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Ni/NiO Core/Shell Nanoparticles for Selective Binding and Magnetic Separation of Histidine-Tagged Proteins

In Su Lee,[†,‡] Nohyun Lee,[†,‡] Jongnam Park,[†,‡] Byung Hyo Kim,[†,‡] Yong-Weon Yi,[§] Taeuk Kim,[§] Tae Kook Kim,[§] In Hwan Lee,[‡] Seung R. Paik,[‡] and Taeghwan Hyeon*[,†,‡]

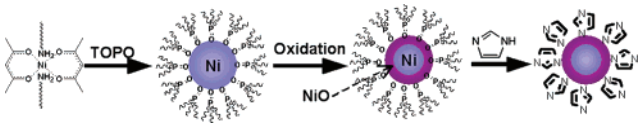
National Creative Research Initiative Center for Oxide Nanocrystalline Materials and School of Chemical and Biological Engineering, Seoul National University, Seoul 151-744, Korea, and Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Korea

Received May 6, 2006; E-mail: thyeon@snu.ac.kr

Nanometer-sized magnetic particles, such as superparamagnetic iron oxide particles, have been extensively used for both in vitro and in vivo applications, such as magnetic resonance imaging (MRI) contrast enhancement, hyperthermia treatment, and gene and drug delivery.[1−3] Another application area of importance is the separation and purification of cells and biomolecules in bioprocesses.[4] Due to their small size and high surface area, magnetic nanoparticles have many superior characteristics for these bioseparation applications compared to those of the conventional micrometer-sized resins or beads, such as good dispersability, the fast and effective binding of biomolecules, and reversible and controllable flocculation. Very recently, Xu and co-workers synthesized nickel−nitrilotriacetic acid (Ni−NTA) complex-conjugated magnetic nanoparticles and reported their excellent performance in manipulating histidine-tagged (His-tagged) proteins.[5] Mirkin and co-workers demonstrated the efficient and selective separation of His-tagged proteins using Ni-containing nanorods with a diameter of about 300 nm.[6] Some of us synthesized uniform-sized Ni nanoparticles with controlled sizes of 2−7 nm from the thermal decomposition of a Ni−oleylamine complex and showed that the nanoparticles were readily oxidized to NiO nanoparticles.[7] On the basis of this discovery, we envisioned that the synthesis of large-sized Ni nanoparticles and their subsequent oxidation would provide an NiO shell having high affinity for polyhistidine (His × 6) with a superparamagnetic Ni portion remaining in the core. We herein report the synthesis of Ni/NiO core/shell nanoparticles and demonstrate their successful utilization for separating and purifying His-tagged proteins from a multicomponent solution including a cell lysate.

For the preparation of the Ni/NiO core/shell nanoparticles, the previously reported procedure was modified (Scheme 1).[7] Ni−oleylamine complex synthesized from $Ni(acac)_2$ and oleylamine was injected into a trioctylphosphine oxide (TOPO) solution with a small amount of trioctylphosphine (TOP) at 80 °C and was reacted by slowly increasing the temperature up to 250 °C. The addition of excess ethanol followed by centrifugation enabled the isolation of the nanoparticles in the form of black powder. The resulting nanoparticles were re-dispersed into a hexane suspension and oxidized in air. Transmission electron microscopy (TEM) revealed the generation of nanoparticles having an average size of 13 nm and a well-defined core−shell structure (Figure 1a,b). The measured lattice distances in the HRTEM image (Figure 1b), 2.40 Å for the shell and 2.04 Å for the core, are quite consistent with the known parameters for NiO and Ni, respectively, and indicate the occurrence of oxidation at the surface of the initially generated Ni particles. The magnetic measurement of the nanoparticles showed that they exhibit a saturated magnetization value of 0.9 emu/g and superparamagnetic behavior at 298 K (Figure 1d). To replace the hydrophobic surfactants present on the surface and to endow the nanoparticles with water compatibility, they were reacted with imidazole in a $CHCl_3$ dispersion and isolated by the addition of hexane and subsequent centrifugation (Figure 1c). The superparamagnetic characteristics were preserved more than 2 months in the powder form. The imidazole-stabilized Ni/NiO nanoparticles can be dispersed in water by vigorous shaking, vortexing, or sonication, resulting in a clear dark-brown-colored dispersion, and they can be easily attracted by using a magnet (Figure 1e). Most of the nanoparticles were aggregated and precipitated within 10 min. The precipitates can be easily re-dispersed by shaking, vortexing, or sonication.

