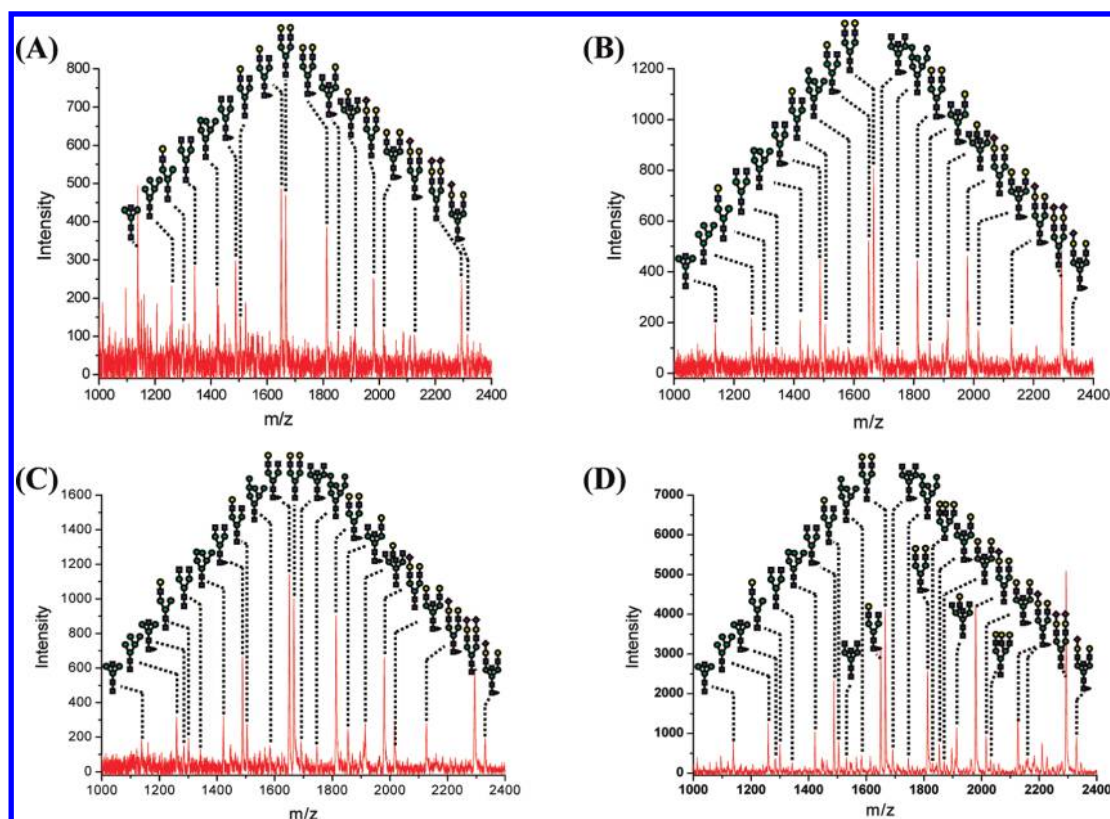
Figure 6. Comparison of glycan profiling of a serum mixture from 100 healthy volunteers by MALDI-TOF MS. N-linked serum glycans released by PNGase F and analyzed (A) by direct analysis, (B) after precipitation with 75% cold ethanol, (C) after enrichment by active carbon, and (D) after enrichment by O-CMK-3, respectively. Serum mixture was ultrafiltrated with MWCO of 10 kDa: galactose (yellow ●), sialic acid (green ●), GlcNAc (yellow ■), fucose (red ▲), sialic acid (pink ◆), and GlcNAc (blue ■). (The glycan structures were searched with the same manner as Figure S6B in the Supporting Information.)

Table 1. Signal to Noise (S/N) of Four of the N-Linked Glycans Released from Serum for Direct Analysis and Analysis after Extraction Using Organic Precipitation, Active Carbon (AC), and O-CMK-3, Respectively

No	m/z	Direct	Organic	AC	O-CMK-3	Structure
			S/N			
1	1485.5	4.9	9.0	10.1	38.5	
2	1647.2	8.9	10.3	22.2	52.3	
3	1663.0	8.6	14.4	18.0	67.2	
4	1809.0	6.5	9.0	17.6	42.2	

enrichment treatment, showing 16 glycan peaks with weak peak intensities and low signal-to-noise (S/N) ratios. After protein precipitation using 75% cold ethanol, 18 N-glycans can be detected with moderately improved peak intensities and S/N ratios. When extracted by active carbon (Figure 3C) and O-CMK-3 (Figure 3D) materials, the numbers of detected N-glycans increase to 24, along with the enhanced peak intensities. This indicates the carbon materials are the promising adsorbents in capturing N-glycans from protein digest.[13,14] While comparing O-CMK-3 and active carbon material, the

