Preparation of Magnetic Silica Nanospheres with Metal Chelate Ligands and Application in Recovery of Protein

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Abstract: Novel magnetic silica nanospheres with metal chelate ligands were prepared for protein recovery. First, Magnetic silica nanospheres were prepared by the aqueous deposition of silica onto ultrafine (about 8 nm) magnetite particles via controlled hydrolysis of sodium silicate. Then the conjugates of iminodiacetic acid (IDA) and 3-glycidoxypropyltrimethoxysilane (GLYMO) were coupled to the nanospheres. After charged with Cu^{2+}, magnetic silica nanospheres were used to recover a model protein, bovine hemoglobin (BHb) from solution. Magnetic silica nanospheres are low cost and have extensive potential for large scale protein separation and other applications requiring rapid and easy operation.

Key words: magnetic silica nanospheres, metal chelate affinity ligand, protein recovery

1. Introduction

Magnetic carriers have been extensively applied in the fields of biotechnology and biomedicine, such as cell separation, enzyme immobilization and protein separation (Šafarik et al., 1999; Bahar et al., 1998; Khng et al., 1998). In recent years, magnetic silica carriers have been investigated in nucleic acid separation and enzyme immobilization (Goetz et al., 1991; Taylor et al., 2000) due to their good mechanical properties, rigidity, better stability and rapid separation. However, little work has been reported on the magnetic affinity separation of proteins with magnetic silica supports. The silica surface is covered with silanol groups (Si-OH), which facilitates its chemical modification by organofunctional silane coupling agents to immobilize specific ligands.

The application of immobilized metal affinity separation method has been developed rapidly since its first introduction by Porath and co-workers in 1975 (Porath et al., 1975). It is based on the interaction between metal ions and the histidine or cysteine exposed on the surface of protein. Compared with classical biological affinity ligands, they are small, inexpensive, chemically and physically stable, and can be easily coupled to matrices at high density resulting in high capacity adsorbents. Furthermore, the elution of target protein can be effected under quite mild conditions and separation selectivity tailed by the choice of metal ion and buffers employed.

In this study, novel magnetic silica nanospheres with metal chelate lignads were prepared for application in recovery of protein. Magnetic silica nanospheres were first prepared by the deposition of silica onto ultrafine (about 8 nm) magnetite particles via controlled hydrolysis of sodium silicate. Then the reaction product of iminodiacetic acid (IDA) and 3-glycidoxypropyltrimethoxysilane (GLYMO) were coupled to the nanospheres. After charged with Cu^{2+}, magnetic silica nanospheres were used to recover a model protein, bovine hemoglobin (BHb) from solution.

2. Experimental

2.1 Materials

3-Glycidoxypropyltrimethoxysilane (GLYMO) and iminodiacetic acid (IDA) were purchased from Acros Organics (USA). BHb was obtained from Beijing Chemical Reagent Company. All other materials were of analytical grade and commercially available, including ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), ammonium hydroxide (25% [w/w]), sodium silicate, and $CuSO_4 \cdot 5H_2O$.

2.2 Preparation of magnetic silica nanospheres

Magnetic silica nanospheres were prepared by the deposition of silica from solution of silicic acid onto the magnetite nanoparticles according to a previously described procedure (Liu et al., 2004).

2.3 GLYMO-IDA modification and Cu^{2+} charged to the magnetic silica nanospheres

Magnetic silica nanospheres were modified with GLYMO-IDA using a procedure described by (Anspach, 1994). To charge Cu^{2+} to magnetic silica nanospheres, the nanospheres were mixed with $CuSO_4 \cdot 5H_2O$ solution (50 mg/ml) with continuous shaking at room temperature for 2 h. Then the excess unbound Cu^{2+} was removed with water.

2.4 Characterization of magnetic silica nanospheres

The size and morphology of magnetic silica nanospheres were determined by transmission electron microscopy (TEM, Hitachi 8100, Japan). The magnetic properties were analyzed by vibrating sample magnetometry (VSM, model-155, Digital Measurement System, Inc.).

