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Kapok Fiber: A Natural Biomaterial for Highly Specific and Efficient Enrichment of Sialoglycopeptides

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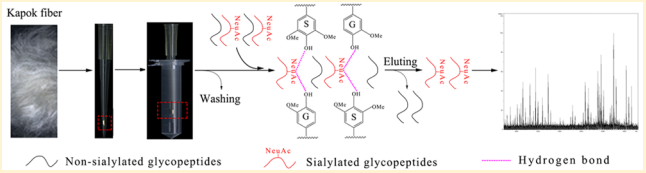
Supporting Information

ABSTRACT: Cancer development and chronic diseases are associated with the overexpression of sialoglycans terminated to the surface proteins and lipids of cancer cells compared with normal cells. The isolation and detection of sialoglycopeptides from complex peptides mixture still remain challenges due to their low abundance, low ionization, and losses of sialic acid residues and water molecule during analytical processes. In this study, kapok fiber, a natural fiber derived from the kapok tree (*Bombax ceiba* L.), has shown excellent capability to specifically and efficiently enrich sialoglycopeptides, without losses of sialic acid residues and water molecule from sialoglycans. The main components on the surface of kapok fiber are syringyl and guaiacyl lignins which play an important role in isolating sialoglycopeptides from complex peptide mixtures.



Sialylation of glycoproteins, the most frequent post-translational protein modification, plays numerous important roles in protein folding, protein stability, and protein functions.[1] Sialic acid (SA) frequently terminates glycan chains and contributes to numerous biological functions, such as cell−cell interactions, inflammation, immune response, and cancer metastasis.[2] All living cells are surrounded by terminal sialoglycans attached to cell surface glycoproteins and glycolipids. Previous studies have shown that heavily sialoglycans are closely associated with cancer and cancer metastasis,[3,4] and the occupancy alteration of sialylation sites has been involved in many human diseases and cancers.[5] High expression of SA is proposed to protect cancer cells from recognition by immune system.[6] Increased expression of SA on the surface of cancer cells may lead to its definition as potential therapeutic targets.[5] However, because of a nontemplate driven biosynthesis of the numerous and diverse glycan structures and high heterogeneity of sialylation sites, as well as their low abundance, low ionization efficiency, and losses of SA residues and water molecule during analytical processes,[7,8] these factors have made glycomic analysis to face great challenge.[2]

A number of efforts to enrich and detect sialoglycopeptides and/or glycopeptides from complex biological samples have been made, such as lectin,[9−11] serotonin-bonded silica,[12] hydrazine chemistry,[13,14] titanium dioxide chromatography,[8,15,16] hydrophilic interaction chromatography,[17] and strong cation exchange chromatography.[18] However, some shortages of these strategies, including low specificity, time-consuming, and potential losses of SA residues and water molecule from sialoglycopeptides during analytical processes, have limited their applications in deciphering correctly sialylation of glycoproteins. A detailed knowledge of sialoglycan structures can aid research efforts in developmental biology, plant sciences, agricultural research. Recently, because of the tremendous need and awareness of environmental impact, the utilization of natural resources is strongly encouraged. Here, we introduce a natural biomaterial, kapok fiber derived from the kapok tree (*Bombax ceiba* L.), to efficiently and selectively enrich sialoglycopeptides.

EXPERIMENTAL SECTION

Materials and Reagents. Bovine fetuin, transferrin, and bovine serum albumin were purchased from Sigma-Aldrich (Steinheim, Germany). PNGase F, α2-3,6,8 Neuraminidase, and RNase B were from New England Biolabs (Massachusetts). Trypsin was acquired from Roche Diagnostics (Mannheim, Germany). Alkaline phosphatase was from Promega (Madison, WI). Kapok fiber was collected from kapok tree (*Bombax ceiba* L.) in Xiamen, China. All other reagents were of analytical reagent grade.

Enzymatic Digestion. Protein was digested by trypsin followed by alkaline phosphatase as previously described.[8] Briefly, before enzymatic digestion, 20 μL of protein (1 μg/μL) was denatured for 6 min in boiling water. The denatured protein was reduced with 0.6 μL of 1 M dithiothreitol at 56 °C for 1 h, followed by alkylation with 5.6 μL of 500 mM iodoacetamide at room temperature for 45 min. The resulting protein was digested with 12 μL of 50 ng/μL trypsin at 37 °C for 12 h, and then dephosphorylated with 1.5 units of alkaline

