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Recent advances in the application of core–shell structured magnetic materials for the separation and enrichment of proteins and peptides

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ABSTRACT

Many endogenous proteins/peptides and proteins/peptides with post-translational modifications (PTMs) are presented at extremely low abundance, and they usually suffer strong interference with highly abundant proteins/peptides as well as other contaminants, resulting in low ionization efficiency in MS analysis. Therefore, the separation and enrichment of proteins/peptides from complex mixtures is of great importance to the successful identification of them. Core–shell structured magnetic microspheres have been widely used in the enrichment and isolation of proteins/peptides, thanks to unique properties such as strong magnetic responsiveness, outstanding binding capacity, excellent biocompatibility, robust mechanical strength and admirable recoverability. The aim of this review is to update the advances in the application of core–shell structured magnetic materials for proteomics analysis, including the separation and enrichment of low-concentration proteins/peptides, the selective enrichment of phosphoproteins and the selective enrichment of glycoproteins, and to compare the enrichment performance of magnetic microspheres with different kinds of functionalization.

1. Introduction

Peptide mapping based on mass spectrometry (MS) along with database searching has become an indispensible tool in proteomics analysis [1–4], which is the premise of shotgun proteomics strategy. Though MS is highly sensitive to trace amount of proteins or peptides, it appears to be insufficient for the detection of low-concentration proteins/peptides that exist in real biological samples. Proteins/peptides extracted from biological samples are not only expressed at extremely low concentrations (less than 1 nM), but also suffer strong interference with highly abundant proteins/peptides as well as contaminants like buffer salts or surfactants that are introduced into the samples during pretreatment process [5]. Therefore, the enrichment of low-abundance peptides from complex mixtures is prior to MS analysis in most cases involving peptides identification.

On the other hand, proteins/peptides with post-translational modifications (PTMs) have stimulated a tremendous amount of research interest in proteomics, since PTMs of a protein are involved in many cellular processes and can determine its activity state, localization, turnover and interactions with other proteins [6]. Furthermore, the aberrant modification of proteins may induce the dysfunction of cells, which is associated with certain human diseases [7]. However, effective analysis of these modified peptides with MS is still a challenging task because of their low abundance and low ionization efficiency than non-modified peptides [8].

Over the past years, core–shell structured magnetic materials, composed of an inorganic magnetic core and a functionalized shell (or a functionalized outer shell together with a hydrophilic intermediate shell), have been widely utilized in various sample preparation procedures thanks to their strong magnetic responsiveness, small diameters advantageous for high sensitivity, excellent biocompatibility, outstanding binding capacity and admirable recoverability [9]. Iron-oxide particles (Fe_3O_4 and γ-Fe_2O_3) are the most ubiquitous inner contents because they are easy to prepare and convenient to modify. Functionalized magnetic materials facilitate the isolation of nanomaterial–target molecule conjugates from sample solutions with the help of a magnetic field. After proper modifications, they may possess satisfactory dispersibility in aqueous solution. In a typical magnetic solid-phase extraction process, magnetic microspheres are dispersed in the sample solution at first. After incubation for an appropriate period of time until the target analytes are adsorbed on the adsorbent, the microspheres can easily be separated from the solution in an external magnetic field. Afterwards, the conjugates are properly rinsed and eluted, followed by MS analysis.

The speciality of core–shell structured microspheres is the combined properties of each component which retains its identity and

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performs its function independently [10]. Multifunctional composite materials have shown improved enrichment efficiency in reduced time. In addition, the unique structure offers the possibility to accurately control the spatial distribution of the added species around the cores. Though physical adsorption is an alternative for modification, the most frequently used functionalization technique is definitely the formation of covalent bonds between the core and the shell. Covalent linkages endow composites with greater mechanical strength and stability enough for reuse, which is crucial to practical application [11,12].

This review mainly focuses on the present advances in the syntheses and applications of core–shell structured magnetic microspheres in sample preparation for proteomics analysis, including the enrichment of low-concentration proteins/peptides, the selective enrichment of phosphoproteins/phosphopeptides and the selective enrichment of glycoproteins/glycopeptides.

2. Enrichment of low-concentration proteins/peptides

Endogenous peptides such as hormones, cytokines are shown to contain potential biomarkers for recording the physiological and pathological states of human beings. Unfortunately, most of them are low-concentration peptides [13,14]. Continuous efforts have been devoted to qualitative and quantitative study of endogenous peptides in biological environment by employing simple, rapid, convenient and universal protocols. Undoubtedly, concentration of low-abundance proteins/peptides from complex mixtures to inhibit the interference from unfavorable substances is necessary for successful MS analysis. In this section, advances in the application of core–shell structured magnetic materials for the enrichment of low-concentration proteins/peptides will be discussed. Core–shell structured magnetic microspheres for the enrichment of low-concentration proteins/peptides published in recent years are summarized in Table 1.

2.1. Hydrophobic materials

Magnetic microspheres modified with n-alkyl chains have normally been used to enrich hydrophobic peptides through hydrophobic–hydrophobic interactions.

