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Fluorous modified magnetic mesoporous silica composites-incorporated fluorous solid-phase extraction for the specific enrichment of N-linked glycans with simultaneous exclusion of proteins

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ABSTRACT

Taking advantage of fluorine-fluorine interactions, fluorous solid-phase extraction (FSPE) is emerging as a novel approach in proteomics research. Notably, silica gel bound with perfluoroalkyl groups was applied to the FSPE of N-linked glycans. Based on previous studies, mesoporous silica coated magnetic nanoparticles bound with perfluoroalkyl groups were synthesized for the specific enrichment of N-linked glycans in this study. The magnetic nanoparticles-incorporated FSPE strategy successfully identified 22 N-linked glycans from the OVA digest with a concentration of 0.5 μg/μL, and achieved a detection limit of 5 ng/μL (with 16 N-linked glycans identified). It also showed good day-to-day reproducibility. Its selectivity towards BSA protein is 1:200 (molar ratio), showing excellent size-exclusion effect. In addition, the present method proved to be effective for the analysis of the human serum digest, opening up new prospect for the identification of glycans and proteins with other post-translational modifications in biological environment.

1. Introduction

The term 'fluorous' was coined to represent the kind of highly fluorinated (or perfluorinated) compounds. The unique interaction between fluorous compounds and fluorous compounds shows their distinct property of being fluorophilic. Fluorous chemistry approach is based on such fluorine-fluorine interactions as described above. Fluorous chemistry approach flourished for its use in biphasic catalysis [1,2], targeted synthesis [3,4], removal of intermediate products [5,6] and copurification for the reaction products of parallel syntheses [7,8]. More recently, fluorous chemistry begins to blossom in biochemical research ranging from microarrays [9–12] to proteomics [13–21]. Among these studies, the most frequently used technique was fluorous solid-phase extraction (FSPE). It is based on the following principle: perfluoroalkyl moieties are firstly appended to target analytes through covalent addition, and then the fluorous labelled molecules will bind strongly to a fluorous modified solid phase through the specific non-covalent interactions that occur between fluorine atoms, while the unlabelled species remain unbound [22]. Eventually, the fluorous labelled species are to be eluted with fluorophilic solutions, thus being selectively extracted.

As is known to all, solid-phase extraction based on functionalized magnetic materials is ubiquitous in biological chemistry due to the large surface-to-volume ratio of magnetic nanoparticles, excellent biocompatibility, easy functionalization, great recoverability, convenient handling and suitability for large-scale analyses [23,24]. Being modified with perfluoroalkyl moieties, mesoporous silica coated magnetic nanoparticles were successfully employed in the FSPE of phosphopeptides [21] and toxic perfluorinated substances in water samples [25]. The combination of FSPE and magnetic separation preserves the particular affinity towards fluorous compounds and the facile operation. On the other hand, silica gel bound with perfluoroalkyl groups were used in the enrichment of N-linked glycans [21]. Similarly, silica coated magnetic particles bound with perfluoroalkyl groups are considered to be promising as adsorbents for the FSPE of N-linked glycans.

Glycans play a vital role in numerous physiological and pathological processes, such as cell growth, intracellular and intercellular signalling, and tumor growth and metastasis [26–28]. The structural alterations of glycans are also regarded as biomarkers for serious diseases including cancers, inflammations and immunodeficiency diseases [29]. As one kind of structurally altered glycans, N-linked glycans are especially confirmed to be closely

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linked with the occurrence of diseases [30]. Currently, mass spectrometry (MS) is the most popular tool for glycan profiling owing to its high sensitivity and throughput. Nevertheless, the low abundance of glycans in complex biological samples and the interference from highly abundant proteins or other contaminants greatly inhibit their MS signals [31]. Moreover, the intrinsic hydrophilicity of glycans and lack of basic sites for protonation result in extremely low ionization efficiency [20]. To settle this issue, glycans are generally derivatized prior to MS analysis for the increase of concentration, improvement of hydrophobicity and enhancement of ionization efficiency [32–34]. In addition to common derivatization strategies, fluorous derivatization is arising as a new way of glycan derivatization. As the key point of this method, perfluoroalkyl compounds are desirable derivatization reagents thanks to the low polarizability, high hydrophobicity and good chemical stability, increasing the hydrophobicity of glycans. After pretreatment, the high selectivity for perfluorinated species through fluorine-fluorine interactions allows FSPE to isolate perfluorinated glycans from nonfluorinated molecules.

In this work, mesoporous silica coated magnetic microspheres with perfluorooctyl moieties functionalized interior pore-walls (designated as Fe_3O_4@$mSiO_2$-C_8F_{17}) are synthesized via a surfactant-templated sol-gel reaction, and serve as the adsorbent for the FSPE of fluorous derivatized N-linked glycans. The synthetic route to Fe_3O_4@$mSiO_2$-C_8F_{17} is illustrated in Scheme S1. Possessing hydrophilic mesopores and fluorophilic pore channels modified with perfluoroalkyl groups, the core-shell structured composites are endowed with large specific area, agreeable dispersibility in aqueous solutions and special affinity towards molecules bearing perfluoroalkyl groups. Taking advantage of these extraordinary properties, Fe_3O_4@$mSiO_2$-C_8F_{17} microspheres are expected to enrich N-linked glycans directly from the fluorous-derivatized peptide N-glycosidase (PNGase F)-deglycosylated glycoprotein solutions and simultaneously exclude large biomolecules.