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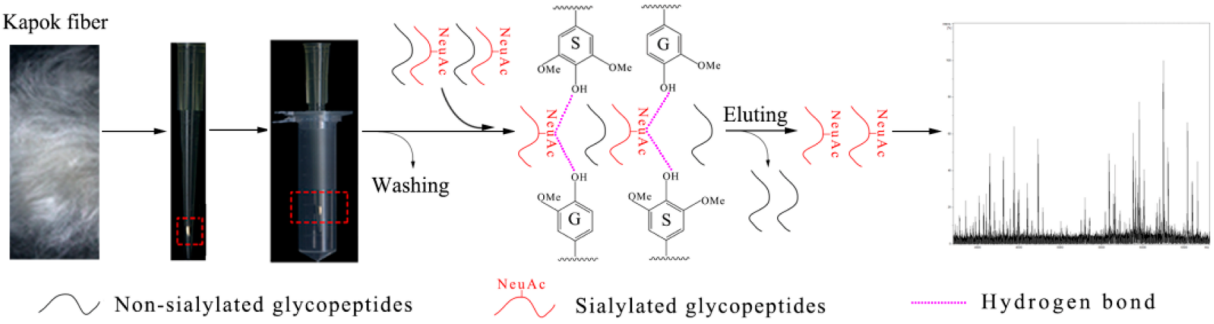
Scheme 1. Workflow of Sialoglycopeptide Enrichment Using kapfib-tip



phosphatase at 37 °C for 4 h. The resulting enzymatic digest was lyophilized and then stored at −80 °C until analysis.

Enrichment of Sialoglycopeptides Using Kapok Fiber. Kapok fiber was washed successively with water, 100% acetonitrile (ACN), and 80% ACN using ultrasonic generator. The cleaned kapok fiber was packed into a 200 μL pipet tip to make a kapfib-tip and then was washed successively with 50 μL of water, 50 μL of 100% ACN, and 50 μL of 80% ACN. The enzymatic digest dissolved in 80% ACN was loaded into the kapfib-tip followed by washing with 50 μL of 80% ACN. Finally, the enriched sialoglycopeptides were eluted using eluting solution. To obtain an optimal eluting condition, different eluting solutions (30 μL) including 0.1% trifluoroacetic acid (TFA), 0.2% formic acid (FA), H_2O, 5 mM NH_4HCO_3, and 0.25% $NH_3{\cdot}H_2O$ solutions were used to enrich sialoglycopeptides, respectively. The resulting solutions were lyophilized and then were redissolved with 2 μL of 0.1% TFA for mass spectrometric analysis.

Mass Spectrometric Analysis. All experiments were performed using an 9.4 T Apex-ultra hybrid Qh-FTICR MS (Bruker Daltonics, Billerica, MA) equipped with a 355 nm Nd:YAG Smartbeam II laser. Precisely 0.3 μL of solution was spotted onto a MALDI target plate and then was air-dried at room temperature before the addition of 0.3 μL of 20 μg/μL 2,5-dihydroxybenzoic acid (DHB) (50% ACN/0.1% TFA). The instrument resolution was 490 000 at m/z 400 over the m/z range of 1500−7000 in positive ion mode. Tandem mass spectra of some sialoglycopeptides were obtained using UltrafleXtreme MALDI-TOF/TOF MS. GlycoMod tool (http://web.expasy.org/glycomod/) was used to predict possible glycan structures based on the experimentally determined masses of glycopeptides combined with their individual tandem mass spectra.

Reproducibility and Stability. Five different amounts of bovine fetuin (i.e., 1, 2, 4, 8, and 16 pmol) were used to evaluate the limit of detection. The intraday and interday assays were also used to evaluate reproducibility and stability of the method.

Glycopeptide Enrichment Approaches of GA-ZipTip, Titanium Dioxide ZipTip (TiO_2-ZipTip), C18 Disk, and Cotton HILIC ZipTip. The procedures of GA-ZipTip, TiO_2-ZipTip, C18 Disk, and cotton HILIC ZipTip were employed to enrich glycopeptides as described previously.[15,19−21] Briefly, GA-ZipTip contains 1:1 ratio of graphite carbon to activated charcoal (w/w) with about 8 mm length. GA-ZipTip was washed sequentially with 30 μL of 80% ACN and 30 μL of 0.2% FA. Enzymatic digest was resuspended with 20 μL of 1% FA and then was loaded into the ZipTip. After washing with 30 μL of 0.2% FA, the enriched glycopeptides were eluted by 30% ACN/0.2% FA. The eluting solution was lyophilized and then was redissolved for mass spectrometric analysis.

TiO_2 was packed into a 200 μL pipet tip to make a TiO_2-ZipTip with about 5 mm length. The TiO_2-ZipTip was washed with 50 μL of ACN and 50 μL of water followed by 80 μL of 100 mg/mL DHB in 80% ACN/5% TFA. The enzymatic digest was resuspended with 20 μL of 100 mg/mL DHB in 80% ACN/5% TFA. The TiO_2-ZipTip was washed with 80 μL of 80% ACN/2% TFA and 20% ACN/0.1% TFA, respectively. The enriched glycopeptides were eluted by 80 μL of 0.8% phosphoric acid buffer. The eluting solution was desalted by a C18 column.