2.5 Protein recovery

BHb with various concentrations were incubated with magnetic silica nanospheres charged with Cu^{2+} (approximately 2 mg) in 4 ml binding buffer (20 mM phosphate saline, pH 8.0, 1 M NaCl) for 2 h. The magnetic nanospheres were magnetically separated and the supernatant was assayed for the protein concentration using a spectrophotometer at 406 nm. The adsorbed mass of protein was calculated by mass balance.

3. Results and discussion

Magnetite nanoparticles were prepared by the co-precipitation method from ferrous and ferric ion solutions with a molecular ration of 1:2. TEM results showed the nanoparticles had an average particle size 8 nm (Liu et al., 2004), which suggested they exhibited superparamagnetism. Silica was precipitated from sodium silicate solution with the addition of hydrochloric acid and then deposited on the magnetite to

form silica coating layer. The coating prevents the core magnetite from degradation influenced by the outside environment and formation of the large aggregates. Moreover, the silica surface is biocompatible and can be functionalized with a variety of known surface chemistry. TEM image showed that the magnetic silica nanospheres were well dispersed with an average size of ~200 nm (Fig. 1). To a certain degree, the particles size of the magnetic silica can be adjusted by changing the ratio of silica to magnetite or by repeating the coating procedure.

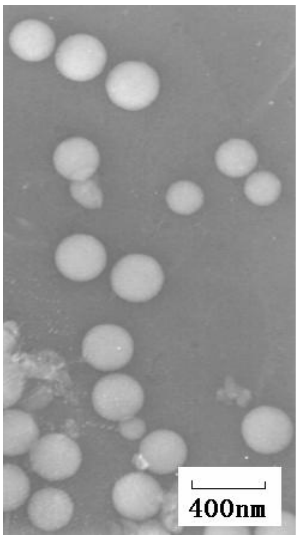

Fig. 1. TEM image of magnetic silica nanospheres

VSM results showed that the magnetic silica nanospheres were superparamagnetic (no hysteresis) and the saturation magnetization was 18.0 emu/g (data not shown). The superparamagnetic property is critical for the magnetic supports used in bioseparation, which prevents magnetic particles from aggregation and enables them to redisperse rapidly when the magnetic field is removed. With such a high magnetization, the nanospheres could be easily and rapidly separated from the solution.

Adsorption isotherms were measured under a pH with the surface histidines of the proteins were at least partially unprotonated and free to co-ordinate bound metal ions. Therefore, pH for the protein adsorption was selected as 8.0. Fig. 2 shows the adsorption isotherm of BHb on magnetic silica nanospheres. The adsorption capacity was as high as 483.6 mg/g.

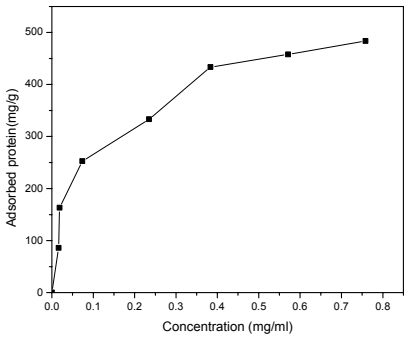

Fig. 2. Adsorption isotherm of BHb on magnetic silica nanospheres charged with Cu^{2+}

4. Conclusions

Magnetic silica nanospheres with the average size of ~200 nm were prepared by the deposition of silica onto magnetite nanoparticles via the controlled hydrolysis of sodium silicate. The magnetic nanospheres exhibited superparamagnetism and high saturation magnetization value of 18.0 emu/g. Modified with GLYMO-IDA and charged with Cu^{2+}, the magnetic nanospheres could efficient recover a model protein (BHb) from the solution with a high adsorption capacity of 483.6 mg/g. These magnetic silica nanospheres have extensive potential for large scale protein affinity separation or purification.

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