In Situ Synthesis of Magnetic Mesoporous Phenolic Resin for the Selective Enrichment of Glycopeptides

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Supporting Information

ABSTRACT: Protein glycosylation is a significant participant in a mass of biological processes, which is a pivotal protein post-translational modification. Due to the low contents of glycopeptides compared with nonglycopeptides and the microheterogeneity of glycosylation sites, highly selective enrichment methods for the purification of glycopeptides are required for the comprehensive characterization of glycoproteomics. In this work, a type of magnetic mesoporous phenolic resin (MMP) was prepared using branched polyethylenimine (PEI) as a cross-linker from a homogeneous magnetic Fe₃O₄@SiO₂ solution in a resorcinol/formaldehyde monomer aqueous system via an in situ emulsion polymerization procedure. The results showed that MMP exhibited good biocompatibility, a mesoporous structure, nitrogen-containing functionality, excellent hydrophilicity, and solvent resistance by using multiple characterization methods. By taking advantage of the interaction between hydrophilic groups on the MMP and glycan components on the glycopeptides, the acquired MMP was utilized to the selective capture of N-glycopeptides (human IgG or HRP tryptic digests/BSA proteins = 1:50), good recovery yield (70.18−97.23%), superior binding capacity (400 mg g⁻¹), and excellent reproducibility. Based on the outstanding performance in standard glycoproteins tryptic digests enrichment, MMP was further used to capture N-glycopeptides from tryptic digests of human serum. A total of 15 unique N-glycopeptides were identified from an ultralow sample volume (0.025 µL) of human serum. Overall, we identified 356 unique N-glycopeptides corresponding to 119 glycoproteins from human serum (0.35 µL) in the overlap of three replicate analyses. All the results have demonstrated that MMP has great potential in large-scale N-glycoproteomics research.

Glycosylation is a kind of the most common, prevalent and important protein post-translational modifications (PTMs), which is a significant participant in many cell biological events, such as tumor immunology, cell−cell recognition, and signal transduction.¹⁻³ Changes in the glycosylation profiles are related to the development of tumors, and several cancer-related biomarkers are found to be glycoproteins or glycopeptides.⁴ Thus, efforts have been made to develop glycoproteomics analysis. Mass spectrometry techniques including matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and electrospray ionization mass spectrometry (ESI-MS) are key technologies for the biological functions research of glycosylation.⁵ While the ionization efficiency of glycopeptides in MS analysis is limited by their relatively low abundance, and the microheterogeneity of each glycosylation site still makes MS-based glycoproteomics analysis challenging. Therefore, the enrichment of glycopeptides become the prerequisite for the successful glycoproteomics analysis.

In recent years, efforts have been made to develop diverse techniques for glycopeptide capture, which basically include hydrazine chemistry,⁶ size exclusion,⁷,⁸ hydrophilic interaction liquid chromatography (HILIC),⁹⁻¹² boronate-functionalized matrix¹³,¹⁴ lectin affinity chromatography,¹⁵ and mesoporous material enrichment.¹⁶⁻¹⁹ Among these methods, HILIC-based enrichment has been efficiently developed due to its simple operating process, excellent compatibility with MS, high glycopeptide coverage, and good reproducibility.

Porous materials have drawn widespread attention of scientists in many fields because they can capture small-size targets from complex samples not only at their surfaces but also at the channels of the materials due to their porosity. During the past few years, various types of porous materials including metal−organic frameworks (MOFs),²⁰−²² covalent organic frameworks (COFs),²³−²⁵ porous organic polymers (POPs),²⁶ conjugated microporous polymers (CMPs),²⁷ magnetic nanoparticles,²⁸,²⁹ and mesoporous polymers (MPs)³⁰ have emerged. Among them, mesoporous polymers have attracted increasing attention because of their wide range of promising applications in adsorption, drug delivery and catalysis due to unique characteristics including well-defined porosity, diverse synthetic routes, and chemical functionalities in their porous.

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framework, which allow the rapid and effective binding of biomolecules. Simultaneously, many biocompatible mesoporous materials were developed by hydrophilic groups modified on mesoporous channels and the surface of the carrier, which endowed separation media with a size-exclusion effect on glycoproteomics research. Therefore, the employment of glycopeptide enrichment expands the application field of mesoporous polymers.