The C18 disk was transferred into an eppendorf tube. The enzymatic digest was resuspended with 20 μL of 20% ACN and then was mixed with the C18 Disk. After 3 h, the C18 disk was washed with water three times. The C18 disk was eluted using 5 μL of 20% ACN/0.1% TFA to obtain the enriched glycopeptides.

Cotton wool was packed into a 200 μL pipet tip to make a cotton HILIC ZipTip, washed with 50 μL of water three times and then 50 μL of 80% ACN three times. The enzymatic digest was loaded into the cotton HILIC ZipTip, followed by washing with 80% ACN three times. The glycopeptides were eluted in 30 μL of 0.25% $NH_3{\cdot}H_2O$ solution.

Scanning Electron Microscopy (SEM) and Fourier Transform-Infrared (FT-IR) Spectroscopy. Morphology was obtained by a Hitachi S-5500 ultrahigh resolution cold field emission scanning electron microscope at 20 kV in high vacuum mode, and FT-IR spectroscopy was obtained using Thermo Nicolet 5700 with 8000 resolution.

Lignin Isolation. Lignin was extracted with 96:4 dioxane/water solution. Approximately 2 g of kapok fiber was directly suspended in 10 mL of 96:4 dioxane/water and heated at 85 °C for 2 h. The resulting solution was filtered and washed with 10 mL of 96:4 dioxane/water until the filtrate was clear. The filtrate was concentrated to about 3 mL with nitrogen gas, and then 3 volumes of 95% ethanol was added into the filtrate. After filtration using a 20 nm nylon membrane filter, the lignin was obtained, followed by precipitation at pH 1.5−2.0 adjusted with HCl. Precipitation was washed with the acidified water adjusted with HCl (pH 2.0) and then was lyophilized. The resulting material was analyzed by FT-IR spectroscopy.

Wiesner and Mäule Staining Assays. Wiesner staining assay for guaiacyl lignin: about 10 mg of kapok fiber was treated with 600 μL of staining solution including 2% (w/v) phloroglucinol, 49.5% (v/v) ethanol, and 2.5% (v/v) HCl for 5 min at room temperature. Mäule staining assay for syringyl lignin: about 10 mg of kapok fiber was treated with 600 μL of staining solution (1% $KMnO_4$ in 50% ACN) for 5 min at room

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temperature, followed by washing with 1 mL of 19% HCl three times, and then 500 μL of $NH_3{\cdot}H_2O$ was added.

Delignification of Kapok Fiber. Six equal amounts (approximately 10 mg) of kapok fiber were treated with 2% H_2O_2 (pH 11.5 adjusted by NaOH solution) in 60 °C for different treatment time (i.e., 0, 5, 15, 30, 45, and 60 min), respectively. The treated kapok fiber was washed with water to remove H_2O_2 and NaOH.

RESULTS AND DISCUSSION

In this study, we introduce a simple, low cost, eco-friendly, highly selective, and highly efficient enrichment approach of sialoglycopeptides based on a natural biomaterial, kapok fiber, which is derived from the kapok tree (*Bombax ceiba* L.). First, approximately 10 mg of kapok fiber was packed into a 200 μL pipet tip to make a kapfib-tip. Herein, we take commonly used bovine fetuin as a model sialoglycoprotein for a sialoglycopeptide enrichment experiment using the kapfib-tip (Scheme 1). Mass spectra of the enriched sialoglycopeptides of 8.0 pmol of bovine fetuin using different eluting solutions indicate that 0.25% $NH_3{\cdot}H_2O$ solution as an eluting solution has highly efficient and selective enrichment of sialoglycopeptides (Figure S1). It should be noted that the signals from the larger sialoglycopeptides eluted using 0.25% $NH_3{\cdot}H_2O$ solution were significantly increased compared with other four eluting solutions. In total, 2.0 pmol of bovine fetuin could generate more than 32 sialoglycopeptides with high intensities over the high m/z region of the mass spectrum, and 8.0 pmol of bovine fetuin could produce more than 45 sialoglycopeptides with high detection sensitivity over the m/z range of 5000−7000 (Figure S2). The coefficient of variation for intra- and interday reproducibility of the number of the detected sialoglycopeptides from 8.0 pmol of fetuin was less than 2.6% (Figure S3) and 7.1% (Figure S4), respectively.

Mass spectra of the enzymatic digest derived from 8.0 pmol of fetuin (Figure 1A) is significantly different from that of the kapfib-tip-enriched glycopeptides of 8.0 pmol of fetuin, which has more than 45 sialoglycopeptides observed (Figure 1B). It should be noted that no glycopeptides were detected in the washing solution (Figure 1C). Figure 1D obtained from the kapfib-tip-enriched glycopeptides of 2,6,8 neuraminidase-treated enzymatic digest from 8.0 pmol of fetuin shows a few glycopeptides detected around the m/z region of 4000, while the mass spectrum of the kapfib-tip-enriched glycopeptides of PNGase F-treated enzymatic digest from 8.0 pmol of fetuin indicates that these glycopeptides disappeared after the PNGase F-treatment (Figure 1E). These results indicate that the kapfib-tip is a SA-specific enrichment material. Compared with Figure 1E, the peaks at around m/z 4000 shown in Figure 1D may be generated from glycopeptides without terminal SA residues.

