Mesoporous Materials

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# Mesoporous Materials in Peptidome Analysis**

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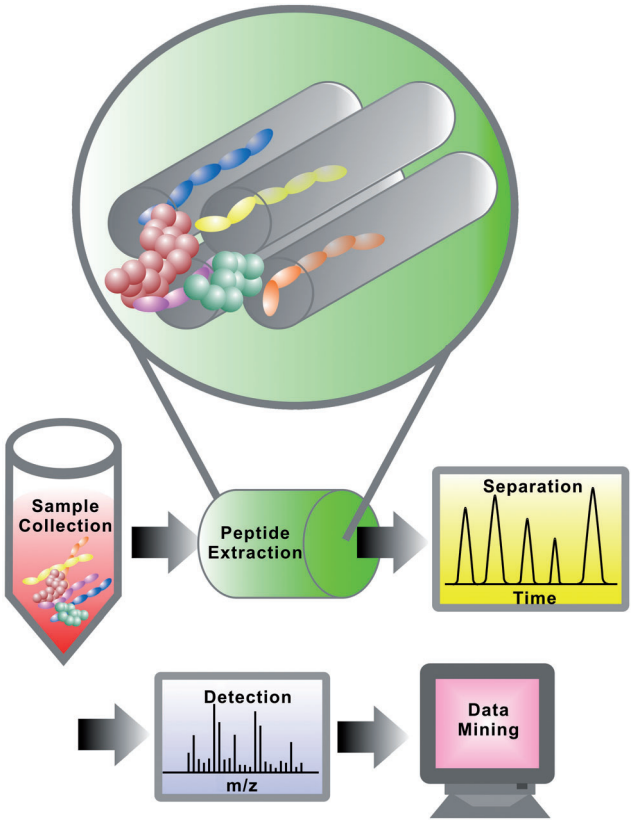
Biologically active peptides, such as peptide hormones and cytokines, play pivotal roles in human health and disease development, as these peptides are important regulators for diverse functions, which range from the endocrine to the inflammatory and the nervous systems.[1] Endogenous peptides can result from the proteolytic cleavage of proteins, and are indicative of protease activity, degradation, and degeneration. The overall concentrations of peptides in biological samples reflect certain biological events and may provide useful information for clinical diagnosis.[2] Therefore, the comprehensive analysis of peptides in complex biological mixtures, termed peptidomics, can contribute to a better understanding of the biochemical functions of endogenous peptides, as well as to the development of diagnostic and prognostic biomarkers.[2]

To profile the peptidome in complicated samples, such as human serum, typical procedures involve sample collection, extraction, separation, detection, and data mining (Scheme 1). Of the five key steps, the selective extraction of peptides has been identified as an important obstacle to overcome for progress in peptidome research,[2,3] because of the complexity and high dynamic range of biomolecules present in the samples from which the peptides must be extracted. For example, the detection of low-abundance peptides in human serum is very challenging because they are overwhelmed by larger and more abundant proteins, salts, and lipids.[3] To confront this challenge, Zou's group has recently developed a highly efficient and selective method to extract peptides from human serum by using ordered mesoporous carbon (OMC).[4] The 4.8 nm pore size of the OMC material enables a molecular-weight cut-off of approximately 10 kDa, which is sufficient to remove the majority of larger interfering serum proteins. The use of OMC greatly enhances the overall extraction efficiency of peptides, which includes peptides in the low mass range (< 2 kDa) that are traditionally difficult to extract. When combined with two-dimensional liquid chromatography and tandem mass spectrometry (2D LC-MS/MS), the researchers were able to identify 3402 unique endogenous peptides from 20 μL of serum.[4] The significantly greater number of serum peptides identified by using this technique demonstrates the advantageous efficiency of mesoporous-based extraction over other sample treatment techniques.




***Scheme 1.*** Typical steps involved in profiling peptidomes from complicated samples. The use of new mesoporous materials improves the efficiency and selectivity of peptide extraction.

Mesoporous materials, such as OMC, have the desirable properties of an ideal extraction material, which include a high surface area, large pore volume, chemical inertness, and good mechanical stability.[5] In recent years, various mesoporous materials have been synthesized to possess a well-defined pore-size distribution and controllable surface functionality.[5] The narrow size distribution of the ordered mesopores facilitates size-selective extraction of endogenous peptides and size exclusion of larger proteins. For example, the highly ordered mesoporous-silica particles in MCM-41, have a pore size of 2 nm and a molecular-weight cut-off of

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12 kDa.[6] This mesoporous material was used to selectively enrich low-molecular-weight peptides from human plasma, which enabled the identification of 988 unique peptides.[6]

Another important property of the mesoporous materials is the versatile incorporation of surface functionalities, which allows different types of peptides to be differentiated. Mesoporous MCM-41 modified with a strong cation-exchange group was able to extract 1328 unique peptides; whereas MCM-41 modified with a strong anion-exchange group extracted 204 peptides, and the unmodified MCM-41 extracted 849 peptides.[7] Only 28 (or 1%) of the 2381 endogenous peptides were identified in all three extracts. These results suggest that the surface functionalities of mesoporous materials play an important role in the selectivity of the extraction. Other surface modifications of mesoporous materials with amino,[8] $Cu^{2+}$,[9] and $C_8$ groups[10] have also shown diversity for the selective enrichment of different types of peptides. In addition, these latter materials have enabled easy magnetic separation because the microspheres consist of a magnetic core and a mesoporous shell.[8–10] The mesoporous silica microspheres modified with $Cu^{2+}$ ions enabled the efficient enrichment of peptides by chelating with the carboxylic acid and amino groups of the peptides.[9] The magnetic mesoporous-silica microspheres modified with $C_8$ groups did not retain salt, which constitutes a desirable property for subsequent mass spectrometry analysis.[10] The recent work by Qin et al.[4] took advantage of the accurate molecular-weight cut-off property of ordered mesopores with the strong hydrophobicity of carbon. Their success in identifying a record number of peptides from human serum is attributed to the enhanced overall extraction efficiency, which includes the hydrophobic peptides that benefited from the combined size-selective effect and the strong adsorption of OMC.[4]

Recent research on the synthesis, modification, and applications of mesoporous materials has shown great potential for these materials to contribute to peptidomic research by improving the existing techniques for peptide enrichment, such as organic precipitation, ultrafiltration, solid-phase extraction, and magnetic-particle-based sample processing. Advantageous features of mesoporous materials include an accurate and controllable molecular-weight cut-off to separate peptides from interfering proteins, a strong retention factor to enrich peptides, a salt exclusion capability to facilitate the subsequent mass spectrometry analysis, and the ability to utilize a minute amount of sample with simple protocols. These features of mesoporous materials can also be applied to other analytical processes, as demonstrated in enzymatic reactors for protein digestion,[11] the enrichment of glycans,[12] and the capture of peptides/proteins that contain post-translational modifications.[12,13] In principle, different mesoporous materials (for example, Si, C, Ti, core-shell) with different pore sizes could be synthesized and tailored for specific applications. The attractive properties of mesoporous materials and the successful applications that have been demonstrated in peptidomic analysis suggest that these materials could also be very useful for proteomic and metabolomic analyses.

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