O-CMK-3 demonstrates the better performance over active carbon material due to the greater efficiency in the removal of residual protein because of the size-exclusion mechanism of highly ordered mesoporous structure of O-CMK-3 against macromolecular proteins.[22]

To further distinguish the performance of O-CMK-3 and active carbon materials, glycan analysis was carried out by adding different amounts of BSA in ovalbumin digest as the interference protein. The spectra obtained MALDI-TOF mass of N-glycans (Figure 4) show that the peak intensities of N-glycans decrease dramatically for active carbon material with the increase of the ratio of ovalbumin/BSA from 1:0 to 1:50, while the peak intensities of N-glycans remain at a pretty stable level for O-CMK-3. This indicates that the highly ordered mesopores of O-CMK-3 have a promising efficacy in excluding proteins for glycan analysis as compared with active carbon, even for more complex samples with different amounts of proteins. With O-CMK-3, 24 N-linked glycans of ovalbumin can be detected with good repeatability ($p >$ 0.18, t test) and low LOD (16 glycans from 10 ng ovalbumin) (Figure S6, Supporting Information).

Profiling of N-Linked Glycans of Human Serum Samples. Inspired by the high efficient removal of residual proteins by O-CMK-3, it was thus applied in the selective capture of N-glycans for human serum. Before the analysis of glycans from serum proteins, the removal of serum residual proteins by O-CMK-3 was performed with the comparison to active carbon extraction and organic solvent precipitation methods (Figure 5)
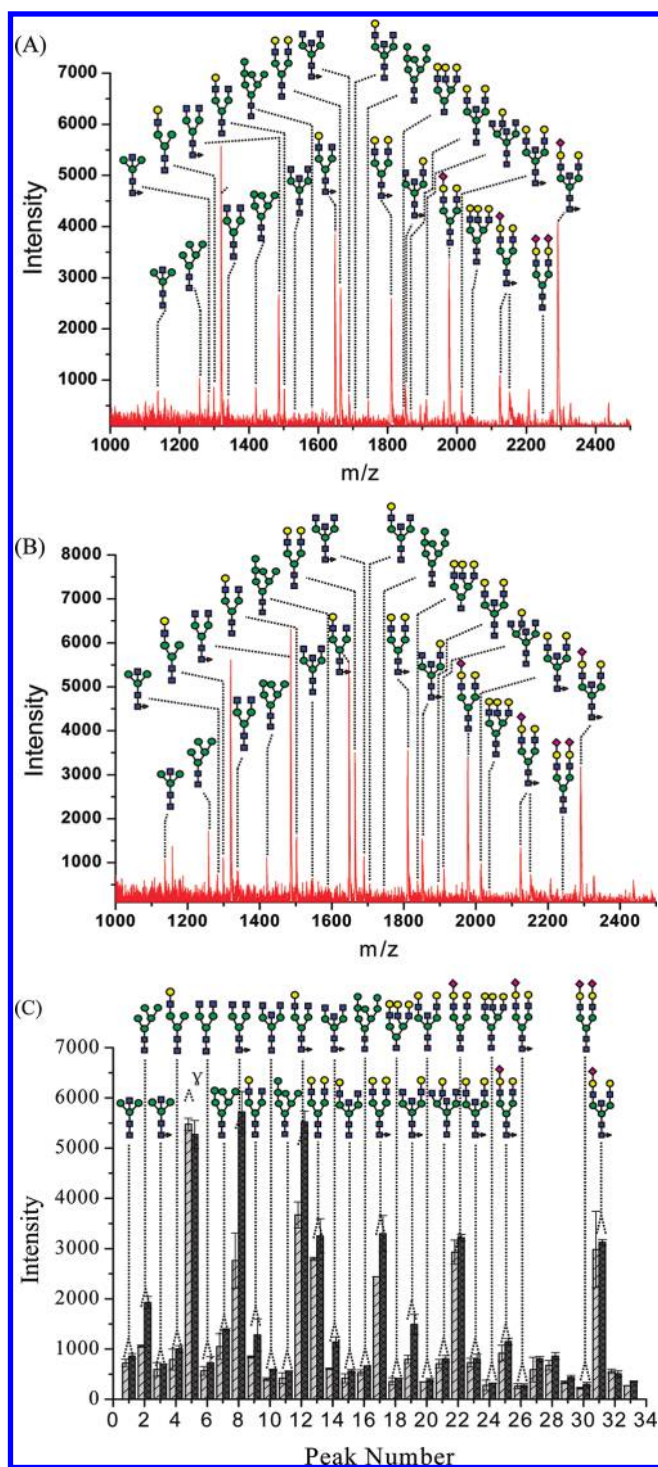
Figure 7. N-linked glycan profiling of serum mixtures from (A) 100 healthy volunteers and (B) 6 liver cancer patients; (C) the comparison of average peak intensities of 32 N-linked glycans enriched from the serum mixtures from 100 healthy volunteers and 6 liver cancer patients (each sample was repeated three times). (N-linked glycans were released by PNGase F digestion, and glycan structures were searched with the same manner as for Figure S6B in the Supporting Information.)

