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Surface modification of iron oxide nanoparticles with polyarginine as a highly positively charged magnetic nano-adsorbent for fast and effective recovery of acid proteins

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ABSTRACT

Polyarginine has been successfully bound onto iron oxide nanoparticles via carbodiimide activation as a highly positively charged magnetic nano-adsorbent for protein separation. They were nearly superparamagnetic with a mean diameter of 10.3 ± 2.36 nm, and the binding process did not change the spinel structure of iron oxide. From the analyses of FTIR spectra and zeta potential, the binding of polyarginine on the surface of iron oxide was confirmed and the resultant polyarginine-coated magnetic nanoparticles (PA-MNPs) were positively charged even up to pH 11. By thermogravimetric analysis, the typical product contained about 7.1 wt% of polyarginine. From the adsorption of the proteins with different pI values, the resultant PA-MNPs were found to be quite efficient for the fast and effective adsorption of acid proteins. For the typical acid protein, bovine serum albumin (BSA), the adsorption equilibrium was achieved within few minutes and obeyed the Langmuir isotherm equation. At pH 7 and 25 °C, the maximum adsorption capacity and equilibrium constant were 67.6 mg/g and 0.0623 L/mg, respectively. Moreover, by SDS–polyacrylamide gel electrophoresis, the capability of PA-MNPs for the separation of BSA-lysozyme mixture and egg white was further confirmed. Accordingly, the PA-MNPs were useful for the fast and effective magnetic recovery of acid proteins.

1. Introduction

In the past decade, the magnetic nano-adsorbents which were composed of magnetic cores and polymeric shells received considerable attention with the development of nanotechnology [1–9]. As compared to the traditional adsorbents, they not only can be manipulated or recovered rapidly by an external magnetic field but also possess quite good performance owing to high efficient specific surface area and the absence of internal diffusion resistance [3,4]. Furthermore, by the choice and chemical modification of polymeric shells, the surface functionality of magnetic nanoparticles (MNPs) can be tailored for various applications in the fields of high-density data storage, magnetic resonance imaging (MRI), drug delivery, therapy, diagnosis, bioseparation, and enzyme immobilization [8–21].

The magnetic cores were usually iron oxide nanoparticles because of their easy-synthesis, low cost, and good chemical stability. For the application in biomedicine, the low cytotoxicity and the accompanied function as a contrast agent of MRI made them more useful in drug delivery and hyperthermia [9,11,13–16]. For the polymer shells, the most attractive functional groups were the carboxyl and amino groups, which were particularly useful for the adsorption of biological molecules such as proteins and genes. The carboxylic groups bearing magnetic nano-adsorbents usually had low isoelectric points (pI) near pH 2, and hence were effective for the adsorption of many basic proteins [3,22]. However, the isoelectric point of amino groups-bearing magnetic nano-adsorbents depended on the selected amino groups-donors and was usually below pH 7 [22,23]. So, for the effective recovery of various acid proteins, it is necessary to develop a magnetic nano-adsorbent with a higher isoelectric point.

Arginine is a well-known amino acid with three pK values of 2.18, 9.09, and 13.2 [24]. It may be used as an amino groups-donor to result in a higher isoelectric point. Many works revealed that the arginine-modified dendrimers and natural or synthetic polymers exhibited enhanced transfection efficiency and could be used as the gene delivery carriers [25–27]. Also, the polyarginine-coated diamond nanoparticles could be successfully used for the selective extraction and enrichment of multiphosphorylated peptide [28]. In addition, some efforts have also been made on the synthesis of arginine-capped magnetic nanoparticles [24,29,30]. Their further binding with antibodies was found to be useful in immunomagnetic assay [30]. However, these arginine-capped magnetic nanoparticles have not been used for the recovery of acid proteins.

