Peptide Enrichment

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Selective Extraction of Peptides from Human Plasma by Highly Ordered Mesoporous Silica Particles for Peptidome Analysis**

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The peptidome of human plasma has attracted increasing interest for its role in the elucidation of biological and pathological variation and discovery of useful biomarkers.[1] Recently, the development of mass spectrometry techniques has increased efforts to broadly characterize its peptide constituents. However, the complexity and high dynamic range of human plasma make the characterization a challenging task.[2] Toward this end, two main methodologies are currently used to enrich peptides from plasma samples. Centrifugal ultrafiltration with accurate molecular weight (MW) cutoff is the most widely used method to extract peptides by removing proteins with larger MWs based on a size-exclusion mechanism.[3] However, as a result of the high protein content of human plasma (60–80 $mg\,mL^{-1}$), the ultrafiltration time will increase sharply if a large amount of plasma sample is applied. Furthermore, other low-MW contaminants (for example, salts) will also be concentrated. Another option is to use adsorbents carrying charged or hydrophobic groups for peptide adsorption.[4] While the peptides are enriched, various proteins will be adsorbed equally. Therefore, it is necessary to develop a general method to overcome the limitations of the state-of-the-art methods.

Silica particles with highly ordered mesostructures (for example, M41s[5] and SBA-15[6]) have been widely applied in the fields of separation and adsorption.[7] The unique properties of these silica materials include high in-pore surface areas, extremely narrow pore size distribution, perfectly adjustable pore size, and the presence of silanol groups and siloxane bridges, which make them effective for selective adsorption of standard proteins with different MWs based on the size-exclusion mechanism.[8] In this study, attempts were made to use these materials with critical pore sizes to selectively enrich peptides from human plasma, but exclude other proteins by an accurate MW cutoff like that of centrifugal ultrafiltration.

Three types of highly ordered mesoporous silica particles with different pore sizes were synthesized according to reported procedures.[5,6,9] A representative TEM image and the pore size distribution diagram for MCM-41 silica particles (Figure 1) demonstrate the existence of highly ordered cylindrical mesopores running along the same direction. This property is critical for selectively adsorbing peptides with accurate MW cutoff and enhanced mass transfer for fast adsorption/desorption equilibration. The characteristics of the three kinds of mesoporous silica particles with similar mesostructures are listed in Table 1. All of the silica particles

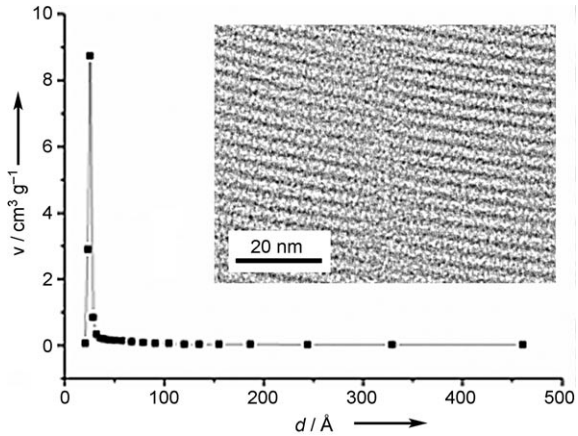

Figure 1. Barrett–Joyner–Halenda (BJH) pore size distribution calculated from the adsorption branch of the nitrogen adsorption/desorption isotherm. Inset: TEM image of corresponding MCM-41 mesoporous silica particles.

Table 1: Comparison of the material properties of three types of mesoporous silica particles and their adsorption efficiency for standard protein lysozyme.

Silica particles	Surface area [$m^2\,g^{-1}$][a]	Pore size [Å][a]	Amounts of lysozyme adsorbed [$mg\,g^{-1}$][b]
MCM-41	871	20.5	63.6
C-SBA-15	645	84.5	191.3
L-SBA-15	639	120	420.8

[a] The surface area and pore size were determined by N_2 adsorption. [b] The amounts of lysozyme adsorbed on the silica particles were determined by UV adsorption at 280 nm (see the Supporting Information).

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have large surface areas to ensure enough capacity for peptide adsorption.