including human serum albumin (HSA) are still present after the organic solvent precipitation. With active carbon extraction, the HSA could not be detected but residual proteins around 12 kDa were present yet. This result indicates that the removal of residual proteins remains a problem for either solvent precipitation or active carbon material. Interestingly, these residual proteins are not detected by MALDI-TOF MS after the glycan enrichment treatment using O-CMK-3 (Figure 5D), indicating the high efficiency removal of serum residual proteins by O-CMK-3. Moreover, the analysis of N-glycans enriched by O-CMK-3 and active carbon materials was performed, where O-CMK-3 enrichment approach demonstrates its merits in analysis of N-glycans for real complex human serum sample as compared to active carbon as well as solvent precipitation methods. As shown in Figure 6, the peak intensity and the ratio of signal-to-noise (S/N) was obviously higher than the other three analysis, and the S/N of 4 of the identified glycans were displayed in Table 1, indicating the higher efficiency for the enrichment of glycans obtained using O-CMK-3 than the approach reported before.

Furthermore, we applied the O-CMK-3 in the analysis of N-glycans for liver cancer patients.[6,29] Here, a human serum mixture from 100 healthy volunteers was used as the control (Figure 7A), and the cancer serum sample was the serum mixture from 6 liver cancer patients (Figure 7B). In order to decrease the variation during the enrichment, γ-CD (m/z 1319.6) was chosen as an internal standard. 32 human serum N-glycans with 27 known were detected from both control and cancerous samples (Figure 7C). By comparing to the control sample, peak intensity of 5 N-linked glycans from the cancer sample were significantly increased ($p < 0.01$, t test), and 4 of them, including glycans of #8, #12, #14, and #19, were core-fucosylated. It is in the accordance with the previous report by Callewaert et al. that the level of the core-fucosylated glycoproteins would increase in the serum of liver cancer patients.[30]

■ CONCLUSION

In summary, we have developed an effective approach to enrich the N-linked glycans from serum samples using the oxidized mesoporous carbon materials of CMK-3. Due to the high surface area, specific interaction between carbon and oligosaccharides, and suitable mesopore structure of O-CMK-3, the N-linked glycans can be captured by the carbon material along with size-exclusion effect against the residual proteins which thus circumvent the problem in removal of residual proteins by other approaches including organic solvent protein precipitation and extraction with active carbon material. Using ovalbumin as the standard glycoprotein, 24 N-linked glycans could be detected with good repeatability and low LOD to 10 ng. For complex real human serum, the distribution of N-linked glycans from sera could be simply determined by MALDI-TOF MS. The profiling change of N-linked glycans between healthy serum and liver cancer patients has thus been discriminated, with 5 N-linked glycans up-regulated and 4 of which were core-fucosylated. It can be concluded that the glycan enrichment using O-CMK-3 will be a simple and promising approach for glycan profiling by MALDI-TOF MS, which has the great potential in glycan analysis for early disease diagnosis, such as liver cancer.

and the residual proteins after the ethanol precipitation and active carbon extraction detected by MALDI-TOF MS, respectively (Figure 5B,C). It is clear that the serum residual proteins

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■ ASSOCIATED CONTENT

ⓢ Supporting Information. Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

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