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Tris(hydroxymethyl)aminomethane-modified magnetic microspheres for rapid affinity purification of lysozyme

Guoqing Zhang, Qing Cao, Na Li, Kean Li, Feng Liu*

Beijing National Laboratory for Molecular Sciences, Key Laboratory of Bioorganic Chemistry and Molecular Engineering of the Ministry of Education, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China

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ABSTRACT

A novel affinity purification method for lysozyme (LZM) based on functionalized magnetic microspheres was developed. Tris(hydroxymethyl)aminomethane (Tris)-modified magnetic microspheres with specific affinity toward LZM were prepared using Tris as ligand and silica-coated magnetic microshperes as support. Transmission electron microscopy and magnetic property measurement results showed that the Tris-modified magnetic microspheres have a very good core-shell structure and high magnetization.The maximum binding capacity of LZM was about 108.6 mg/g magnetic microspheres. LZM purified from chicken egg white had high purity and well-maintained activity of 8140 U/mg. This magnetic-mediated LZM purification strategy has advantages of high efficiency, low cost and easy operation.

1. Introduction

Functionalized magnetic microspheres have been extensively applied in various fields, such as separation and purification [1–4], immunoassay [5], targeted drug delivery [6] and magnetic resonance imaging [7]. Functionalized magnetic microspheres are ideal biomacromolecule carriers in separation and purification process with several unique advantages: various functional groups can be immobilized on the surfaces of magnetic microspheres according to different target analytes, and the process of functionalization is generally simple; magnetically driven separation is much easier and faster in liquid medium than filtration and centrifugation; and the functionalized magnetic microspheres can be regenerated by releasing the target analytes from the microsphere surface. In magnetic affinity purification, however, the classical affinity ligands, including antibodies, dyes, metal ions, etc., are expensive, toxic or unstable. Therefore, there is always a need to develop novel affinity ligands.

Protein purification is vital for the characterizations of the function, structure, physico-chemical properties and industrial application of the proteins of interest. Lysozyme (LZM) is a commercially valuable enzyme and has widespread application [8,9] as cell-disrupting reagent, antibacterial agent, food additive, etc. Practically, LZM is vastly obtained from chicken egg white in which the content of LZM is about 0.34%. A large number of coexisting proteins make it challenging to purify LZM from chicken egg white. LZM purification methods have been developed based on various techniques including ultrafiltration [10], ion-exchange [11,12], crystallization [13,14], and affinity precipitation [15]. However, these separation and purification methods are usually complicated and time-consuming. A simple, rapid and efficient purification method for LZM is demanded both in laboratory and industry.

This work aims at combining the advantages of affinity ligand and easy operation of magnetic microspheres to develop an inexpensive, simple and rapid LZM purification method. The specific interaction between tris(hydroxylmethyl)aminomethane and LZM was proved in our previous work [16,17]. Inspired by former studies, novel magnetic microspheres with high magnetization and specific affinity toward LZM were prepared, using Tris as the affinity ligand to functionalize magnetic microspheres synthesized by solvothermal method. The effect of pH and initial LZM concentration on the binding capacity, the repeatability and reproducibility of Tris-modified magnetic microspheres are investigated. Subse-

* Corresponding author. Tel.: +86 1062761187; fax: +86 1062751708.
E-mail address: liufeng@pku.edu.cn (F. Liu).

