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Nitrilotriacetic Acid-Modified Magnetic Nanoparticles as a General Agent to Bind Histidine-Tagged Proteins

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This communication reports synthesis and applications of nitrilotriacetic acid (NTA)-modified magnetic nanoparticles that can act as a general agent to separate, transport, and anchor a protein. To manipulate proteins effectively is an important step for research and applications in the area of proteomics. Among many current protocols, metal−chelate affinity chromatography (MCAC),[1] which employs an NTA-attached resin to immobilize nickel ions (Ni^{2+}) and to separate recombinant proteins that are engineered to have six consecutive histidine residues (6xHis), is the most frequently used for separating recombinant proteins. Although MCAC is easily adaptable to any protein expression system, it still requires pretreatment to remove cell debris and colloid contaminants. It is also limited by a relatively long operation time, solvent consumption, and protein solubility.[1] To develop a simpler and more versatile platform, we create surface-modified magnetic nanoparticles as the binder, carrier, and anchor for histidine-tagged proteins.

Magnetic nanoparticles[2−4] (less than 10 nm in diameter) should perform better than micrometer-sized resins or beads used in MCAC because (1) their high surface/volume ratio and good solubility result in a higher binding rate, (2) their nanometer sizes lead to faster movement and easy entry into cells, and (3) their magnetically controllable aggregation behavior allows them to be anchored onto a solid support for further usage. Despite increasing attention to their applications in biomedicine,[4−6] magnetic nanoparticles have yet to be rendered with metal−chelate affinities for manipulating proteins. Using NTA to attach to FePt magnetic nanoparticles covalently, we show here a simple procedure to obtain pure proteins directly from the mixture of lysed cells (Figure 1) within 10 min. The binding capacity reaches 2−3 mg of protein/per mg of FePt−NTA nanoparticles (**1**), and the lowest concentration of proteins that can be separated is about 0.5 pM.[7] Superior to microbead-based MCAC columns, this magnetic nanoparticle−NTA system essentially exhibits no nonspecific bindings. In addition, this system also allows proteins to be anchored reversibly on a solid support by a small magnet. Because of the extensive usage of 6xHis-tagged proteins in biological research, this system will be a useful alternative for handling the histidine-tagged recombinant proteins.

Scheme 1 illustrates the synthetic route for making the FePt−NTA agents. Protecting the thiol group of mercaptoalkanoic acids (**2**) with acetyl chloride followed by activating the carboxylic group of **2** with *N*-hydroxysuccinimide yielded **3**. N_α,N_α-bis(caboxymethyl)lysine (**4**) reacted with **3** to give **5** after deprotecting the thiol group. FePt nanoparticles[3] (in hexane phase) reacted with **5** (in aqueous solution) under vigorous stirring[6,8] for 2 h to form Pt−S and Fe−S bonds that linked **5** to FePt. After the reaction, product **6** became water soluble and was easily separated from the organic phase. Product **6** reacted with excess $NiCl_2·6H_2O$ (10 times the amount of **5** used to make **6**) to give **1**. After separation from the Ni^{2+} solution and washed by Tris buffer solution, **1** can be used directly or stored for later use.

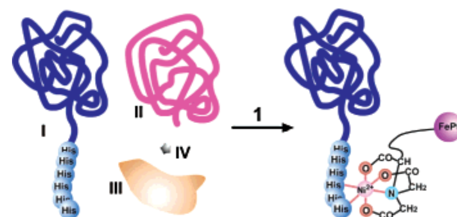
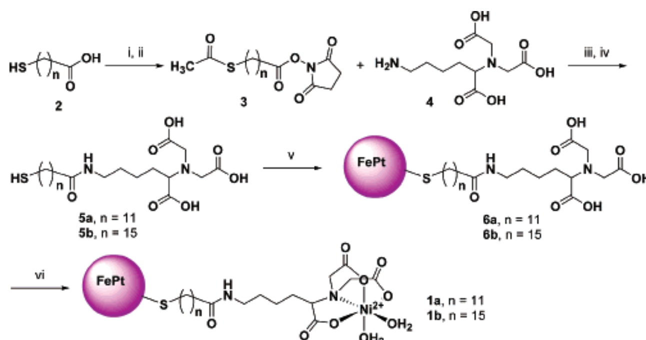


Figure 1. The surface-modified magnetic nanoparticles selectively bind to histidine-tagged proteins in a cell lysate (I: 6xHis tagged protein; II: other proteins; III: cell debris; IV: colloid contaminants).

Scheme 1



The UV−vis spectra of the aqueous solution of **1** exhibit absorption at 205 nm (originated from the carbonyl groups of **5**), indicating that **5** attaches to the FePt nanoparticles.[7] X-ray photoelectron spectroscopy (XPS) of **1** shows peaks at 712/710 and 72.8 eV, corresponding to the energies required to break Fe−S and Pt−S bonds.[7] Time-of-flight second ion mass spectra (ToF−SIMS) also confirm the coordination between **5** and Ni^{2+}.[7] A broad peak around 3435 cm^{-1} and a strong, sharp peak around 1603 cm^{-1} in Fourier transformed infrared (FT-IR) spectra of **1** further confirm the presence of **5** on the surface of FePt.[7] Each FePt nanoparticle has 108 molecules of **5a** or 75 molecules of **5b** on its surface, calculated according to the calibration curves generated using the solutions of **5** and FePt with known concentrations.[7] Transmission electron micrographs (TEM) indicate that the morphology of the as-prepared FePt nanoparticles changes little except for slightly increased aggregation after **5** binds to the FePt nanoparticles (Figure 2, A and B). Magnetic measurements reveal superparamagnetic behavior (no hysteretic behavior) of **1b** at 298 K (Figure 2C). The curve in Figure 2C also shows that the magnetization of **1b** is 7−8 emu/g

