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Preparation of anionic polyelectrolyte modified magnetic nanoparticles for rapid and efficient separation of lysozyme from egg white

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ABSTRACT

Poly(sodium 4-styrenesulfonate) modified magnetic nanoparticles (PSS–MNPs) were successfully synthesized and characterized by transmission electron microscopy, scanning electron microscopy, zeta potential, vibrating sample magnetometry, and Fourier-transform infrared spectrometry. The PSS–MNPs were found to enable effective separation of lysozyme from egg white. The impacts of solution pH, ionic strength, and contact time on the adsorption process were investigated. The adsorption kinetic data were well fitted using a pseudo-second-order kinetic model and the adsorption equilibrium can be reached in 3 min. The adsorption isotherm data could be well described by the Langmuir equation. The maximum adsorption capacity of PSS–MNPs for lysozyme was calculated to be 476.2 mg g^{-1} according to the Langmuir adsorption isotherm. The fast and efficient adsorption of lysozyme by PSS–MNPs was mainly based on electrostatic interactions between them. The adsorbed lysozyme can be eluted using 20 mM phosphate buffer (pH 7.0) containing 1.0 M NaCl with a recovery of 96%. The extracted lysozyme from egg white demonstrated high purity, retaining about 90.7% of total lysozyme activity.

1. Introduction

Lysozyme is a commercially valuable enzyme. Since lysozyme has bactericidal and bacteriostatic properties, it is widely applied in food industry as an anti-microbial agent and a food preservative [1]. In recent years, lysozyme has been reported to be used for wound healing [2] and inhibiting angiogenesis and tumor growth [3]. Due to the industrial and medical importance of lysozyme, the lysozyme separation techniques have attracted more and more attention. Lysozyme is mainly extracted from chicken egg white because it is rich in chicken egg white.

Some conventional methods including precipitation [4], ultrafiltration [5], and liquid chromatography [6,7] have been developed for separation of lysozyme from chicken egg white. The primary limitation of precipitation method is the low selectivity for lysozyme. In comparison with precipitation method, ultrafiltration and chromatography methods may yield better quality of lysozyme, but need corresponding special equipment. Recently, some adsorbents were synthesized and used for separation of lysozyme, such as human serum albumin-coated gold nanoparticles [8], nitrogen-doped carbon materials [9], dye functionalized poly(2-hydroxyethyl methacrylate) nanoparticles [10], poly(methacrylic acid) grafted chitosan beads [11], silica nanoparticles [12], and collagen fiber [13]. Among these methods, centrifugation, column-packing or filtration steps were needed and these steps usually take more than 20 min.

Magnetic separation technology based on magnetic particles (MPs) could take the place of the filtration or centrifugation step by an external magnetic field to achieve solid-liquid separation due to the magnetic responsiveness of MPs. MPs can be separated from aqueous solutions in less than 20 s by applying an external magnetic field [14]. MPs have drawn a great deal of interest from biological, biomedical and environmental research fields [15–18]. Some functional MPs were applied in targeted drug delivering [19], magnetic resonance imaging [20], metal ions removal [21], and polycyclic aromatic hydrocarbons detection [22]. Some MPs functionalized with different active moieties were reported to be applied in protein separation [23–26]. Nitrilotriacetic acid/Co^{2+}-linked, silica/boron-coated magnetite nanoparticles were used to purify 6 × histidine-tagged proteins [23]. Poly(hydroxyethyl methacrylate) based magnetic nanoparticles were employed to purify lysozyme from chicken egg

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white [24]. Fe_3O_4/polymethyl methacrylate/SiO_2 nanorattles were synthesized and applied for adsorption of standard lysozyme [25]. Recently, magnetic poly(lactic-co-glycolic acid)/Fe_3O_4 composite microspheres were prepared to extract lysozyme, in which the adsorption time was 24 h and desorption time was 24 h [26].

Poly(sodium 4-styrenesulfonate) (PSS) with negatively charged sulfonate groups is a water-soluble anionic polyelectrolyte. It is often combined with cationic polyelectrolyte to build up polyelectrolyte multilayers [27–30]. The polyelectrolyte multilayer films were reported to adsorb protein mainly based on electrostatic interactions [30]. PSS was also used to improve capacity of metal cations removal by grafting chitosan based on ion-exchange mechanism [31]. The successful study about the adsorption of protein by polyelectrolyte multilayer film intrigued us to synthesize PSS functionalized magnetic nanoparticles (PSS–MNPs) and investigate the feasibility of the particles as adsorbents for separation of protein. PSS–MNPs can combine the favorable attributes of MPs and PSS. So far, there is no report about the application of PSS–MNPs in lysozyme separation.

In this work, PSS–MNPs were synthesized by two reaction steps. They were characterized by transmission electron microscopy (TEM), scanning electron microscopy (SEM), zeta potential, vibrating sample magnetometry (VSM), and Fourier-transform infrared spectrometry (FT-IR). The as-prepared PSS–MNPs were used for adsorption and separation of lysozyme. The positively charged lysozyme enables them to be rapidly adsorbed by negatively charged PSS–MNPs from aqueous solution based on electrostatic interaction. And PSS–MNPs can be easily separated from aqueous solution by an external magnetic field because of their magnetic properties. The adsorption kinetics and isotherm of PSS–MNPs for lysozyme were studied. The performance of the prepared PSS–MNPs for adsorption and separation of lysozyme from egg white was investigated.