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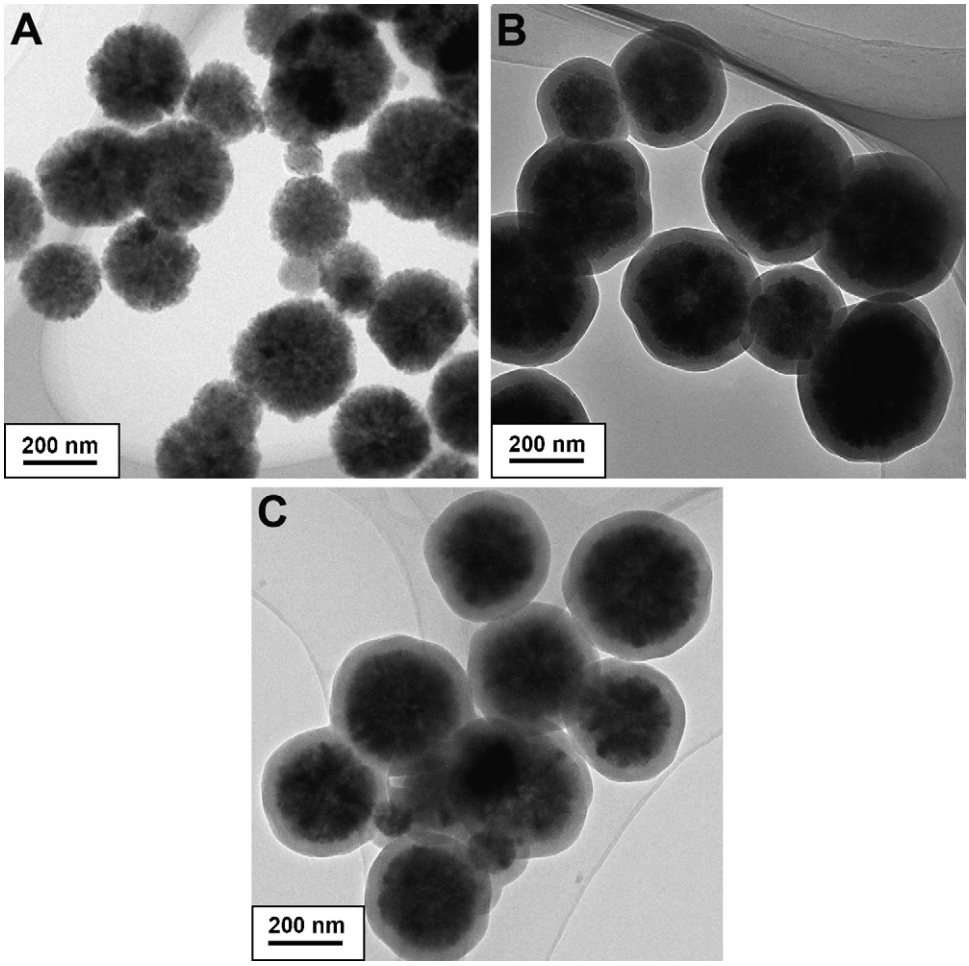

Fig. 1. TEM images of (A) Fe_3O_4, (B) Fe_3O_4@SiO_2 and (C) Fe_3O_4@SiO_2@GPS@Tris.

quently, such microspheres were used to purify LZM from chicken egg white.

2. Experimental

2.1. Chemicals

Tris(hydroxylmethyl)aminomethane (Tris) and protein marker were bought from Amresco (Solon, OH, USA). Chicken egg white lysozyme, oval albumin (OVA) and 3-glycidoxypropyltrimethoxysilane (GPS) were obtained from Sigma (St. Louis, MO, USA). $FeCl_3{\cdot}6H_2O$ was bought from Shanghai Chemical Reagents Company (Shanghai, China). Tetraethoxysilane (TEOS), $NH_3{\cdot}H_2O$ (25 wt%), methanol, ethanol, toluene and glycol were purchased from Beijing Chemicals Plant (Beijing, China). The LZM activity assay kit was acquired from Jiancheng Institute of Bioengineering (Nanjing, China). All other chemicals were of analytical grade, and deionized water was used for aqueous solution preparation.

2.2. Preparation of submicron-size Fe_3O_4 particles

Submicron-size Fe_3O_4 particles were prepared by a solvothermal method [18]. In a typical synthesis, 1.35 g of $FeCl_3{\cdot}6H_2O$ was added into 50 mL of glycol, and the mixture was stirred vigorously to acquire a transparent solution. The solution was sealed in a stainless-steel autoclave and heated at 200 °C for 8 h. This reaction led to the formation of submicron-size Fe_3O_4particles. Fe_3O_4microspheres were separated with a magnet and washed 3 times with ethanol and hot water, sequentially. The obtained Fe_3O_4microspheres were dried at 40 °C under vacuum for 24 h.

2.3. Preparation of Tris-modified Fe_3O_4microspheres

100 mg of submicro-size Fe_3O_4particles were firstly treated by 100 mL of 2 M HCl aqueous solution under ultrasonic vibration for 5 min, and then washed with deionized water. The microspheres were transferred into a solution consisting of 200 mL ethanol, 50 mL deionized water and 10 mL concentrated ammonia (25 wt%). A stable dispersion was obtained after ultrasonic vibration for 20 min. Subsequently, 0.3 mL of TEOS was added and the reaction mixture was stirred for 8 h at 40 °C. The resulted silica-coated magnetic particles with core-shell structure were expressed as Fe_3O_4@SiO_2.

0.2 g of dried Fe_3O_4@SiO_2 was redispersed into a mixture of 30 mL of anhydrous toluene and 1.5 mL GPS. This suspension was refluxed for 8 h to synthesize particles Fe_3O_4@SiO_2@GPS. The obtained microspheres were separated from the mixture, and washed with methanol for three times. Then the epoxy-activated Fe_3O_4@SiO_2@GPS reacted with 0.2 g Tris in 10 mL of potassium phosphate buffer (2.5 M, pH 7.9) at 60 °C for 48 h. Finally, 10 mL of Tris-HCl (1.0 M, pH 8.0) was used to block the residual reacting sites for 3 h, the obtained Tris-modified magnetic microspheres were denoted as Fe_3O_4@SiO_2@GPS@Tris.