We have used transferrin as another example to evaluate the above-mentioned phenomena. Mass spectrum of the enzymatic digest derived from 4.0 pmol of transferrin (Figure 2A) differs significantly from that of the kapfib-tip-enriched glycopeptides from 4.0 pmol of transferrin, which has 25 sialoglycopeptides detected (Figure 2B), while a few signals of peptides such as m/z 2228 were observed in the washing solution (Figure 2C). Compared with Figure 2B, it is found that no glycopeptides was detected in the kapfib-tip-enriched peptides of 2,6,8 neuraminidase-treated enzymatic digest from 4.0 pmol of transferrin (Figure 2D) and the kapfib-tip-enriched peptides of PNGase F-treated enzymatic digest from 4.0 pmol of transferrin (Figure 2E). These results again suggest that the kapfib-tip has excellent ability to selectively capture sialoglycopeptides.

RNase B as a high mannose glycoprotein without terminal SA residues was used to confirm the capability of kapfib-tip to enrich sialoglycopeptides. Mass spectra of the enzymatic digest (Figure 3A), the kapfib-tip-enriched species (Figure 3B), the washing solution (Figure 3C), and the kapfib-tip-enriched species of PNGase F-treated enzymatic digest from 8 pmol of RNase B (Figure 3D) have no significant difference, while mass

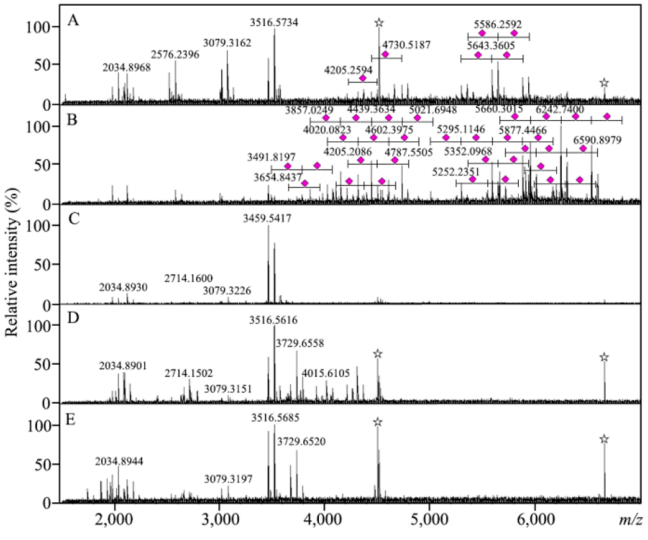

Figure 1. Mass spectra of the glycopeptides derived from the enzymatic digest of 8.0 pmol of bovine fetuin: (A) the enzymatic digest, (B) the kapfib-tip-enriched glycopeptides, (C) the washing solution, (D) the kapfib-tip-enriched peptides of α2-3,6,8 neuraminidase-treated enzymatic digest, and (E) the kapfib-tip-enriched peptides of PNGase F-treated enzymatic digest. Purple diamond, sialic acid residue; star symbol, electric noise.

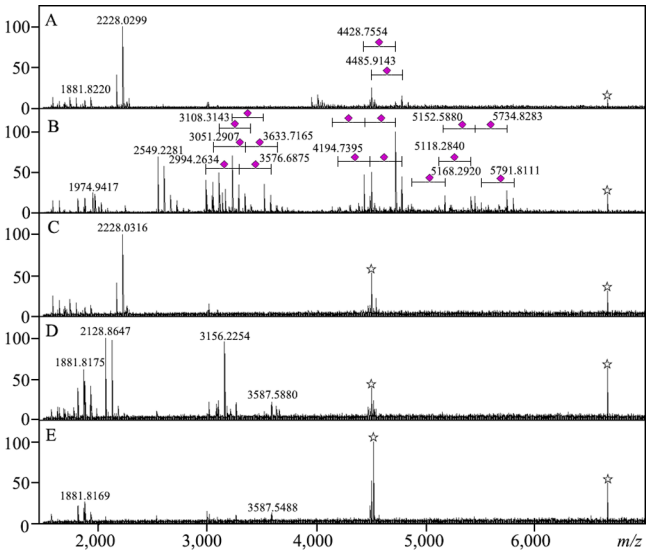

Figure 2. Mass spectra of the glycopeptides derived from the enzymatic digest of 4.0 pmol of transferrin: (A) the enzymatic digest, (B) the kapfib-tip-enriched glycopeptides, (C) the washing solution, (D) the kapfib-tip-enriched peptides of α2-3,6,8 neuraminidase-treated enzymatic digest, and (E) the kapfib-tip-enriched peptides of PNGase F-treated enzymatic digest. Purple diamond, sialic acid residue; star symbol, electric noise.

