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Dendrimer modified magnetite nanoparticles for protein immobilization

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Abstract

A cascading polyamidoamine (PAMAM) dendrimer was synthesized on the surface of magnetite nanoparticles to allow enhanced immobilization of bovine serum albumin (BSA). Characterization of the synthesis revealed exponential doubling of the surface amine from generations one through four starting with an amino silane initiator. Furthermore, transmission electron microscopy (TEM) revealed clear dispersion of the dendrimer-modified magnetite nanoparticles in methanol solution. The dendrimer-modified magnetite nanoparticles were used to carry out magnetic immobilization of BSA. BSA immobilizing efficiency increased with increasing generation from one to five and BSA binding amount of magnetite nanoparticles modified with G5 dendrimer was 7.7 times as much as that of magnetite nanoparticles modified with only aminosilane. There are two major factors that improve the BSA binding capacity of dendrimer-modified magnetite nanoparticles: one is that the increased surface amine can be conjugated to BSA by a chemical bond through glutaraldehyde; the other is that the available area has increased due to the repulsion of surface positive charge.

Keywords: Polyamidoamine (PAMAM) dendrimer; Protein immobilization; Magnetite nanoparticle; Bovine serum albumin (BSA)

1. Introduction

Magnetite particles (microspheres, nanospheres, and ferrofluids) are widely studied for their applications in biology and medicine such as enzyme and protein immobilization, magnetic resonance imaging (MRI), RNA and DNA purification, magnetic cell separation and purification, and magnetically controlled transport of anticancer drugs, as well as hyperthermia generation [1–5]. These magnetite beads are generally of core–shell type: the biological species cells, nucleic acids, and proteins are connected to the magnetite core through an organic or polymeric shell. The shells either are biocompatible in general (such as dextran and PEG) or possess active groups which can be conjugated to biomolecules such as proteins and enzymes [6–8]. Therefore, the investigation of magnetite nanoparticles with organic coating is of significance for applications.

A polyamidoamine (PAMAM) dendrimer can introduce a dense outer amine shell through a cascade type generation [9]. A PAMAM coating may be used to reduce magnetite agglomeration, and the increased cationic contribution will be useful for generating a colloidal suspension with increased surface area for protein immobilization [10]. Serum albumins are the most abundant proteins in plasma. As the major soluble protein constituents of the circulatory system, they have many physiological functions and play a key role in the transport of many endogenous and exogenous ligands. For many drugs, binding to serum albumin is a critical determinant of their distribution and pharmacokinetics [11]. This paper describes the first report of direct formation of a cascading PAMAM dendrimer on the surface of aminosilane-modified magnetite nanoparticles for BSA immobilization. TEM, element analysis, FT-IR, TGA, zeta potential analysis, and UV spectroscopy were used to characterize the structure, composition, and protein-binding capacity of the dendrimer-modified magnetite nanoparticles.

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2. Materials and methods

2.1. Materials

Ferric chloride, ferrous sulfate, ammonia, methylacrylate, and ethylenediamine were obtained from the Shanghai Chemical Reagent Corporation (Shanghai, China). 3-Aminopropyltrimethoxysilane ($NH_2(CH_2)_3$–Si–$(OCH_3)_3$, APTS) and bovine serum albumin (BSA) were purchased from Sigma (USA). All chemicals were of analytical grade and used as received. BSA was used without further purification.

2.2. Synthesis of magnetite nanoparticles

Magnetite was made according to the method of Molday [12]. Typically, a solution of mixture of $FeCl_3$ (0.085 M) and $FeSO_4$ (0.05 M) at pH 1.7 was prepared under N_2 protection. Then, ammonia aqueous solution (1.5 M) was dropped into it with violently stirring until the pH of the solution rose to 9. The obtained magnetite was washed immediately with water five times and ethanol two times by magnetic separation. Finally, magnetite nanoparticles were dispersed in ethanol with concentration of 0.0128 M.