Scheme 1. Preparation of Imidazole-Stabilized Ni/NiO Nanoparticles



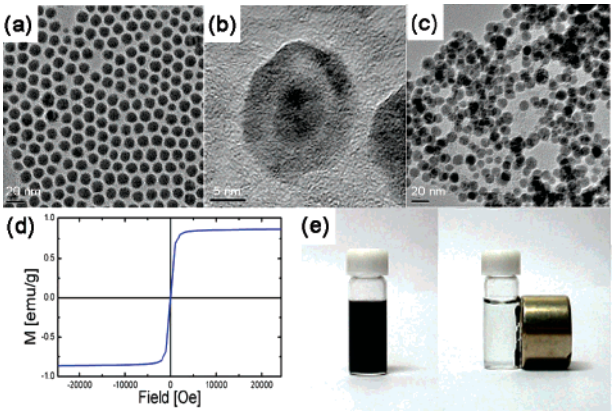


Figure 1. (a) TEM and (b) high-resolution TEM (HRTEM) images of as-synthesized Ni/NiO nanoparticles. (c) TEM image of an imidazole-stabilized Ni/NiO nanoparticle. (d) A hysteresis loop showing superparamagnetic properties of Ni/NiO nanoparticles. (e) Pictures showing magnetic attraction of imidazole-stabilized Ni/NiO nanoparticles.

The protein separation efficiency of the Ni/NiO nanoparticles was investigated by interacting them with His-tagged green fluorescent protein (GFP). In a typical experiment, the nanoparticles were incubated with His-tagged GFP for 30 min at room temperature and then separated from the supernatant by applying a magnet. The binding of His-tagged GFP on the nanoparticles could be monitored by observing the reduction in the fluorescent emission from the solution (Figure 2a). The fluorescent emission intensity of the supernatant solution after removing the His-tagged GFP-bound nanoparticles decreased to 10% of the initial intensity, indicating the efficient binding and separation of the His-tagged GFP. Fluorescent imaging of the isolated nanoparticles confirmed

[†] National Creative Research Initiative Center for Oxide Nanocrystalline Materials, Seoul National University.
[‡] School of Chemical and Biological Engineering, Seoul National University.
[§] Korea Advanced Institute of Science and Technology.

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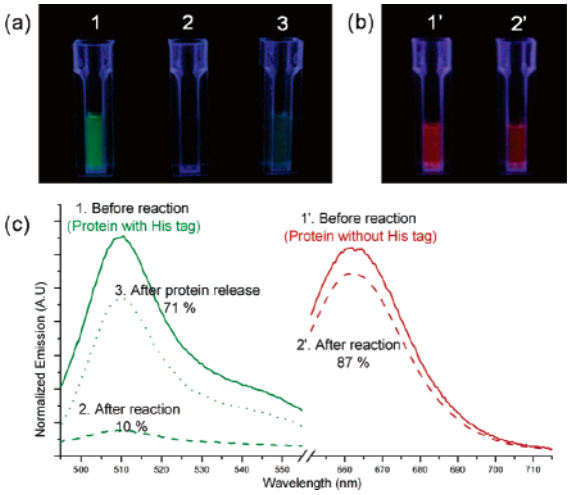
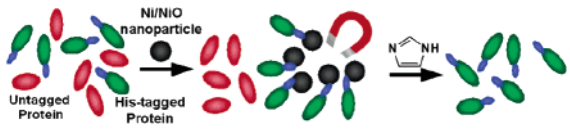


Figure 2. (a) Fluorescent images from the solutions of His-tagged GFP (1) before and (2) after treating with Ni/NiO nanoparticles, and (3) after treating protein-bound Ni/NiO nanoparticles with imidazole solution. (b) Fluorescent images from the solutions of normal mouse IgG labeled by Cy5 (1′) before and (2′) after treating with Ni/NiO nanoparticles. (c) Fluorescence spectra showing the change of emission intensity of the solutions in panel a (green lines) and panel b (red lines).