Herein, a template-free synthesis method was employed for the preparation of the magnetic mesoporous phenolic resin (MMP) with good magnetic properties and the high efficiency enrichment of glycopeptides using branched polyethylenimine (PEI) as a cross-linker and mesoporous structure-directing agents from a homogeneous magnetic Fe3O4@SiO2 solution in a resorcinol/formaldehyde monomers aqueous system via an in situ emulsion polymerization procedure. Here, the magnetic component Fe3O4@SiO2 was dispersed homogeneously in the monomer solution due to the intermolecular hydrogen bonds between hydroxyl groups and phenolic hydroxyl groups. Because the copolymer PEI was employed in the polymerization system, the hydrophilicity of the prepared magnetic mesoporous phenolic resin could be enhanced, indicating a better enrichment efficiency than traditional phenolic resin fabricated by polymerization in hydroquinone acidic solution accompanied by formaldehyde. Afterward, the resultant MMP with good biocompatibility, nitrogen-containing functionality, and superior hydrophilicity were utilized to specifically and efficiently enrich glycopeptides from complex biosamples.

## Experimental Section

### Materials

FeCl3·6H2O, ethanol, ethylene glycol, and sodium acetate were of analytical grade and purchased from Shanghai Chemical Reagent (Shanghai, China). Trifluoroacetic acid (TFA) and acetonitrile (ACN) were purchased from Merck (Darmstadt, Germany). Resorcinol and formaldehyde solutions (37 wt % in water) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Iodoacetamide (IAA) and dithiothreitol (DTT) were obtained from BioRad (Hercules, CA, U.S.A.). Branched polyethyleneimine (PEI) Mw = 70000 was purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Human immunoglobulin G (IgG), ammonium bicarbonate (NH4HCO3), trypsin (from bovine pancreas, TPCK treated), bovine serum albumin (BSA), 2,5-dihydroxyl benzoic acid (DHB), tetraethylanethoxysilane (TEOS), and concentrated ammonia aqueous solution (28 wt %) were purchased from Sigma (St. Louis, MO, U.S.A.). Human serum samples were offered by healthy volunteers at Zhongshan Hospital and stored at −80 °C before analysis. Distilled water was purified with a Milli-Q system (Milford, MA, U.S.A.).

### Preparation of the Magnetic Mesoporous Phenolic Resin

The magnetic mesoporous phenolic resin (MMP) were fabricated by an in situ synthesis process. In detail, 11.5 g sodium acetate and 5.20 g FeCl3·6H2O were mixed into 100 mL of ethylene glycol by the use of magnetic stirring for 30 min. After stirred, stainless-steel autoclave was employed to seal the mixture. And then the autoclave was located into the drier oven followed by heating 12 h under 200 °C. The autoclave was picked out until cooled to room temperature. In order to get cleaner Fe3O4 particles, the resulting products were washed with ethanol and Milli-Q water three times separately and dried at 60 °C in vacuum. Next, Fe3O4@SiO2 microspheres with core–shell structure were synthesized with the self-assembly method. Briefly, 200 mg dried Fe3O4 nanoparticles was dispersed in the solvents (40 mL of H2O, 160 mL of ethanol, 1.5 mL of concentrated ammonia aqueous solution) with the help of supersonic dispersion for 0.5 h, followed by 30 min mechanical stirring. Then, it is worth to mention that 1.5 mL of TEOS must be injected in the dispersion dropwise. The mixture was mechanically stirred for 12 h at room temperature. The process was repeated twice to produce a moderately SiO2 shell. The obtained Fe3O4@SiO2 microspheres were washed with ethanol and Milli-Q water three times separately and dried at 60 °C in vacuum.