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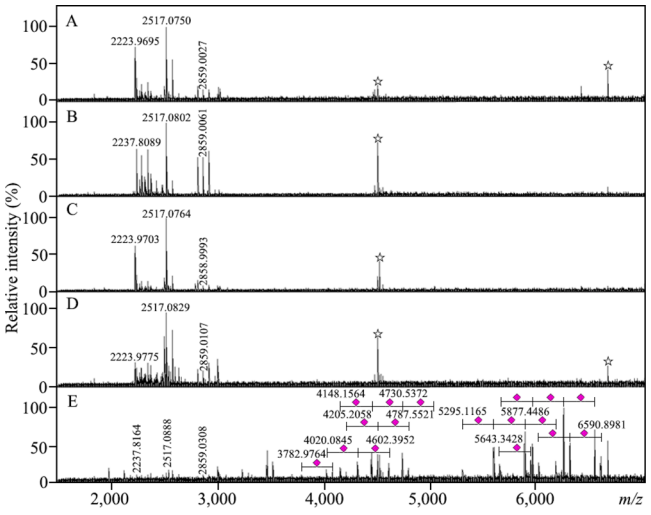

Figure 3. Mass spectra of glycopeptides: (A) the enzymatic digest derived from 8.0 pmol of RNase B, (B) the kapfib-tip-enriched glycopeptides of the enzymatic digest derived from 8.0 pmol of RNase B, (C) the washing solution, (D) the kapfib-tip-enriched glycopeptides of PNGase F-treated enzymatic digest derived from 8.0 pmol of RNase B, (E) the kapfib-tip-enriched glycopeptides of the enzymatic digest derived from a mixture of RNase B (8 pmol) and fetuin (8 pmol). Purple diamond, sialic acid residue; star symbol, electric noise.

spectrum of the kapfib-tip-enriched species from the enzymatic digest of a mixture of 8.0 pmol of fetuin and 8.0 pmol of RNase B shows abundant signals of sialoglycopeptides (Figure 3E). These results indicate that the kapfib-tip is a powerful tool to efficiently and selectively enrich sialoglycopeptides.

We have further used a mixture of 8.0 pmol of fetuin, 4.0 pmol of transferrin, and 4.0 pmol of albumin as a complex model to evaluate sialoglycopeptide enrichment performance of kapfib-tip. As shown in Figure 4, the spectral profile of the

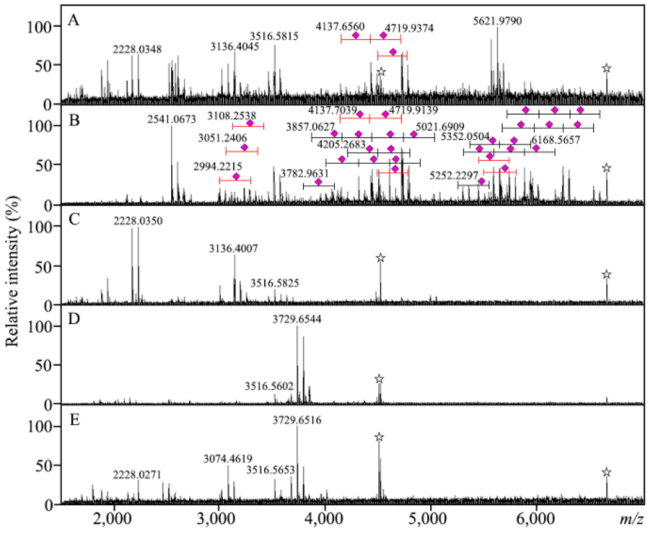

Figure 4. Mass spectra of the glycopeptides derived from the enzymatic digest of the mixture of 8.0 pmol of bovine fetuin, 4.0 pmol of transferrin, and 4.0 pmol of albumin: (A) the enzymatic digest, (B) the kapfib-tip-enriched glycopeptides, (C) the washing solution, (D) the kapfib-tip-enriched peptides of α2-3,6,8 neuraminidase-treated enzymatic digest, and (E) the kapfib-tip-enriched peptides of PNGase F-treated enzymatic digest. Purple diamond, sialic acid residue; star symbol, electric noise.