Compared with conventional adsorbents for peptide enrichment, a unique character of these materials is their highly ordered mesopores. The size-exclusion effect of the pore structure was investigated with standard protein lysozyme (MW 14400 Da), because its MW is near the cutoff to sample pretreatment of human plasma for peptidome analysis (that is, approximately 15 kDa[10]). As shown in Figure 2, equilibrium adsorption of lysozyme within MCM-41 can be almost reached in about 30 min even with a pore size of 20.5 Å. The equilibration times for the other two materials were also investigated in our previous study under the same conditions.[11] Here, 2 h was selected as the time point to ensure enough adsorption. The amounts of lysozyme adsorbed on the three types of silica particles vary significantly from 63.6 mg g^{-1} silica for MCM-41 to 420.8 mg g^{-1} silica for L-SBA-15 (see Table 1). The reason for this is that lysozyme ($30 \times 30 \times 45$ $Å^3$)[12] is larger than the pores of MCM-41 but well suits the pore size of C-SBA-15 and L-SBA-15. Therefore, it can be concluded that MCM-41 can efficiently exclude lysozyme and other proteins with larger MWs.

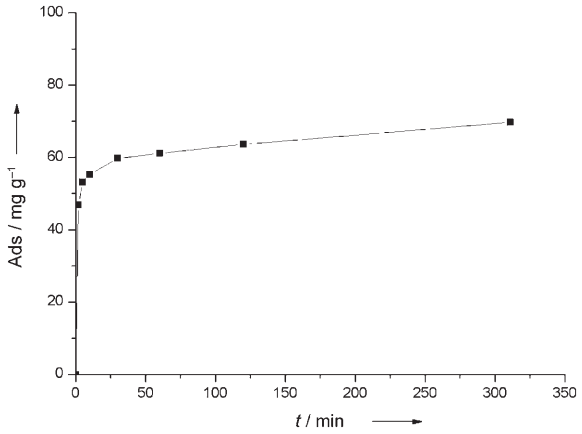

Figure 2. Equilibrium adsorption isotherms for lysozyme on MCM-41 determined by UV adsorption at 280 nm. Ads = Amount adsorbed.

In contrast to the ultrafiltration-based method, the peptide enrichment by these materials is realized by adsorbing peptides inside with their rich surface property. Hence, unlimited amounts of samples can be applied and low-MW contaminants, especially salts, can be removed. To further confirm its superior efficiency for peptide enrichment, MCM-41 was used to extract peptides from native human plasma. Most of the silanol groups were converted to hydrophobic siloxane bridges by calcination, and therefore most of the peptides should be adsorbed by hydrophobic interaction. Then, the extracted peptides were eluted by 50% acetonitrile (CH_3CN) and analyzed by MALDI-TOF mass spectrometry