The mesoporous polymers were encapsulated on the Fe3O4@SiO2 particles via an in situ synthesis method by using resorcinol, branched polyethylenimine and formaldehyde solution as precursors. The dried Fe3O4@SiO2 nanomaterials (200 mg) were dispersed in the solvents (9 mL of H2O, 11.4 mL of ethanol). After supersonic dispersion for 0.5 h, 600 mg of resorcinol was mixed in the solution and mechanically stirred at 40 °C for 30 min. Afterward, 47 mg of PEI (Mw = 70000, 50% in water) was injected into the above solution and stirred at 40 °C for 30 min. The formaldehyde aqueous solution (816 μL) was then quickly injected into the solution at 40 °C and mechanically stirred for another 30 min. Subsequently, stainless-steel autoclave was used to seal the mixture and heated to 120 °C in the drier oven. After 16 h, the autoclave was picked out until cooled to room temperature. The resulting brown gel was washed, washed with Milli-Q ethanol and water three times separately, and dried at 60 °C in vacuum. The obtained MMP was sealed in a centrifuge tube for further use.

### Characterization of the Magnetic Mesoporous Phenolic Resin

The morphology and elemental analysis of the MMP particles were characterized by using transmission electron microscopy (JEOL 2011, Tokyo, Japan). A drop of the MMP particles solution were dried onto a Cu grid (Lacey Formvar/Carbon #01883-F, Ted Pella Inc., U.S.A.). Fourier transform infrared spectroscopy (FT-IR) characterization was conducted by a Thermo Nicolet 380 spectrometer with a KBr pellet (Nicolet 6700, Wisconsin, U.S.A.). Thermogravimetric analysis (TGA) was detected under a nitrogen atmosphere, and the temperature was ranged from 25 to 800 °C (Netzsch, Selb, Germany). The pore size distribution and nitrogen adsorption/desorption isotherms were detected with nitrogen adsorption (Micromeritics, AsAp2010, GA, U.S.A.) system, the liquid nitrogen temperature was −196 °C in the application of static-volumetric method. The Brunauer–Emmett–Teller (BET) equation was used for the calculation of the surface area value which was obtained from the nitrogen adsorption isotherm and a relative pressure (P/P0) 0.01–0.98 was described. The calculation of pore diameter and distribution curves from the adsorption branch were obtained from Barrett–Joyner–Halenda (BJH) method. PerkinElmer PHI 5000C ESCA system was employed for the performance of X-ray photoelectron spectroscopy (XPS) using a hemispherical electron energy analyzer. The Mg Kα (hv = 1253.6 eV) anode operated at 14 kV and 20 mA. A Bruker D8 Advance X-ray diffractometer with Ni-filtered Cu Kα radiation (40 kV, 40 mA) collected powder X-ray diffraction (PXRD) data. Water contact angle measurement was carried out on JC 2000A optical contact angle meter at ambient conditions. A superconducting quantum interference device (SQUID) determined magnetic properties. The magnetization loops were measured at 300 K by sweeping the applied magnetic field between 15 and −15 kOe. The size distributions of MMP material with

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Glycopeptides Enrichment by Magnetic Mesoporous Phenolic Resin. At first, tryptic digests of IgG and HRP were applied as standard glycopeptides samples to verify the enrichment efficiency of MMP. A total of 300 μg of the synthesized MMP particles were cleaned by loading solution, consisting of 94% ACN–H2O and 0.5% TFA, in a microcentrifuge tube (600 μL) and then dispersed in 400 μL of loading solution with the help of a supersonic dispersion for 5 min. The tryptic digests of IgG or HRP (20 μg) were injected, then at room temperature, the mixture was incubated for 6 h. The supernatant of MMP was pipetted out with the help of a magnet and that of MP was pipetted out with centrifugation at 15000 g for 5 min. Afterward 20 μL of desorption solution (30% ACN–H2O, 0.1% TFA) was used to elute the captured glycopeptides under powerful shaking for 6 h in a new tube. A total of 0.5 μL of the final elution was detected by MALDI-TOF-MS (matrix is consisted of 25 mg/mL of 2,5-DHB in 70% ACN–H2O, 1% H3PO4).

For glycopeptide enrichment from the tryptic digests of human serum sample, 5 and 20 μL human serum tryptic digests were treated with 3 mg and 100 μg of the synthesized MMP particles according to the above procedure. A total of 20 μL of the obtained eluate was lyophilized using a vacuum centrifuge and redispersed with 25 μL of the NH4HCO3 solution (10 mM), and 50 units of PNGase F was used to release glycans at 37 °C, which reacted for 16 h. The solution was detected by nano-LC-MS/MS.