enzymatic digest from the protein mixture (Figure 4A) is remarkably different from that of the kapfib-tip-enriched glycopeptides from the mixture, which has 46 sialoglycopeptides detected (Figure 4B), while no sialoglycopeptides was detected in the washing solution (Figure 4C), and also no sialoglycopeptides was observed in mass spectra of the kapfib-tip-enriched peptides of 2,6,8 neuraminidase-treated enzymatic digest (Figure 4D) and the kapfib-tip-enriched peptides of PNGase F-treated enzymatic digest from the protein mixture (Figure 4E). These results suggest that the kapfib-tip also could selectively capture sialoglycopeptides from the complex peptide mixture. It should be noted that some low-abundance sialoglycopeptides, such as m/z 3576, m/z 3654, and m/z 3946, have not been observed due to ion suppression in the complex peptide mixture (Figure 4B). The detailed m/z values and their corresponding proposed structures of the detected sialoglycopeptides from fetuin, transferrin, and the protein mixture are shown in Figure S5 (Table S1), Figure S6 (Table S2), and Figure S7 (Table S3), respectively. The enrichment efficiency as ratio between number of peaks of the identified sialoglycopeptides to the total number is follows: 79% (45/57) for fetuin, 63% (25/40) for transferrin, and 78% (46/59) for the protein mixture. Some sialoglycopeptide structures (e.g., m/z 4074.126 from fetuin in Figure S8 and m/z 4720.05 from transferrin in Figure S9) were confirmed based on their tandem mass spectra. Taken together, all above-mentioned results further confirm that the kapfib-tip can specifically and efficiently enrich sialoglycopeptides from the complex peptide mixture.

To further evaluate kapfib-tip selective enrichment performance toward sialoglycopeptides, a comparison of kapfib-tip approach with GA-ZipTip,[19] TiO_2,[15] C18 disk,[21] and cotton HILIC ZipTip[20] were performed using fetuin as a model protein. As shown in Figure 1B and Figure 5, the kapfib-tip approach could provide highly selective and efficient enrichment of sialoglycopeptides, along with larger sialoglycopeptides detected over the m/z region of 5000−7000, as compared with other three approaches. These results indicate that the kapfib-tip approach not only enriches selectively and efficiently

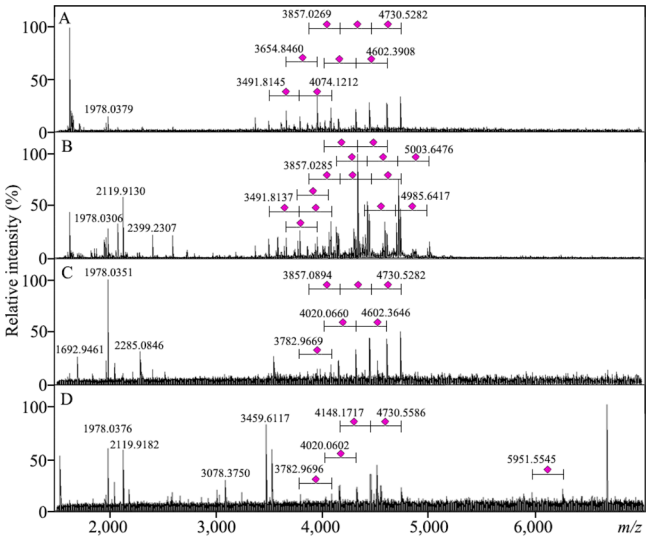

Figure 5. Mass spectra of the enriched sialoglycopeptides derived from 8.0 pmol of bovine fetuin: (A) GA-ZipTip, (B) TiO_2-ZipTip, (C) Empore C18 disk, and (D) cotton HILIC ZipTip. Purple diamond, sialic acid residue.

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sialoglycopeptides but also avoids loss of SA residues and water molecule from sialoglycopeptides.

The excellent capturing sialoglycopeptide performance of kapok fiber has pushed us to investigate its structural characteristics. The scanning electron microscopy analysis indicates that the surface morphology of the kapok fiber significantly differs from that of cotton wool which has been used for N-linked glycopeptide enrichment.[20] As shown in Figure 6A,B, the diameter of the kapok fiber (~8 μm) is much smaller than cotton wool (~20 μm), suggesting that the kapok fiber has more surface area than cotton wool for the capture of glycopeptides. In addition, the former has cylindrical and smooth morphology and the latter has flat, twisted ribbon with a rough surface. Previous study has indicated that cellulose is the main component of cotton (~100%).[20] To reveal the sialoglycopeptide enrichment mechanism of the kapok fiber, Fourier transform infrared (FT-IR) spectroscopy analysis was also performed. As shown in Figure 6C, the absorption spectrum of the kapok fiber (a) obviously differs from that of the cotton wool (b). The band at 2917.8 cm^{-1} corresponds to the asymmetric and symmetric aliphatic CH_2 and CH_3 stretching, which is associated with the presence of plant wax, such as *n*-alkanes and fatty acids, aldehydes, ketones, and *n*-alkyl esters.[22] The bands at 1740.4 and 1243.2 cm^{-1} are attributed to the presence of the carbonyl group (C═O) in the ester bonds.[23] The bands at 1595.3, 1507.2, and 1425.4 cm^{-1} are associated with the aromatic skeletal vibrations. And the spectrum of the dioxane-water extracts of the kapok fiber (c) with the characteristic absorption bands at 1594.1, 1507.5, 1422.2, 1328.2, 1268.2, 1226.1, 1126.6, 1031.6, and 834.8 cm^{-1} suggests the presence of guaiacyl (G) and syringyl (S) units,[24] suggesting that its feature is sensitive criterium for GS-type lignin. Taken together, the results indicate that lignin is the main structural component on the outer layer of the kapok fiber.