Scheme 2. Purification of His-tagged Proteins from Mixture Solution



the efficient binding of the His-tagged GFP on the nanoparticles, presumably through the interaction between the Ni^{2+} ions on the NiO shell and polyhistine.[8] The incubation of the His-tagged GFP-captured nanoparticles with concentrated imidazole solution caused the proteins to be released from the nanoparticles, resulting in a 71% recovery of the fluorescent emission intensity (Figure 2c). The nanoparticles were retrieved from the imidazole solution by applying a magnet and were reused for the next cycle. In the second cycle, 93% of the His-tagged GFP was separated by using the nanoparticles, and 55% of the His-tagged GFP was recovered.

As a control experiment, when normal mouse IgG without His-tag and labeled by red-emitting Cy5 was treated with the Ni/NiO nanoparticles under a nearly identical condition, only a 13% decrease in the fluorescent emission intensity was observed from the supernatant, indicating the small extent of the binding of the normal mouse IgG with the nanoparticles (Figure 2b,c). When the Ni/NiO nanoparticles were incubated with a mixture solution containing both the His-tagged GFP and normal mouse IgG labeled by Cy5 and then separated from the mixture, the supernatant exhibited red emission. This indicates the selective removal of the His-tagged GFP from the mixture solution, which initially showed a yellow emission. The His-tagged GFP was released by treating the bound nanoparticles with concentrated imidazole solution, generating a green emitting solution (Figure 3a). To demonstrate their practical utility, the Ni/NiO nanoparticles were incubated with *E. coli* cell lysate containing His-FcBD proteins and isolated using magnetic attraction. SDS−PAGE analysis of proteins released by treatment with a concentrated imidazole confirmed specific purification of His-tagged proteins (Figure 3b).

In conclusion, we demonstrated the synthesis of NiO-coated Ni nanoparticles and their successful application to the magnetic separation of His-tagged proteins. We believe that our current approach will provide a more convenient method for efficiently purifying His-tagged proteins, as compared with the current method, which needs various steps to synthesize and conjugate NTA

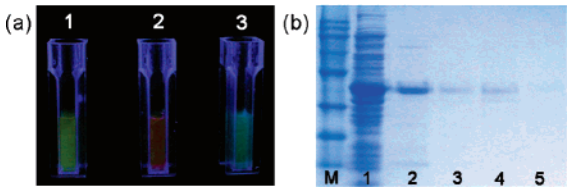


Figure 3. (a) Fluorescent images from the mixture solutions of His-tagged GFP and normal mouse IgG labeled by Cy5 (1) before and (2) after treating with Ni/NiO nanoparticles, and (3) after treating protein-bound Ni/NiO nanoparticles with imidazole solution. (b) SDS/PAGE analyses of the cell lysate containing His-FcBD (lane 1) and proteins released from nanoparticles treated twice with PBS containing 0.5 M imidazole (lanes 2 and 3) and twice with 1 M imidazole (lanes 4 and 5) solutions. Lane M is molecular weight markers.

derivatives on support materials.[9] We also believe that our approach can be employed to anchor biomolecules on nanoparticles, producing novel biomedical nanomaterials.

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Supporting Information Available: Experimental procedures for the syntheses of nanoparticles and protein separation, confocal laser microscopy image of His-tagged GFP capturing Ni/NiO nanoparticles, fluorescent spectra showing the change of emission intensity of the solutions in Figure 3a, and detailed SDS/PAGE analyses of purification of His-FcBD from cell lysate with Ni/NiO nanoparticles. This material is available free of charge via the Internet at http://pubs.acs.org.

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