2.1.1. C_8-functionalized magnetic microspheres

Among them, C_8-functionalized magnetic particles received the most wide-spread concern. Several kinds of C_8-functionalized magnetic microspheres prepared via different methods have been applied to the efficient enrichment of low-concentration peptides [15–19]. C_8-functionalized core–shell structured magnetic microspheres were synthesized for the first time via a conventional silanization method [15]. C_8 chains were grafted onto the surface of silica-coated Fe_3O_4 microspheres with the aid of n-octyldimethylchlorosilane. The resulting $Fe_3O_4@nSiO_2@C_8$ microspheres displayed well-defined core–shell–shell structure and high dispersibility, and enriched hydrophobic peptides in the solutions of a standard peptide Angiotensin II, bovine serum albumin (BSA) and myoglobin (MYO) tryptic digest, and human serum. The enrichment factor was estimated to be nearly 100 times. Twenty-six peptides with sequence coverage of 42% could be identified for 5 nM BSA tryptic digest, and 11 peptides with sequence coverage of 79% could be identified for 5 nM MYO digest after treatment with $Fe_3O_4@nSiO_2@C_8$. Additionally, many endogenous peptides in human serum came into detection after enrichment with $Fe_3O_4@nSiO_2@C_8$.

Considering the synthetic route mentioned above was time-consuming and low-yield, C_8-functionalized magnetic microspheres were synthesized by a one-pot process where Fe_3O_4 microspheres were modified with amine and chloro(dimethyl) octylsilane in succession [16]. After being enriched by $Fe_3O_4–NH_2@C_8$ microspheres for only 30 s, matched peptides with high intensity dominated the MS spectra for 5 nM MYO digest. The enrichment approach based on $Fe_3O_4–NH_2@C_8$ also showed good reproducibility and a detection limit of 20 nM.

Another option to avoid the complex silica coating process was to coat Fe_3O_4 nanoparticles with carbonaceous polysaccharide [17]. The as-synthesized $Fe_3O_4@CP@C_8$ microspheres could identify 22 peaks with sequence coverage of 35% in 5 nM BSA tryptic digest solution which contained abundant contaminants (100 mM urea), thus obviating the need for a desalting step. The feasibility of employing $Fe_3O_4@CP@C_8$ composites in real proteomic applications was further confirmed by direct analysis of peptides present in the digestion mixture of a protein spot that was obtained via 2D-PAGE analysis of human-eye lens. The protein identified with $Fe_3O_4@CP@C_8$ got a score of 96.

In all of the above-mentioned reports, C_8-functionalized magnetic microspheres suffered from limited specific area. Hence, silica-coated magnetic microspheres were fabricated with an ordered mesoporous hybrid C_8 layer to settle this issue. The as-prepared $Fe_3O_4@nSiO_2@$meso-hybrid-C_8 microspheres possessed high surface area and large pore volume, and achieved better performance than $Fe_3O_4–NH_2@C_8$ when treating BSA tryptic digests [18]. The detection of limit (LOD) for BSA digest was 1 nM. Detectable peptides in a tryptic digest of rat cerebellum proteins increased in number from 3 to 15, and numerous endogenous peptides in human serum were successfully detected after pretreatment with $Fe_3O_4@nSiO_2@$meso-hybrid-C_8.

However, the fabrication of $Fe_3O_4@nSiO_2@$meso-hybrid-C_8 microspheres still involved the introduction of silica layer. C_8-functionalized magnetic mesoporous silica (designated as $Fe_3O_4@mSiO_2@C_8$) microspheres preserved the merits of distinguished surface area and pore volume and avoided the time-consuming silica coating. The enrichment efficiency of the novel material was superior to that of $Fe_3O_4@nSiO_2@C_8$ [19]. Originated from the unique mesoporous structure, $Fe_3O_4@mSiO_2@C_8$ could selectively capture proteins with low molecular weight (MW) and exclude BSA and other large proteins simultaneously. $Fe_3O_4@mSiO_2@C_8$ microspheres were also demonstrated to be capable of capturing endogenous peptides from human serum and mouse brain extract. Notably, 267 peptides were finally identified from mouse brain extract.

2.1.2. Other carbon-based magnetic microspheres

Other carbon-based composites like Fullerene (C60)-functionalized magnetic microspheres ($Fe_3O_4@nSiO_2@$C60), carbon nanotube (CNT)-decorated magnetic microspheres and graphene-encapsulated magnetic microspheres ($Fe_3O_4@nSiO_2@$G) were demonstrated to have extraordinary capability for the enrichment of low-concentration peptides thanks to the strong hydrophobic interactions between the carbon skeletons and peptides [11,20,21]. $Fe_3O_4@nSiO_2@$C60 microspheres were synthesized by radical polymerization of C60 molecules on the surface of Fe_3O_4 microspheres. C60 was anchored onto the surface of $Fe_3O_4@nSiO_2$ by the use of 3-(trimethoxysilyl) propylmethacrylate (MPS), and the MPS-modified $Fe_3O_4@nSiO_2$ was then polymerized with C60 by using 2,2-azobisiso-butyronitrile (AIBN) as the polymerization initiator [20]. The enrichment factor for Angiotensin II was estimated to be over 100 times and the enrichment yield was about 0.12 ng peptide per microgramme of $Fe_3O_4@nSiO_2@$C60 microspheres. The material not only remarkably concentrated peptides in BSA tryptic digest solutions, but also proved to be effective for the enrichment of Cytochrome c (Cytc) protein at a concentration of 0.2 ng/μL. What is more, $Fe_3O_4@nSiO_2@$C60 could also directly analyze human urine sample without additional desalting step.

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