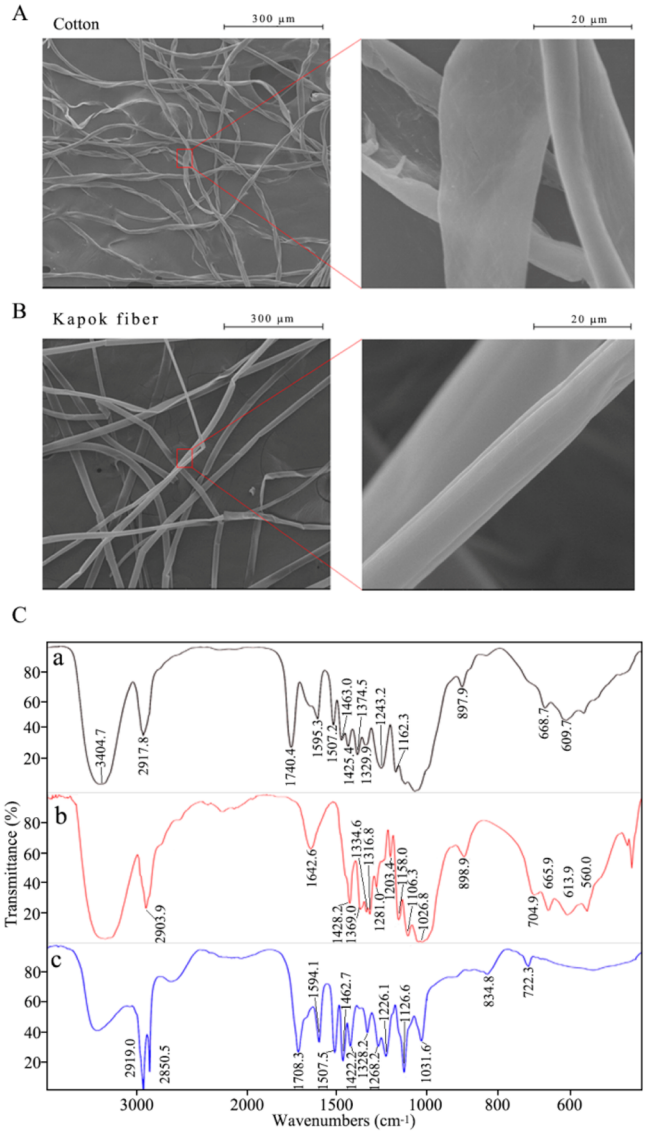

Figure 6. Scanning electron microscopy (SEM) and Fourier transform infrared (FT-IR) spectroscopy: (A) SEM morphology of cotton wool, (B) SEM morphology of kapok fiber, (C) FT-IR spectra of kapok fiber (a), cotton wool (b), and the isolated lignin of kapok fiber (c).

To evaluate the main factors that specially enrich sialoglycopeptides, Mäule and Wiesner staining assays were further performed (Figure S10A). It is found that cotton wool is hydrophilic and unstained, while kapok fiber is clumps in water and can be stained, suggesting that the kapok fiber is hydrophobic material. The delignified experiments of the kapok fiber indicated that the delignified kapok fiber shows weaker capability to capture sialoglycopeptides with an increase of the treatment time of delignification (Figure S10B). Taken together, these results suggest that the lignins on the surface of the kapok fiber play an essential role in capturing sialoglycopeptides.

The proposed enrichment mechanism of the kapfib-tip is as follows: GS-type lignin has a mass of hydroxyl group and oxygen atom, and SA residues have also hydroxyl group and oxygen atom. Under weak polar solution environment, such as 80% ACN in water, GS-type lignins can interact with the SA residues to form hydrogen bonds, resulting in attaching strongly sialoglycopeptides to lignins on the surface of the kapok fiber, while glycopeptides without terminal SA residues and peptides had none of these interactions and were washed away. However, under stronger polar and alkaline solution environment, such as 0.25% $NH_3{\cdot}H_2O$ in water, these hydrogen bonds were easily broken, resulting in these sialoglycopeptides being removed from the surface of the kapok fiber.

