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Preparation of magnetic microspheres functionalized by lanthanide oxides for selective isolation of bovine hemoglobin

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ABSTRACT

The isolation and depletion of highly-abundant proteins and peptides is of vital importance for the screening and monitoring of biomarkers which are low stoichiometry in proteomics and protein-based biotechnologies. Here we have designed and synthesized a series of magnetic microspheres $MnFe_2O_4$ @SiO_2 @ Lanthanide Oxides (La = Ce, Sm, Er, Tm, Yb) whose surface were functionalized by lanthanide oxides that could successfully isolate bovine hemoglobin (BHb), and $MnFe_2O_4$ @SiO_2 @Er_2O_3 microspheres were found to be most efficient in separating BHb with excellent selectivity and a binding capacity as high as 238.2 mg/g. The resulting microspheres demonstrated the favorable performance for isolation of BHb from bovine serum as confirmed by SDS-PAGE, which would open a promising strategy in the pretreatment of proteomic analysis.

1. Introduction

Proteomics, one of the researching focus in post-genomic era, aims to study the structures globally, translation, functions and expression patterns of proteome as well as interactions among proteome, which is helpful for understanding physiological and pathological changes related to human diseases and cell metabolism process [1,2]. Recently, researchers find some proteins biomarkers special to pathologies are difficult to analyze them directly mainly due to at the low stoichiometry and submerged by highly abundant histidine (His)-rich proteins in complex biological samples [3,4]. Therefore, development of novel strategies for highly-abundant proteins (like heme proteins) selectively separation and removal prior to analysis has been intimately associated with the proteomics studies [5].

So far, a plethora of strategies have been developed for separation of highly-abundant proteins, including metal affinity [6], lectin affinity [7], hydrophilic [8] and electrostatic interactions [9], and so on. Among these techniques used for enrichment strategies, immobilized metal ion affinity chromatography (IMAC) [10–13], which takes advantage of the interaction between the electron donor groups such as histidine exposed on the surface of proteins and an immobilized metal ion, is the versatile strategies. Many metal ions have been successfully immobilized on the matrix to trapping the target proteins and peptides including Ti^{4+} [14], Cu^{2+} [15], Fe^{3+} [16], and so on. However, these methods based on IMAC require complex synthesis steps to conjugate proper ligands on the matrix, nevertheless, these ligands are either inconvenient to obtain or expensive [17]. For example, Xu et al. reported Nitrilotriacetic acid-modified magnetic particles charged them with Ni^{2+} for selective enrichment His-rich protein [18]. Zhang et al. proposed a series of strategies that iminodiacetic acid (IDA) immobilized magnetic nanoparticles (MNPs) bound with Cu^{2+} or Ni^{2+} for highly efficient isolation of bovine hemoglobin [19,20]. Moreover, the cheated metal ions may lose during loading and washing [21]. Therefore, developing a facial method for selective capturing His-rich proteins is urgently needed. Compared with IMAC, metal oxide affinity chromatography (MOAC) has received considerable attention for the separation of proteins or peptides [22] attributing to abundant interaction sites and good stability such as SnO_2 [23], TiO_2 [24], and Al_2O_3 [25], which also depends on the coordination between the electron donor groups exposed on the surface of proteins and metal ion.

Over the past decades, considerable efforts have been spent in the synthesis and characterization of magnetic nanoparticles (MNPs) and the improvement of their applicability in many different areas [26]. Owing to their unique magnetic responsivity, biocompatibility and low toxicity, these MNPs gained wide interest for biomedical applications [27–29]. Many of the complex manipulations in extraction protocols may be avoided such as centrifugation and filtration when using magnetic nanostructures as adsorbents. For example, Wang et al. fabricated the magnetic nanospheres encapsulated by mesoporous copper oxide shell to selective isolation of hemoglobin [30]. Jia et al. built an on-chip

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magnetic system with In_2O_3-coated Fe_3O_4 MNPs for selective enrichment of phosphopeptides [31]. Furthermore, Lanthanide oxides have received great attention on account of their more coordination sites, the characteristics of being hard Lewis acids and highly electroposition in diverse fields such as biological applications [32], photocatalysis [33] and luminescent [34]. The materials based on rare earth metal species can coordinate with oxygen, aliphatic nitrogen and phosphor containing ligands [35]. Therefore, the maximum selectivity and sensitivity may be achieved when using lanthanides-based materials as adsorbents to isolate or enrichment proteins. However, very few proposals that magnetic microspheres functionalized by Lanthanide Oxides for isolating proteins from complex biological samples are reported.