2. Experimental section

2.1. Materials and reagents

Iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ethylene glycol, sodium acetate anhydrous (NaAc), ethanol and sodium hydroxide (NaOH) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Hexadecyltrimethylammonium bromide (CTAB) was purchased from Sigma Chemical (St. Louis, MO, USA). Tetraethylsilicate (TEOS) was purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). 1H,1H,2H,2H-perfluorodecyltriethoxysilane was purchased from Alfa Aesar (Tianjin, China). The NdFeB magnet was purchased from PCCW Ltd. (Beijing, China), 4 cm long, 4 cm wide, 1 cm high, with surface magnetic field strength of 4000 Gauss.

Chicken egg ovalbumin (OVA), peptide N-glycosidase (PNGase F, Genetimes Technology), bovine serum albumin (BSA), 1H,1H,2H,2H-perfluorooctane-1-thiol, 2,5-dihydroxybenzoic acid (DHB) and trifluoroacetic acid (TFA) were purchased from Sigma Chemical (St. Louis, MO, USA). 1H,1H-perfluorooctylamine (PFOA) was purchased from J&K Chemical (Shanghai, China). Acetonitrile (ACN) was purchased from Merck (Darmstadt, Germany). Ammonium bicarbonate (NH_4HCO_3) was purchased from Chinasun Specialty Products Co., Ltd (Jiangsu, China). Dimethyl sulfoxide (DMSO) and hydrogen peroxide (30%, w/w) were purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). Barium hydroxide ($Ba(OH)_2$) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The human serum sample originated from a hepatocellular carcinoma patient was acquired from Shanghai Zhongshan Hospital. Deionized water was purified by a Milli-Q system (Milford, MA, USA).

All other chemicals and reagents are of the highest grade commercially available and used as received.

2.2. Preparation of Fe_3O_4@$mSiO_2$-C_8F_{17} composites

Prior to the synthesis of Fe_3O_4@$mSiO_2$-C_8F_{17}, the Fe_3O_4 microspheres were prepared via a hydrothermal reaction frequently used by our groups. Briefly, 1.35 g of $FeCl_3 \cdot 6H_2O$ was dissolved in 75 mL of ethylene glycol under magnetic stirring. After complete dissolution, 3.60 g of NaAc was added. After the mixture was adequately stirred for another 1 h, the resulting solution was sealed in a Teflon-lined stainless-steel autoclave and heated at 200 °C for 16 h. The Fe_3O_4 microspheres obtained were washed with deionized water and ethanol several times.

The Fe_3O_4@$mSiO_2$-C_8F_{17} microspheres were synthesized through a surfactant-mediated sol-gel process. Detailedly, 0.075 g of the Fe_3O_4 prepared beforehand and hexadecyltrimethylammonium with a weight ratio of 75 mg/750 mg were dispersed in 75 mL of deionized water and ultrasonicated for 30 min. The resultant dispersion was mixed with 675 mL of NaOH solution (with a concentration of 10^{-3} M) and ultrasonicated for additional 5 min to obtain a stable dispersion. The mixture was then mechanically stirred at 60 °C for 30 min. Afterwards, 3.75 mL of TEOS/ethanol (v/v, 1:4) solution was added drop by drop under vigorous stirring, followed by heating at 60 °C for 30 min. After that, 225 μL of 1H,1H,2H,2H-perfluorodecyltriethoxysilane/TEOS (v/v, 1:2) solution was injected into the dispersion. The dispersion was further heated at 60 °C for 12h. The products were collected by magnetic separation and refluxed in 100 mL of ethanol at 60 °C for 2 h to remove the CTAB template. The concentration of the intermediate product was about 1 mg/mL. Eventually, the as-synthesized Fe_3O_4@$mSiO_2$-C_8F_{17} microspheres were dried in vacuum at 50 °C for characterization and future use.

2.3. Fluorous derivatization of N-linked glycans

The enzymatic deglycosylation products (100 μL) of the OVA protein and the human serum sample were dissolved in 400 μL of 50% ACN/5% acetic acid (v/v) buffer and were allowed to react with 1H,1H-perfluorooctylamine (the molar ratio of the derivatization reagent to the glycoprotein was 1:10) at 65 °C for 2h, with $NaBH_3CN$ added as the reducing reagent [20].

2.4. Fluorous solid-phase extraction of fluorous derivatized N-linked glycans

Before studying the enrichment ability of Fe_3O_4@$mSiO_2$-C_8F_{17} for fluorous labelled N-linked glycans, we suspended 10 mg of the magnetic microspheres in 1 mL of 50% ethanol solution (v/v).

For better enrichment performance, 200 μg of Fe_3O_4@$mSiO_2$-C_8F_{17} was activated with 100 μL of 30% methanol (MeOH) containing 10 mM ammonium formate three times. Then, 200 μL of the fluorous derivatized glycoprotein digest was added. The mixture was vibrated in a vortex at 37 °C for 30 min. The magnetic microspheres were isolated from the supernatant under an external magnetic field. After incubation, the magnetic microspheres were rinsed with 30% MeOH containing 10 mM ammonium formate and 60% MeOH containing 10 mM ammonium formate three times sequentially. Next, the fluorous labelled glycans were eluted from the magnetic microspheres by 20 μL of 100% MeOH. The eluent (1 μL) was deposited on a MALDI sample target (Applied Biosystems/MDS SCIEX, Foster City, CA, USA) and dried at room temperature. The glycoprotein solutions without enrichment of equal volume were also deposited on the same sample target for comparison. Eventually, 0.8 μL of DHB matrix was dropped on the sample spots. Four replicate spots were taken for each sample.