Recovery Yield Characterization of Magnetic Mesoporous Phenolic Resin for Standard Glycopeptides Enrichment. Six standard glycopeptides were synthesized according to previous strategy.35 According to a previous method,36 stable isotope dimethyl labeling was used to investigate the recovery yields of six standard glycopeptides. Two batches of glycopeptide mixtures were first tagged with light (each amino group pluses 28 Da) and heavy (each amino group pluses 32 Da) dimethyl isotopes, respectively. A total of 1 μg light-labeled glycopeptides were dispersed into 400 μL of loading solution (94% ACN–H2O, 0.5% TFA) and enriched with 50 μg MMP and MP, respectively. At room temperature, the mixture was incubated for 6 h. With the help of a magnet, the supernatant was pipetted out. Afterward 20 μL of desorption solution (30% ACN–H2O, 0.1% TFA) was used to elute the captured glycopeptides under powerful shaking for 6 h in a new tube. Then the elution was mixed with 1 μg heavy labeled glycopeptides. The mixture was tested directly by MALDI-TOF-MS. As a result, isotope cluster area was used to calculate the recovery yields, which was determined by ratios of light and heavy-tagged glycopeptides.

RESULTS AND DISCUSSION

Synthesis and Characterization of Magnetic Mesoporous Phenolic Resin. In the previous work, many types of mesoporous materials have been reported.31−33 To capture glycopeptides from biological samples, the chemical modification of the surfaces of these materials is common. However, as a new type of material, MMP shows a great advantage during glycopeptide enrichment. As shown in Scheme 1a, an in situ polymerization method was used to build the mesoporous network structure of MMP. Although this type of polymer may not be more facile in applications compared with magnetic materials, the mesoporous structure can be synthesized in one step without any template. During the in situ polymerization process, proper controls on the surface of the magnetic material provide better dispersion in the monomer solution. Specifically, there are many hydroxyl groups on the surface of the Fe3O4@SiO2 microspheres, which hydrogen bonds were formed with the phenolic hydroxyl groups from resorcinol. Thus, the Fe3O4@SiO2 microspheres were dispersed well in the resorcinol solution, and the homogeneous magnetic mesoporous polymer material is obtained without any agitation after in situ polymerization. As shown in Figure S1, whether MMP was grinded (a), (c) or not (b), (d), they both exhibited great magnetism. Then, the glycopeptide enrichment can be achieved by MMP, as shown in Scheme 1b.

The morphology of the MMP was optically detected by TEM. As shown in Figure 1, the MMP materials contain homogeneous Fe3O4@SiO2 microspheres, which consist of a magnetic Fe3O4 core (200−400 nm, Figure S2a) and an encapsulated layer of SiO2 (∼21.9 nm, Figure S2b) generated from TEOS.35,37 The sheet structure in gray and white colors show the pore channels of the phenolic resin component. The...
elemental composition of the MMP by energy dispersive X-ray (EDX) analysis (Figure 1, inset, without determination of N) shows the presence of C, O, Si, and Fe. It is worth to mention that the SiO2 layer is significant for enhancing the hydrophilic of MMP due to the hydroxyl groups on the surface, which also promoted the dispersibility in aqueous system. So the layer should be as dense as possible under conditions that do not affect the magnetic property. Thus, we repeated the coating process twice to build the dense SiO2 layer, and the thickness of SiO2 is suitable for the fabrication of MMP.

The chemical composition and structure of MMP were tested by FT-IR spectroscopy (Figure S3a). The absorption peaks around 680 and 1640 cm⁻¹ can be attributed to N–H deformation vibrations of out-of-plane and in-plane, respectively, demonstrating that amine groups are existing, which play a vital part in the hydrophilic enrichment. Furthermore, peaks of MMP at 1090 cm⁻¹ can be attributed to C–N stretch vibration, 3428 cm⁻¹ (O–H stretch vibration), 1460, 1510 cm⁻¹ (the vibrations of phenyl skeleton), 2850–3090 cm⁻¹ (C–H stretching model of phenyl), and 1240 cm⁻¹ (C–O stretch vibration on phenyl) were also identified. Meanwhile, the FT-IR spectrum of mesoporous phenolic resin (MP), which is synthesized without the magnetic components Fe₃O₄@SiO₂, is shown in Figure S3b, and the characteristic peaks are similar to those in Figure S3a.