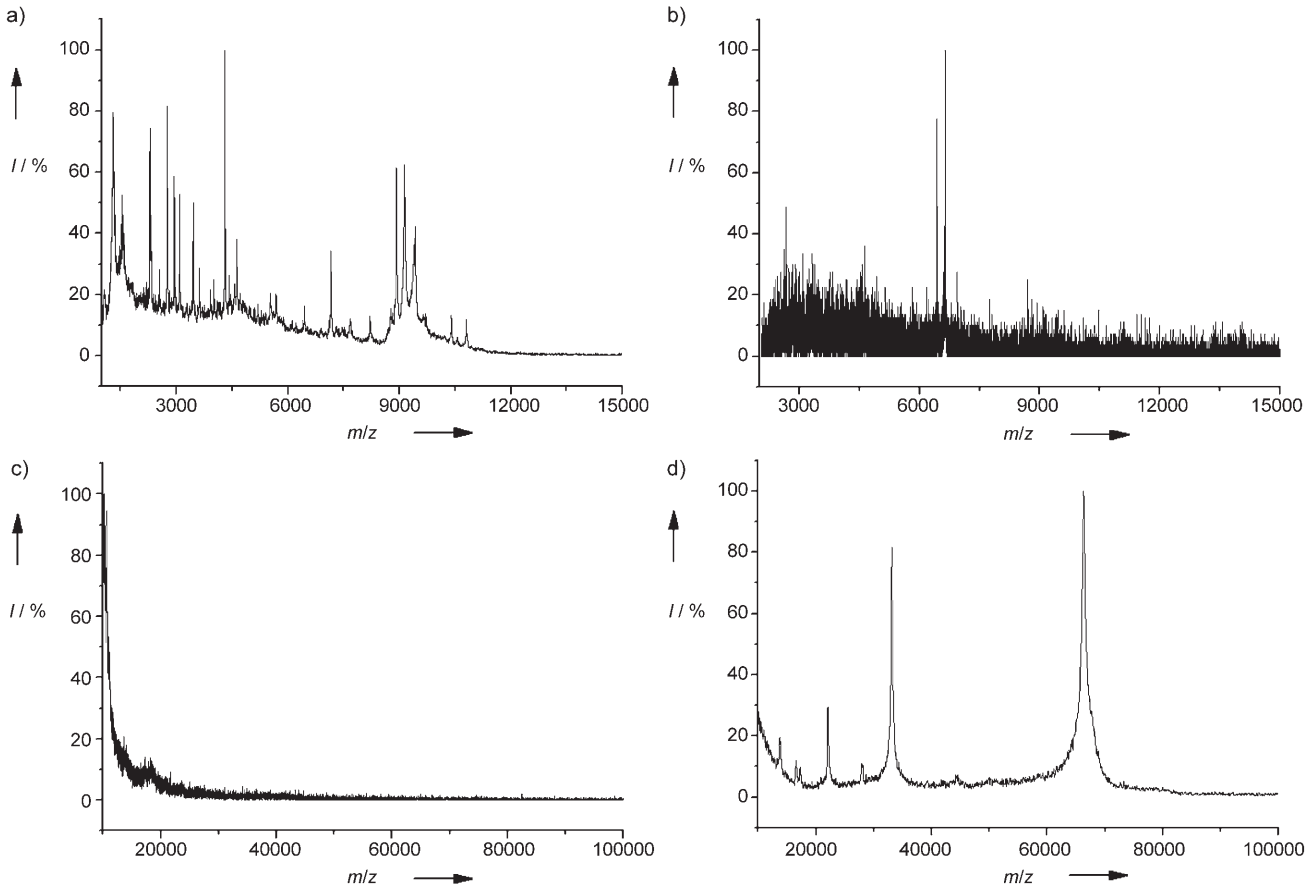

Figure 3. MALDI-TOF MS analysis of human plasma a,c) after and b,d) before exposure to MCM-41 by using α-cyano-4-hydroxycinnamic acid as matrix. Analysis in the MW range of a,b) 1–15 kDa and c,d) 10–100 kDa.