In this study, we have designed and synthesized a series of functionalized magnetic microspheres $MnFe_2O_4$ @SiO_2 @ Lanthanide Oxides (La = Ce, Sm, Er, Tm, Yb) to universally and effectively isolate bovine hemoglobin from complex biological samples, and $MnFe_2O_4$ @SiO_2 @Er_2O_3 microspheres exhibit favorable efficiency and specificity over other microspheres. The resultant microspheres were characterized by the scanning electron microscope (SEM), transmission electron microscope (TEM), X-ray powder diffraction (XRD), Fourier transform infrared spectroscope (FT-IR) and vibrating sample magnetometer (VSM), and the favorable performance for isolation of bovine hemoglobin from bovine serum is opening a novel strategy in the pretreatment of proteomic analysis.

2. Experimental section

2.1. Materials and methods

2.1.1. Chemicals and materials

Ferric chloride ($FeCl_3{\cdot}6H_2O$) was purchased from Xilong Chemical Co., Ltd (Guangdong, China). Manganese chloride tetrahydrate ($MnCl_2{\cdot}4H_2O$), samarium nitrate hexahydrate ($Sm(NO_3)_3{\cdot}6H_2O$), Cerium nitrate hexahydrate ($Ce(NO_3)_2{\cdot}6H_2O$) and Erbium trinitrate pentahydrate ($Er(NO_3)_3{\cdot}5H_2O$) were attained from Aladdin industrical corporation (Shanghai, China). Trulium nitrate hexahydrate ($Tm(NO_3)_3{\cdot}6H_2O$) was bought from Sun chemical Technology Co., Ltd (Shanghai, China). Ytterbium nitrate pentahydrate ($Yb(NO_3)_3$) was bought from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China). sodium acetate (C_2H_5ONa), trisodium citrate, ethylene glycol (EG), Tetrathoxysilane (TEOS) Ammonia solution ($NH_3{\cdot}H_2O$),sodium carbonate anhydrous (Na_2CO_3), sodium hydroxide (NaOH), hydrochloric acid (HCl), phosphoric acid (H_3PO_4) and acetic acid ($C_2H_4O_2$) were acquired from Beijing Chemical Works (Beijing, China). Hexamethylenetetramine (HMT) was bought from shanghai Macklin Biochemical Co., Ltd (Shanghai, China). Sodium phosphate tribasic (Na_3PO_4), sodium dodecyl sulfonate (SDS) and boric acid (H_3BO_3) were bought from Tianjin Fuchen chemical reagents factory (Tianjin, China). Tris (chydroxymethyl) aminomethane (Tris) was purchased from J&K Chemicals (Beijing, China). All these chemicals listed above were of analytical reagent grade and used without further purification or modification.

Proteins including bovine serum albumin (BSA, MW 67 kDa, pI 4.7), bovine hemoglobin (BHb, MW64.5 kDa, pI6.8), Lysozyme(Lyz, MW14kDa, pI11.2) were obtained from Beijing Keao Biological Pharmaceutical Co., Ltd (Beijing, China).α-Lactalbumin (α-Lac, MW14kDa, pI4.2) from bovine milk, β-Lactoglobulin (β-Lac, MW18kDa, pI5.2) from bovine and Fetuin (MW48kDa, pI3.8) from fetal bovine serum were purchased from Sigma-Aldrich (St. Louis, MO, USA). Bovine plasma was from Beijing Keao Biological Pharmaceutical Co., Ltd (Beijing, China).