2. Experimental

2.1. Materials

PSS (average molecular weight 1,000,000 Da), poly(diallyl dimethylammonium chloride) (PDDA, average molecular weight between 400,000 and 500,000 Da), 20% in water, was purchased from Sigma-Aldrich Co. (Saint Louis, USA). Ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), tris (hydroxymethyl) amino methane (Tris), sodium dihydrogen phosphate dehydrate ($NaH_2PO_4{\cdot}2H_2O$), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$), sodium chloride (NaCl), and sodium hydroxide (NaOH) were purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China). Sodium acetate anhydrous (NaAc) was purchased from Tianjin Guangcheng Chemical Reagent Company (Tianjin, China). Ethylene glycol was purchased from Tianjin Damao Chemical Reagent Factory (Tianjin, China). All these reagents were of analytical grade or better. Lysozyme and ovalbumin were purchased from GBCBIO Technologies (Guangzhou, China). Conalbumin was purchased from Sigma-Aldrich. Chicken eggs were purchased from a local market.

All aqueous solutions were prepared with deionized water (18.2 MΩ) purified by an Elga water purification system (ELGA, London, UK).

2.2. Preparation of PSS–MNPs

The procedure for preparation of PSS–MNPs is schematically illustrated in Fig. 1a. The synthesis of PSS–MNPs was divided into two steps. Firstly, amino modified magnetic nanoparticles (NH_2–MNPs) were prepared using a solvothermal method according to a previously reported method [32]. $FeCl_3{\cdot}6H_2O$ (1.0 g) was first dissolved in ethylene glycol (30 mL). Then NaAc (2.0 g) and 1, 6-hexanediamine (6.5 g) were added to the solution and the mixture was stirred vigorously at 50 °C for 30 min to form a transparent solution. The solution was transferred to a Teflon-lined stainless-steel autoclave (50 mL) and heated at 198 °C for 6 h. The black products were cooled to room temperature, and washed several times with ethanol and water respectively. Finally, the products were separated with a magnet and dried at 40 °C under nitrogen atmosphere for further use.

Secondly, NH_2–MNPs were functionalized with PSS. The PSS coating solution was prepared by dissolving PSS at 1% in a 20 mM Tris-HCl (pH 8.0) aqueous solution containing 1.5 M NaCl. Then, 0.3 g NH_2–MNPs were dispersed in 50 mL PSS solution and shaken at 30 °C for 10 h under nitrogen atmosphere. The final black products (PSS–MNPs) were isolated with a magnet and rinsed with water. Finally, they were dried at 40 °C under nitrogen atmosphere for further use.

2.3. Characterization

The FT-IR spectra of NH_2–MNPs and PSS–MNPs were recorded on a Bruker FT-IR spectrometer (Bruker, Germany). TEM images were carried out on a JEM-2100HR transmission electron microscope (JEOL, Tokyo, Japan). SEM micrographs were obtained using a field emission scanning electron microscope (ZEISS Ultra 55, Carl Zeiss, Germany). The magnetization characterization of NH_2–MNPs and PSS–MNPs was performed on a vibrating sample magnetometer (PPMS-9, Quantum Design, San Diego, USA) at room temperature and an applied field of 20 kOe. The zeta potential was tested on Zetasizer Nano ZS (Malvern, Worcestershire, UK).

2.4. Optimization of lysozyme adsorption and desorption conditions

The adsorption of lysozyme by PSS–MNPs was carried out using the following experimental procedure. Firstly, the lysozyme concentration was adjusted to a desired level (1.5 mg mL^{-1}) with the adsorption solution (20 mM phosphate, pH 10.0), then 5 mg PSS–MNPs were added to the lysozyme solution (1.5 mL). The solution was stirred at 100 rpm and room temperature for 5 min; afterwards an external magnet was employed for magnetic separation. The lysozyme concentrations in the initial solution and supernatant were obtained by measuring the UV absorbance at 280 nm on a Micro-spectrophotometer (K5600, Beijing Kaiao Technology Development Company, Beijing, China). The adsorbed amount of lysozyme was calculated according to the following equation.

$$q_e = \frac{(C_0 - C_e)V}{m} \quad (1)$$

where q_e (mg g^{-1}) is the amount of lysozyme adsorbed at equilibrium, C_0 and C_e are the initial and equilibrium concentrations of lysozyme (mg mL^{-1}), respectively, V (mL) is the volume of lysozyme solution, and m (g) is the mass of PSS–MNPs used.

The influence of sample pH and ionic strength on the adsorption of lysozyme was investigated. The pH of sample solution (20 mM phosphate) was adjusted by adding different amounts of sodium dihydrogen phosphate (20 mM) or NaOH (1 M). The ionic strength of sample solution (20 mM phosphate, pH 7.0) was adjusted by adding different amounts of NaCl. The adsorption kinetics and isotherm were also studied.

The pH and ionic strength in eluent were studied to optimize the desorption of lysozyme from PSS–MNPs. The lysozyme-adsorbed particles were washed twice with adsorption solution. Then the adsorbed lysozyme was eluted from the particles by incubation