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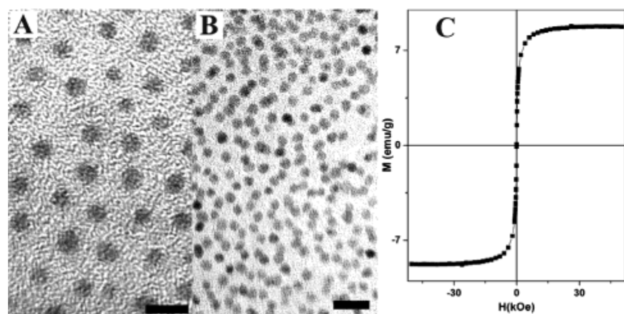

Figure 2. TEM images of the as-prepared FePt nanoparticles (A) before and (B) after being attached to **5** (scale bars = 5 nm) and (C) the hysteresis loop obtained at 298 K for **1b**.

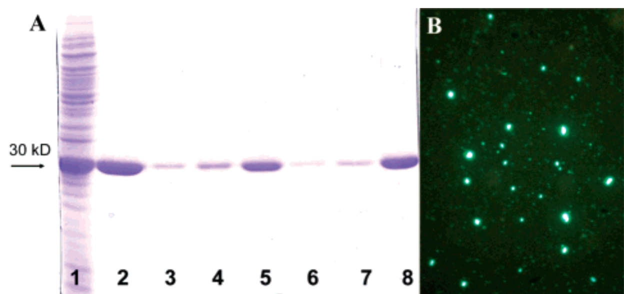

Figure 3. (A) SDS/PAGE analysis of the cell lysate (lane 1), the fraction (lane 2) washed off commercial Ni^{2+}−NTA column according to ref 1; the fraction washed with the fresh-made **1b** using imidazole solution (10 mM, lane 3; 80 mM, lane 4; 500 mM, lane 5); fractions washed off the reused **1b** using imidazole solution (10 mM, lane 6; 20 mM, lane 7; 500 mM, lane 8). (B) Fluorescent image of the conjugate of GFP and **1b** anchored on a glass slide by the magnet.

at 3000 G, which allows the "magnetized" proteins to be attracted by a small magnet (with the surface field of ∼3000 G).[7]

The procedure to use **1** for protein separation consists of three simple steps: (1) adding **1** into the suspension of the lysed cells and shaking for 5 min, (2) using a small magnet to attract the nanoparticles to the wall of the vial and washing them with deionized water to remove the residual protein solution, and (3) using concentrated imidazole solution to wash the nanoparticles to yield pure proteins. After releasing the proteins and being washed sequentially by EDTA, buffer, and $NiCl_2 \cdot 6H_2O$ solution, **1** can be recovered and reused.

To determine the experimental conditions and the efficiency of **1** in binding proteins, we used **1** to separate histidine-tagged green fluorescent protein (6xHis-GFP, ∼30 kD, produced following the procedure in the literature[1]). We also used denative electrophoresis to analyze the purity of GFP, thus defining the optimum concentration of the elutions containing imidazole. After washing away the residual protein solutions or the nonspecifically bound proteins on **1b**, the elutions (obtained by sequentially washing **1b** with 10, 80, and 500 mM imidazole solutions) contain only 6xHis-GFP (lanes 3, 4, and 5, Figure 3A). In addition, the affinity and specificity of **1b** remains unaffected after being recovered and reused, as shown in Figure 3A, lanes 6−8. **1a** exhibits specificity and affinity similar to that of **1b**. Furthermore, nanoparticles also enhance the specificity of NTA−Ni^{2+} to bind with histidine-tagged proteins since there is no other protein being washed off by low-concentration imidazole solutions (Figure 3A, lanes 3 and 6). We also used **6** as the control. When there is no chelation of Ni^{2+} ions with the NTA groups, the amount of GFP absorbed on **6b** is almost negligible.

Using magnetic nanoparticles not only eliminates the preequilibration step required for MCAC but also allows proteins to be transferred to a desired substrate and anchored onto it by a magnet. For example, Figure 3B shows the fluorescent image of the "magnetized" GFP transferred to a glass slide and anchored there by a magnet. We also found the protein binding capacity of **1** (2−3 mg protein/1 mg of **1**) is about 200 times higher than that of the commercial magnetic microbeads (10−12 μg protein/1 mg bead), which is consistent with the high efficiency originating in the high surface/volume ratio of the nanoparticles. In conclusion, by combining FePt magnetic nanoparticles with the specific affinity offered by metal chelation,[9] we have demonstrated that **1** can act as a useful agent for manipulating histidine-tagged proteins. This result suggests that this general and versatile system will be able to bind other biological substrates at low concentrations. This system should also be compatible with other nanoparticle-based systems[10] and offer new possibilities in biological applications.

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Supporting Information Available: The details of the synthesis and characterization of **1** and the conditions for separation of histidine-tagged GFP (PDF). This material is available free of charge via the Internet at http://pub.acs.org.

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