2.3. Magnetite nanoparticles coated by aminosilane

The 25-ml magnetite colloid ethanol solution prepared above was diluted to 150 ml by ethanol. The solution was then treated by ultrasonic waves for 30 min. A 10-ml amount of 3-aminopropyltrimethoxysilane ($NH_2(CH_2)_3$–Si–$(OCH_3)_3$, APTS) was added into it with rapid stirring for 7 h. The result solution was washed with methanol five times by magnetic separation. APTS-coated magnetite nanoparticles were dispersed in methanol with a concentration of 5 wt% (Fig. 1, step 1). G0 represents the magnetite nanoparticle modified only with APTS.

2.4. Surface modification with PAMAM dendrimer

Dendrimer generation was initiated with 50 ml of 5 wt% G0 methanol solution. A 200-ml sample of 20% (v/v) methylacrylate methanol solution was added and the suspension was immersed in an ultrasonicating water bath at room temperature for 7 h. The particles were then collected magnetically and rinsed with methanol five times by magnetic separation. After rinsing, 40 ml of 50% (v/v) ethylenediamine methanol solution was then added and the suspension was immersed in an ultrasonicating water bath at room temperature for 3 h. The particles were rinsed with methanol five times by magnetic separation. Stepwise growth using methylacrylate and ethylenediamine was repeated until the desired number of generations (G1–G5) was achieved (Fig. 1, step 2, step 3). The product was then washed three times with 25 ml methanol and five times with 25 ml water by magnetic separation.

2.5. BSA immobilization

A 50-mg sample of magnetite nanoparticles (G0–G5) was dispersed in 10 ml of 5% glutaraldehyde aqueous solution with stirring for 3 h at room temperature. The suspension was washed with phosphate-buffered saline (PBS: 150 mmol/l NaCl, 1.9 mmol/l NaH_2PO_4, 8.1 mmol/l Na_2HPO_4, pH 7.4, 0.01 M) for three times; then 5 ml of BSA in 0.01 M PBS solution with a concentration of 2 mg/ml was

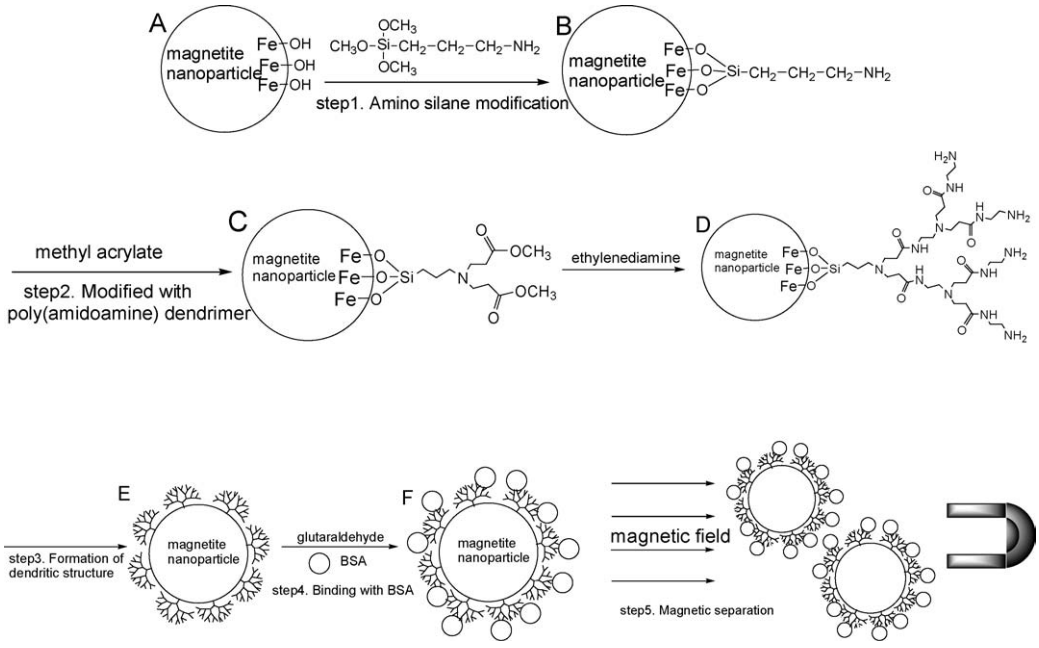

Fig. 1. Magnetite nanoparticle modified with PAMAM dendrimers for BSA immobilization.