CONCLUSIONS

Kapok fiber, a natural material, is first employed in sialoglycopeptide enrichment. The material has highly specific and efficient capability to enrich sialoglycopeptides from the complex peptide mixture under an optimized eluting condition. The main component on the surface of kapok fiber is GS-type lignins which play an essential role in capturing sialoglycopeptides. More importantly, our results suggest that kapok fiber not only provides us a good opportunity for an eco-friendly probe of natural phenomena but also has efficient and selective performance to enrich sialoglycopeptides, without losses of SA residues and water molecules, from complex peptide mixture compared with other human-made materials which are employed to enrich glycopeptides and sialoglycopeptides.

ASSOCIATED CONTENT

Supporting Information

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Experimental details and supporting figures and tables (PDF)

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Notes

The authors declare the following competing financial interest(s): Dr. Zhili Li and Yujie Liu are listed as the inventors on a patent (No. 201510651116.6) filed in 2015 for a material and its uses thereof in sialoglycopeptide enrichment. The patent is owned by the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. No other potential conflict of interest relevant to this article was reported.

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REFERENCES

(1) Moremen, K. W.; Tiemeyer, M.; Nairn, A. V. *Nat. Rev. Mol. Cell Biol.* **2012**, *13*, 448−462.
(2) Cohen, M.; Varki, A. *OMICS* **2010**, *14*, 455−464.
(3) Varki, N. M.; Varki, A. *Lab. Invest.* **2007**, *87*, 851−857.
(4) Varki, A. *Trends Mol. Med.* **2008**, *14*, 351−360.
(5) Bull, C.; Stoel, M. A.; den Brok, M. H.; Adema, G. J. *Cancer Res.* **2014**, *74*, 3199−3204.
(6) Bull, C.; den Brok, M. H.; Adema, G. J. *Biochim. Biophys. Acta, Rev. Cancer* **2014**, *1846*, 238−246.
(7) Nie, H.; Li, Y.; Sun, X. L. *J. Proteomics* **2012**, *75*, 3098−3112.
(8) Li, F. J.; Wang, X. D.; Liu, Y. J.; Liu, H.; Li, Z. L. *Anal. Chim. Acta* **2013**, *787*, 140−147.
(9) Wu, J.; Xie, X. L.; Nie, S.; Buckanovich, R. J.; Lubman, D. M. *J. Proteome Res.* **2013**, *12*, 3342−3352.
(10) Cummings, R. D.; Kornfeld, S. *J. Biol. Chem.* **1982**, *257*, 1235−1240.
(11) Yang, Z. P.; Hancock, W. S. *J. Chromatogr. A* **2005**, *1070*, 57−64.
(12) Yodoshi, M.; Ikuta, T.; Mouri, Y.; Suzuki, S. *Anal. Sci.* **2010**, *26*, 75−81.
(13) Zhang, H.; Li, X. J.; Martin, D. B.; Aebersold, R. *Nat. Biotechnol.* **2003**, *21*, 660−666.
(14) Tian, Y.; Esteva, F. J.; Song, J.; Zhang, H. *Mol. Cell. Proteomics* **2012**, *11*, 11.
(15) Larsen, M. R.; Jensen, S. S.; Jakobsen, L. A.; Heegaard, N. H. H. *Mol. Cell. Proteomics* **2007**, *6*, 1778−1787.
(16) Palmisano, G.; Lendal, S. E.; Engholm-Keller, K.; Leth-Larsen, R.; Parker, B. L.; Larsen, M. R. *Nat. Protoc.* **2010**, *5*, 1974−1982.
(17) Takegawa, Y.; Deguchi, K.; Ito, H.; Keira, T.; Nakagawa, H.; Nishimura, S. I. *J. Sep. Sci.* **2006**, *29*, 2533−2540.
(18) Lewandrowski, U.; Zahedi, R. P.; Moebius, J.; Walter, U.; Sickmann, A. *Mol. Cell. Proteomics* **2007**, *6*, 1933−1941.
(19) Xin, L.; Zhang, H. J.; Liu, H.; Li, Z. L. *Rapid Commun. Mass Spectrom.* **2012**, *26*, 269−274.
(20) Selman, M. H. J.; Hemayatkar, M.; Deelder, A. M.; Wuhrer, M. *Anal. Chem.* **2011**, *83*, 2492−2499.
(21) Chen, G. Q.; Wang, Y. M.; Qin, X. Z.; Li, H. X.; Guo, Y. M.; Wang, Y. Y.; Liu, H.; Wang, X. D.; Song, G. G.; Li, F.; Li, F. J.; Guo, S.; Qiu, L.; Li, Z. L. *Electrophoresis* **2013**, *34*, 2407−2416.
(22) Tserki, V.; Zafeiropoulos, N. E.; Simon, F.; Panayiotou, C. *Composites, Part A* **2005**, *36*, 1110−1118.
(23) Lim, T. T.; Huang, X. *Ind. Crops Prod.* **2007**, *26*, 125−134.
(24) Faix, O. *Holzforschung* **1991**, *45*, 21−27.

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