2.1.2. Characterization

The morphological structures were obtained with a SU-8010 (SEM, Hitachi, Japan) field emission scanning electron microscope. Transmission electron microscopy(TEM) images were performed on an H-7650B (Hitachi, Japan). The magnetization characterization was measured by vibrating sample magnetometer (VSM) (Quantum Design, USA). The Brunauer-Emmett-Teller (BET) specific surfaces areas were performed on an ASAP 2010C analyzer (Micromeritics, USA). Zeta potential data was carried out by a SZ-100 Nanoparticle Analyzer (Horiba, Japan). The X-ray powder diffraction (XRD) patterns were determined on an X′Pert Pro MPD (Peak, Japan) diffractometer (CuK, radiation, = 0.154056 nm). Fourier transform infrared (FT-IR) spectra in KBr was recorded on a Bruker Fourier Transform. Infrared Spectrometer (Horiba, Germany) within a range of 4000–400 cm^{-1}. U-3900 UV–vis spectrophotometer (Hitachi, Japan) was used to determine the absorbance of protein. The pH of buffers were measured using a Delta 320 pH Meter (Mettler Toledo, China). The chromatographic measurements were determined using an Agilent Technologies 1200 Series HPLC and an Agilent Technologies ZORBAX Rx-SIL C18(4.6 × 250 mm,5 μm,300 A) column(USA).proteins were eluted with linear gradient from 10% to 70% buffer B(buffer A, 0.1%TFA in water; buffer B, ACN) at a flow rate of 1.0 mL min^{-1} at 35 °C. Electrophoresis of proteins was carried out by regular Sodium dodecyl sulfate polyacrylamide gel electrophoresis (Beijing Liuyi Biotechnology Co., Ltd, China) with 15% running and 5% stacking. Proteins were stained with Coomassie Brilliant Blue R-250.

2.2. Synthesis of $MnFe_2O_4$ @SiO_2 @ Lanthanide Oxides microspheres

2.2.1. Preparation of $MnFe_2O_4$ nanoparticles

$MnFe_2O_4$ nanoparticles were prepared by a solvothermal method at 200 °C for 10 h according to previously reported procedure with minor modifications [36]. Briefly, a mixture of $FeCl_3{\cdot}6H_2O$ (1.35 g, 5 mmol), $MnCl_2{\cdot}4H_2O$ (0.4925 g, 2.5 mmol), NaAc (2.16 g) and $Na_3Cit{\cdot}2H_2O$ (0.324 g) were dissolved in ethylene glycol (40 mL). the mixture was afterward stired vigorously for 1 h with a magnetic stirring bar, and then transferred to the poly-tetrafluoroethylene (PTFE) lining of a stainless steel reactor (capacity of 100 mL) for heating at 200 °C for 10 h. After the solution was cooled down to room temperature, the black products were collected under a magnet and washed several times with distilled water and ethanol. Finally the particles were dried under vacuum at 30 °C for the next reaction.

2.2.2. Preparation of $MnFe_2O_4$ @SiO_2 particles

$MnFe_2O_4$ @SiO_2 particles were prepared via Stober sol-gel process [37]. In detail, the resulting $MnFe_2O_4$ nanoparticles (100 mg) were dispersed in a mixture of water (40 mL), ethanol (160 mL) and 2 mL of concentrated ammonia aqueous under ultrasonication for 30 min. Then, 1 mL of Tetrathoxysilane (TEOS) was added drop wise to the above-mentioned solution, followed by mechanically stirring at room temperature for 6 h, finally the particles were washed several times with distilled water and ethanol to remove blank silica particles and dried under vacuum at 30 °C for further use.

2.2.3. Preparation of $MnFe_2O_4$ @SiO_2 @ Lanthanide Oxides microspheres

$MnFe_2O_4$ @SiO_2 @Er_2O_3 microspheres were prepared by chemical precipitation method. Typically, 50 mg $MnFe_2O_4$ @SiO_2 particles and 100 mg of $Er(NO_3)_3{\cdot}5H_2O$ were dispersed in 60 mL of ethanol respectively under ultrasonication for 30 min, and mixed with above-mentioned solution under an ultrasonic treatment process for 10 min. Subsequently, 0.4 g of HMT dissolved in 40 mL absolute water was added to the resulting solution with ultrasonic vibrations for another 15 min. The mixture was then stired vigorously for 2 h at 80 °C. The as-prepared microspheres were collected by an external magnetic field and rinsed with distilled water and ethanol to remove the excess ionic remnants, subsequently, the resulting powder was dried under vacuum at 30 °C and calcined at 400 °C for 2 h. The final microspheres, $MnFe_2O_4$ @SiO_2 @Er_2O_3, were used to enrich BHb.

The synthesis of $MnFe_2O_4$ @SiO_2 @CeO_2, $MnFe_2O_4$ @SiO_2 @Sm_2O_3, $MnFe_2O_4$ @SiO_2 @Tm_2O_3 and $MnFe_2O_4$ @SiO_2 @Yb_2O_3 were