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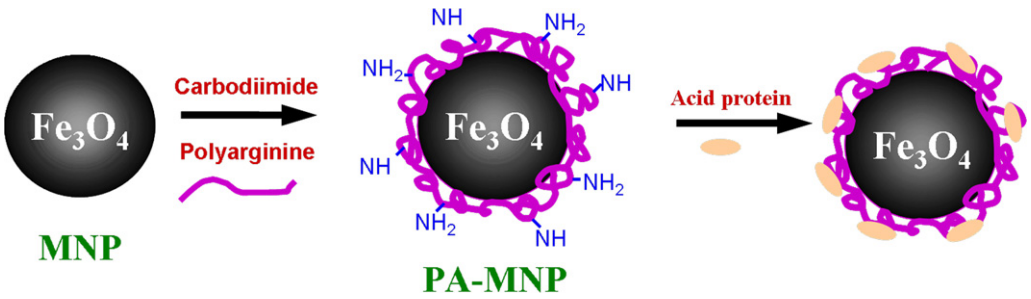

Fig. 1. A scheme for the surface functionalization of iron oxide nanoparticles with polyarginine for magnetic recovery of acid proteins.

In general, the small molecules capped on the particle surface usually existed as a monolayer and hence could not provide plenty of functional groups for the adsorption as the polymers did. So, the above arginine-capped magnetic nanoparticles might be unsuitable as an efficient adsorbent for protein adsorption. The surface modification of iron oxide nanoparticles with polyarginine should be a more practicable strategy. Unfortunately, no corresponding works have been reported until now.

According to the above, in this work, the polyarginine-coated magnetic nanoparticles (PA-MNPs) were fabricated by the covalent binding of polyarginine on the iron oxide nanoparticles via carbodiimide activation as illustrated in Fig. 1 and then further used for the magnetic recovery of acid proteins. The size, structure, and magnetic property were characterized by transmission electron microscopy (TEM), X-ray diffraction (XRD), and the superconducting quantum interference device (SQUID) magnetometer, respectively. The binding of polyarginine on the iron oxide nanoparticles was recognized by the analyses of Fourier transform infrared (FTIR) spectroscopy and zeta potential. The binding amount of polyarginine was determined by thermogravimetric analysis (TGA). The capability for the selective separation of proteins was demonstrated using bovine serum albumin (BSA; pI = 4.7) and lysozyme (pI ~11) as model acid protein and basic protein, respectively.

2. Experimental

2.1. Chemicals

Ferric chlorides, 6-hydrate was purchased from Mallinckrodt (Paris). Ammonium hydroxide (29.6%) was supplied by TEDIA (Fairfield). Ferrous chloride tetrahydrate, poly-L-arginine hydrochloride (P-7762; molecular weight: 15,000–70,000), albumin from bovine serum (BSA; A 8022), crystallized and lyophilized lysozyme (EC 3.2.1.17) from hen's egg white (No. L-6876), carbonic anhydrase from bovine erythrocytes (No. C-3934), myoglobin from horse heart (No. M-1882), α-lactalbumin type III from bovine milk (No. L-6010), cytochrome c from bovine heart (No. C-2037), and carbodiimide (cyanamide, CH_2N_2) were all supplied by Sigma Chemical Co. (St. Louis, MO). Bio-Rad reagent for protein assay was obtained from Bio-Rad Lab. (Hercules). The water used throughout this work was the reagent-grade water produced by Milli-Q SP ultra-pure-water purification system of Nihon Millipore Ltd., Tokyo. All other chemicals were the guaranteed or analytic grade reagents commercially available and used without further purification.

For SDS–polyacrylamide gel electrophoresis (SDS-PAGE), ammonium persulfate was supplied by Sigma Chemical Co. (St. Louis, MO) and all other reagents were obtained from Bio-Rad Lab. (Hercules), including sodium dodecyl sulfate (SDS) solution, acrylamide/bis solution (37.5:1; 2.6% C), laemmli sample buffer (loading dye), coomassie brilliant blue R-250 (staining solution), N,N,N′,N-tetramethyl-ethylenediamine (TEMED; catalyst), 1.5 M Tris–HCl buffer (pH 8.8), 0.5 M Tris–HCl buffer (pH 6.8), 10× Tris/glycine/SDS buffer (running buffer), protein standard (marker).