The mass ratios of different components and thermal stability of MMP were revealed by thermogravimetric analysis (TGA; Figure S4a,b). Compared with the reduction in weight rose from 200 to 950 °C in Figure S4, it can be concluded that the magnetic component was successfully synthesized into the mesoporous phenolic resin. Between 200 and 950 °C, the weight of MMP material decomposed by 41.30 wt %, the weight of MP material decomposed by 56.20 wt %. This result shows the reduction slows down when the temperature reached 700 °C. From 850 to 950 °C, the distance between the two curves are almost constant at 13.90 wt %. So the content of magnetic component Fe₃O₄@SiO₂ in MMP is calculated to be 26.51 wt %.

XPS characterization identifies the surface elements of MMP (Figure S5a). The nitrogen content was determined to be 0.62% (wt %, Figure S5a, inset), and compared with the nitrogen content of MP (0.76%, Figure S5b, inset) in Figure S5b, the presence of nitrogen within MMP was successfully revealed.

A superconducting quantum interference device (SQUID) was used to record the magnetic properties of the materials. The Fe₃O₄@SiO₂ core exhibits a strong saturation magnetization value measured to be 72 emu/g. After an in situ polymerization process, the obtained MMP material still shows a strong saturation magnetization value measured at 62 emu/g. Without the magnetic component, the MP material exhibits a small saturation magnetization value measured at 10 emu/g. In Figure S6, the magnetic hysteresis loops of Fe₃O₄@SiO₂, MP, and MMP material. Due to the homogeneous presence of the magnetic component Fe₃O₄@SiO₂, the MMP material present superparamagnetic property.

Nitrogen adsorption–desorption isotherms of MMP was tested to further prove the porous structure of it (Figure 2). The hysteresis loops with Type IV isotherms of MMP and MP matches the well-constructed mesopores. The surface area of the MMP were determined to be 120 m²/g, suggesting the high surface area of MMP. The pore size distribution of MMP was shown in Figure S7, it is calculated by Barrett–Joyner–Halenda (BJH) model. The result further indicated that the pore size concentrated at 13.2 nm, which can effectively enrich glycopeptides and exclude proteins.

The structures of MMP were characterized by the XRD pattern (Figure S8). Six discernible diffraction peaks at 30.08°, 35.38°, 43.18°, 53.38°, 57.08°, and 62.68°, in the database of the Joint Committee on Powder Diffraction Standards (JCPDS card: 19–0629) file, they were indexed as (220), (311), (400), (422), (511), and (440). This result clearly reflected the presence of the magnetic component in the MMP material. Compared with the MP material, the patterns between 10° and 25° are similar, which exhibit the amorphous characteristics of the mesoporous phenolic resin component.

Figure S9 shows the water contact angles of MMP and MP. The results suggest that the water contact angles of the materials were lower than 40°, indicating both of the two materials were hydrophilic. This result suggesting the existence of hydroxyl groups on the materials ensures the great hydrogen bonding capability between MMP and glycopeptides.

Dynamic light scattering (DLS) analysis verified the homogeneous property of MMP material with different grinding time (Figure S10). The results shows that the size distribution of MMPs range from 6000 to 600 nm, while the grinding time ranges from 10 to 40 min. With the extension of grinding time, the size distribution gets narrow. Indicating that the homogeneous property of MMP material getting more uniform. As shown in Figure S11, the enrichment efficiency of MMP material promoted with the reduction of particle size distribution. Besides, after the particle distribution concentrated at 1000 nm or below, the increment in the enrichment efficiency is not significant.