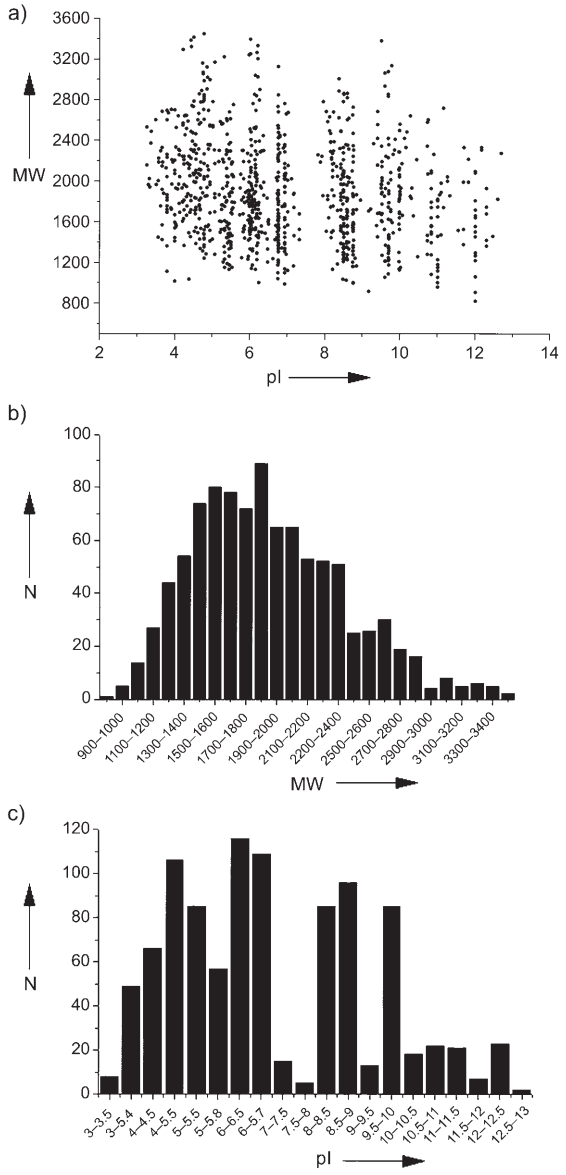

Figure 4. a) Scatter plot of MW versus pI distribution of the peptides enriched with MCM-41 and identified by 2D LC-MS/MS. Peptide abundances within different b) MW and c) pI ranges. *N* = Peptide number.

(MS) in an MW range from 1 to 15 kDa and from 10 to 100 kDa. As a comparison, human plasma without pretreatment was also analyzed in the same MW ranges. As shown in Figure 3a, clear and continuous peptide peaks can be observed in the mass spectrum at MWs less than 12 kDa. However, in Figure 3b for direct analysis of human plasma, only a few peptide peaks were obtained. In the MW range from 10 to 100 kDa (mass spectra shown in Figure 3c and d), the peaks of human serum albumin (HSA) and some other proteins that were clearly observed by direct plasma analysis disappeared after exposure to MCM-41. These results indicate that MCM-41 can be used for selective enrichment of plasma peptides with a wide range of MWs, and simultaneously for effectively removing the high-abundance proteins in human plasma. Microporous silica particles (such as ZSM-5) with a pore size of 5 Å were also investigated, but results no better than those for MCM-41 were obtained (data not shown).

To evaluate the capacity of MCM-41 for peptide enrichment, stepwise elution with 20, 50, and 80% CH_3CN was attempted to completely elute the adsorbed peptides. Sequence information for the identified peptides can be provided for assessing their biological relevance and cleavage pattern, and therefore nanoscale liquid chromatography–tandem mass spectrometry (nano-LC/MS/MS) with database searching was used for peptide separation and identification. It was observed that the numbers of identified unique peptides increased greatly as compared to the one-step elution by 50% CH_3CN (see the Supporting Information). For full separation of the enriched peptides by MCM-41, a two-dimensional chromatographic system with online MS/MS detection (2D LC-MS/MS) was applied to analyze the peptides directly eluted by 80% CH_3CN, and a total of 988 unique peptides were identified (see the Supporting Information). Among these peptides, we also found many regular peptide ladders previously observed.[13] The MW versus isoelectric point (pI) distribution of the identified peptides is depicted in Figure 4; it is clearly clustered into two groups in the acidic and basic pH ranges. As a result of limitations in instrumentation and data processing, the MWs of the peptides identified by 2D LC-MS/MS are less than 4 kDa, which is supported by previous reports.[14]

In summary, highly ordered mesoporous silica particles were successfully applied to the selective enrichment of peptides in human plasma. MCM-41 with a pore size of 20.5 Å was proved to be effective for enriching peptides in human plasma with a wide MW range from 1 to 12 kDa, while repelling most other plasma proteins outside. The unique pore structure of this material makes it superior for peptide enrichment when compared to both adsorbent- and ultrafiltration-based methods. As a result of the rapid and simple procedures for peptide enrichment and the powerful resolution of peptides by 2D separation, this methodology is promising for both comprehensively understanding the peptidome on a global scale and rapidly discovering biomarkers for clinical application.

Experimental Section

Human blood plasma was obtained from one healthy female donor (O type), provided by Zhuanghe Blood Center (Dalian, China). The well-mixed slurry of selected silica particles (10 mg particles in 1 mL deionized water) was mixed with diluted human plasma (1 mL plasma in 3 mL deionized water) and shaken at 25 °C for 2 h. The suspension was centrifuged at 5000 *g* for 2 min, and then the supernatant was removed. The resulting silica particles were washed with deionized water (1 mL) three times. The peptides retained on the silica surface were eluted with solutions (1 mL) of 1 M NaCl and aqueous CH_3CN in turn. The eluted portions were analyzed by either MALDI-TOF MS or nano-LC/MS/MS.

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