2.2. Preparation of PA-MNPs

Iron oxide (Fe_3O_4) nanoparticles were prepared by coprecipitating Fe^{2+} and Fe^{3+} ions with ammonia solution and treating under hydrothermal conditions according to our previous work [3,4]. The ferric and ferrous chlorides (molar ratio 2:1) were dissolved in water at a concentration of 0.3 M iron ions. Chemical precipitation was achieved at 25 °C under vigorous stirring by adding NH_4OH solution (29.6%). During the reaction process, the pH was maintained at about 10. The precipitates were heated at 80 °C for 30 min, then washed several times with water and ethanol, and finally dried in a vacuum oven at 70 °C.

For the binding of polyarginine, iron oxide magnetic nanoparticles were added to buffer A (0.003 M phosphate, pH 6, 0.1 M NaCl) to yield a colloid solution of 10 mg/mL at first. Then, 1.0 mL of colloid solution was mixed with 0.25 mL of carbodiimide solution (2.5 mg/mL in buffer A) and sonicated for 10 min. Finally, 0.5 mL of polyarginine solution (0.4–2.0 mg/mL in buffer A) was added and the reaction mixture was sonicated for another 30 min. The binding process was carried out at a constant temperature of 4 °C. The resultant PA-MNPs were recovered from the reaction mixture by placing the bottle on a permanent magnet with a surface magnetization of 6000 G. The magnetic particles settled within 1–2 min and then were washed with buffer A. Here NaCl was used for the flocculation of magnetic nanoparticles. Its addition could accelerate the magnetic separation, particularly in alkaline solutions. In addition, using arginine to replace polyarginine, the arginine-coated iron oxide magnetic nanoparticles were also prepared according to the above method to confirm that the surface modification with polyarginine is a more practicable strategy than with the small molecules arginine.

2.3. Characterization of PA-MNPs

TEM analysis was carried out using a Hitachi Model H-7500 at 120 kV. The sample was obtained by placing a drop of colloid solution onto a Formvar-covered copper grid and evaporated in air at room temperature. XRD measurement was performed on a Rigaku D/max III.V X-ray diffractometer using CuKα radiation (λ = 0.1542 nm). FTIR spectra were recorded on a Varian FTS-1000 FTIR spectrometer. The zeta potentials were measured on a Malvern ZEN2600 Zetasizer Nano Z. TGA was done on the dried sample in air with a heating rate of 10 °C/min on Shimadzu TA-50WSI TGA. Magnetic measurement was performed on a SQUID magnetometer (MPMS7, Quantum Design).

2.4. Adsorption study

The adsorption of BSA by PA-MNPs was investigated in 0.03 M phosphate buffer solutions at pH 3–9 and 25 °C. In general, about 10.76 mg of PA-MNPs (10 mg iron oxide and 0.76 mg polyarginine) was added to 5 mL of BSA solution (10–400 mg/L). After mixing by vortex and then thermostated at 25 °C for 5 min to achieve the equilibrium, the solution was removed for protein assay from the mixture by using a permanent magnet to hold the magnetic nanoparticles. Unless otherwise specified, the absorption experiments were performed in the 0.03 M phosphate buffer solution at pH 7, 25 °C, and an initial protein concentration of 100 mg/L. Protein assay was conducted using the Bio-Rad reagent. For the effects of pH and BSA concentration, the adsorption experiments were repeated three times to make sure the repeatability and reproducibility. By replacing BSA with carbonic anhydrase, myoglobin, α-lactalbumin, cytochrome c, and lysozyme, a test for the adsorption of other proteins with different isoelectric points (pI) by PA-MNPs in 0.03 M phosphate buffer at pH 7, 25 °C, and an initial protein concentration of 100 mg/L was conducted for comparison. In addition, the egg white samples obtained by diluting the fresh egg white to 100-fold with the phosphate buffer solutions (0.03 M, pH 4–8) were used as the real protein solution to demonstrate the practical application of PA-MNPs.

2.5. SDS-PAGE

The SDS-PAGE of BSA, lysozyme, and egg white was conducted on the 10% SDS–polyacrylamide gel in Tris/glycine/SDS buffer, electrophoresed at 110 V for 120 min, and stained with coomassie brilliant blue R-250 for 30 min. In the case of egg white, the pH of eluate was adjusted from 2 to above 3 with 1 M NaOH solution (2 μl per 20 μl of eluate) for the SDS-PAGE.