All the data above prove that the MMP material with a homogeneous magnetic property was successfully synthesized, and its well hydrophilicity was confirmed which indicating the capability for the enrichment of glycopeptides with high efficiency and selectivity. MMP not only shows excellent selectivity regarding glycopeptide enrichment and size exclusion effect as the previous mesoporous polymer did but also possesses a rapid separation property as a magnetic material. Glycopeptides Enrichment by Magnetic Mesoporous Phenolic Resin. The efficiency of MMP in glycopeptide enrichment was first assessed by employing human IgG and HRP tryptic digests as model biological samples. Before enrichment of the human IgG tryptic digest, nonglycopeptides...
peaks suppressed the ionization of the N-linked glycopeptides (Figure S12), and only two glycopeptides can be detected and the signal-to-noise ratios (S/N) are relatively low, which is shown in Figure 3a. However, after capture with MMP, the spectrum of MALDI-TOF MS showed that 28 N-glycopeptides were detected with strong signals (Table S1 and Figure 3b). As a comparison in Figure 3c, the enrichment with MP showed that 21 N-glycopeptides were detected with strong signals, which is a little less than the MMP result. This might due to the silica layer coated on the magnetic core which have a lot of hydroxyl groups, and this property further improved the hydrophilicity of MMP, which enhanced the enrichment efficiency.16

Before enrichment of the HRP tryptic digest, nonglycopeptides peaks suppressed the ionization of the N-linked glycopeptides and only two glycopeptides can be detected and the signal-to-noise ratios (S/N) are relatively low, which is shown in Figure 3d. However, after capture with MMP, the spectrum of MALDI-TOF MS showed that 20 N-glycopeptides were detected with strong signals (Table S2 and Figure 3e), suggesting the superior affinity of MMP toward glycopeptides. As a comparison, the enrichment with MP showed that 12 strong signals that correspond to the N-glycopeptides are detected (Figure 3f), which is a little less than the MMP result. The glycopeptides are marked with "*".

Figure 3. MALDI mass spectra of the tryptic digest human IgG or HRP without (a, d), with MMP (b, e) or MP (c, f) enrichment. The glycopeptides are marked with "*".

Figure 4. MALDI mass spectra of the mixture of BSA proteins and tryptic digest of human IgG (BSA proteins/IgG tryptic digests = 10:1, 50:1) without (a, d) and with MMP (b, e) or MMP in large particle (c, f) enrichment. The glycopeptides are marked with "*".

The enrichment specificity and size-exclusion effect of MMP toward glycopeptides was further tested by utilizing human IgG and HRP tryptic digest with multiple amount of non-glycosylated protein BSA (IgG/HRP tryptic digests:BSA proteins = 1:10 and 1:50). Due to the BSA proteins’ interference, the signals of glycopeptides declined obviously. Thus, the identification of glycopeptides was impossible by direct analysis. As shown in Figure 4, with ratios of the BSA proteins and tryptic digest of human IgG 10:1 w/w and 50:1 w/w, practically no peaks of glycopeptides could be detected. However, 25 and 22 glycopeptides could be distinctly identified after the capture of MMP. As a comparison, the enrichment with MMP in large particles (grinding for only 5 min, whose size distribution cannot be determined by DLS) showed that 21 and 17 signals corresponding to N-glycopeptides are detected, which is a little less than the MMP result. We detected the MALDI-MS of proteins in the solution before and after enrichment (Figure S15). BSA proteins can be identified in the supernatant, but no peak was found in the elution, indicating that MMP can effective exclusive high molecule proteins and enrichment the low abundant glycopeptides.

As shown in Figure 5, with ratios of BSA proteins and HRP tryptic digest of 10:1 and 50:1 w/w, only seven and five glycopeptides with low signal-to-noise ratios (S/N) were detected. Whereas after the capture of MMP, 17 and 11 glycopeptides could be detected separately. As a comparison, the enrichment with MMP in large particles showed that 11 and 9 signals corresponding to N-glycopeptides are detected, which is a little less than the MMP result, indicating the enrichment capability of the MMP in large particles.

The recovery yields of six synthesized standard glycopeptides with different glycoforms and peptides were tested by stable isotope dimethyl labeling. There are no significant difference among the recovery yields of six synthesized standard glycopeptides (Table S3), which ranged from 70.18% to 97.23%. No bias capture of MMP for glycopeptides and proteins and tryptic digest of human IgG 10:1 w/w and 50:1 w/w, practically no peaks of glycopeptides could be detected. However, 25 and 22 glycopeptides could be distinctly identified after the capture of MMP. As a comparison, the enrichment with MMP in large particles (grinding for only 5 min, whose size distribution cannot be determined by DLS) showed that 21 and 17 signals corresponding to N-glycopeptides are detected, which is a little less than the MMP result. We detected the MALDI-MS of proteins in the solution before and after enrichment (Figure S15). BSA proteins can be identified in the supernatant, but no peak was found in the elution, indicating that MMP can effective exclusive high molecule proteins and enrichment the low abundant glycopeptides.

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superior recovery yields are confirmed. Compared with the recovery yields of MP, which range from 75.62% to 102.64%, there are no significant differences between the two materials. Indicating the Fe₃O₄@SiO₂ components have no visible influence on the recovery yields.

A series quality of materials (50 μg) and IgG digests (1–30 μg) were employed to investigate the binding capacity. After enrichment, the eluted solution from MMP and MP (0.5 μL) were tested by MALDI-TOF-MS. The maximum intensities of two selected glycopeptides appear when the IgG digests amount reached 20 μg. Thus, the calculated binding capacities of MMP and MP were both 400 mg g⁻¹, which is shown in Figure S16. Compared with HILIC materials such as CSMs (100 mg g⁻¹), 38 Fe₃O₄@DA-maltose (43 mg g⁻¹), 39 Fe₃O₄@CS MCNCs (17.5 mg g⁻¹), 40 and boronic acid-functionalized mesoporous silica (40 mg g⁻¹), 41 MMP shows a large binding capacity which is taking advantage of many polar groups on MMP and also due to strong multivalent hydrophilic interactions between MMP and glycopeptides.

To evaluate the applicability of MMP in analysis of large scale glycoproteomics from complex biosamples, a trypsic digest of 0.025 μL human serum was employed for investigation. After enrichment with MMP, the acquired glycopeptides were followed by elution. PNGase F was used to deglycosylate the glycopeptides. Finally the nano-LC-MS/MS was applied to analyze the deglycosylated glycopeptides. N-Glycopeptides and their sites identified by consensus motif of N-glycosylation (N-X-T/S/C, X ≠ P). 35 According to the Human UniProtKB/Swiss-Prot database, from 0.025 μL the human serum, a total of 15 N-glycopeptides assigned to 9 glycoproteins were identified (detailed information regarding the identified N-glycopeptide is shown in Table S4). Compared with the previous hydrophilic strategy using 1 μL of human serum, 34 MMP showed comparable performance in the analysis of glycoproteomics with an ultralow volume of human serum (0.025 μL).

With the loading amount of trypsic digest of human serum increasing to 0.35 μL, a total of 356 unique N-glycospeptides corresponding to 119 glycoproteins were identified in three replicate analyses (Figure S17, Table S5). The enrichment performances of MMP were compared with previous materials in Table S6. 38–41 MMP shows high binding capacity, high enrichment efficiency, and the recovery yield are preferable. The glycopeptides enrichment from complex biosamples is excellent. The results suggest that MMP is able to specifically capture the glycopeptides from complex biosamples.

CONCLUSION

In summary, magnetic mesoporous phenolic resin (MMP) with a superior homogeneous property, magnetism, binding capacity and reproducibility was prepared in a resorcinol/formaldehyde monomer aqueous system via a one-step in situ emulsion polymerization procedure. Owing to the hydrophilic interaction between abundant nitrogen-containing functionalities and glycopeptides and mesoporosity, MMP was used to capture the glycopeptides with remarkable selectivity, excellent sensitivity, high binding capacity, and good biocompatibility with HRP digests, IgG digests, and complex biological sample such as human serum digests. All in all, MMP is a talented enrichment medium for the identification of low abundant glycopeptides in human serum with tiny volume. MMP has the potential to be a new access to the analysis of large-scale glycopeptomies in complex biosamples.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.8b00708.

Sample preparation of HRP, human IgG, and human serum; Parameters for MS analysis and database research; Photographs of the MMP dispersion solution; FT-IR spectra; TGA curves; XPS patterns; Magnetic hysteresis curves; TEM pages; PSD analysis curves; Recovery yield table; Binding capacity curve; PXRD patterns; Water contact angles; Detailed information on glycopeptides identified from human IgG, HRP, and human serum; Comparison table between MMP and previous materials (PDF).

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Notes

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REFERENCES

(2) Ohtsubo, K.; Marth, J. D. Cell 2006, 126, 855–867.