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Immobilization of histidine-tagged proteins by magnetic nanoparticles encapsulated with nitrilotriacetic acid (NTA)-phospholipids micelle ☆

Yong Taik Lim [a], Kun Yeong Lee [a], Kwangyeol Lee [b], Bong Hyun Chung [a,*]

[a] *Bionanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology, 52 Eoeun-dong, Yuseong-gu, Daejeon 305-333, Republic of Korea*
[b] *Department of Chemistry, Korea University, Anam-dong, Sungbuk-gu, Seoul 136-701, Republic of Korea*

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Abstract

We described the development of functionalized magnetic nanoparticles (MNPs) with PEG-modification, a phospholipids micelle coating, and their use in manipulating histidine-tagged proteins. Highly monodisperse MNPs were synthesized in an organic solvent and could be phase-transferred into an aqueous solution by encapsulating the nanoparticles with a phospholipids micelle. The phospholipids micelle coating rendered the nanoparticles highly water-soluble, and the functional groups of the phospholipids coating allowed for the bioconjugation of various moieties, such as fluorescent molecules and engineered proteins. Functionalized phospholipids, such as nitrilotriacetic acid (NTA)-phospholipids, caused the MNPs to bind and allowed for manipulation of histidine-tagged proteins. Due to their high surface/volume ratio, the MNPs showed better performance (about 100 times higher) in immobilizing engineered proteins than conventional micrometer-sized beads. This demonstrates that MNPs coated with phospholipids micelle can be a versatile platform for the effective manipulation of various kinds of engineered proteins, which is very important in the field of proteomics. It is expected that a combination of MNPs with optical fluorescent molecules can find applications in bimodal (magnetic and optical) molecular imaging nanoprobes.

Keywords: Immobilization of proteins; Magnetic nanoparticles; Phospholipids micelle; NTA-phospholipids; Fluorescent proteins

The dimensions of MNPs are comparable to those of a virus (20–500 nm), a protein (5–50 nm) or a gene (2 nm wide and 10–100 nm long). MNPs have a large surface that can be appropriately modified to afford attachment of biological agents [1–5]. Iron oxide nanoparticles have been investigated in the areas of biomedicine and biology due to the inherent biocompatibility of iron oxide [1–5]. However, MNPs with tailored surface chemistry are typically required for specific biological applications.

Recently, highly crystalline and monodisperse MNPs such as Fe_2O_3, FePt, and Co have been fabricated in organic solvents at elevated temperatures [6,7]. However, the nonaqueous method involves a surface coating with long hydrocarbon chains, which renders the MNPs hydrophobic. The biological applications of these MNPs are thus greatly restricted due to their poor solubility in aqueous solution.

Several methods that can increase the aqueous phase dispersion of MNPs through surface modification with Au, polymers, and silica have been suggested [8–10]. Polymer- and silica-based coating processes are difficult

☆ *Abbreviations:* NTA, nitrilotriacetic acid; MNPs, magnetic nanoparticles; EYFP, enhanced yellow fluorescent proteins; TEM, transmission electron micrographs; DSPE-mPEG 2000, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-methoxy(polyethylene glycol) 2000; DOGS–NTA–Ni, 1,2-dioleoyl-*sn*-glycero-3-{[*N*(5-amino-1-carboxypentyl)iminodiacetic acid]succinyl}(Nickel salt); IPTG, isopropylthiogalactoside; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

* Corresponding author. Fax: +82 42 879 8594.
E-mail address: chungbh@kribb.re.kr (B.H. Chung).

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to control, however, and often result in multilayered coatings and multiple nanoparticles in the same encapsulation [9,10]. These approaches have other drawbacks such as low specificity and poor tenability. In situ coating approaches such as a method that utilizes dextran [10] often lead to multilayered coatings, which can result in a heterogeneous size distribution. In situ synthesis conditions also limit the variety of functionalization that can be achieved.

In this communication, we report on the development of highly monodisperse water-soluble MNPs encapsulated by phospholipids micelles and the use of functionalized MNPs with NTA-phospholipids micelle in anchoring, separating, and transporting a target protein. The method described in this study can be widely applicable to encapsulate and functionalize other kinds of nanoparticles that have a hydrophobic surface [11]. Furthermore, the coating of MNPs with various functional phospholipids micelle would be a robust platform for the effective manipulation of other engineered proteins with reactive moiety. This is of particular significance, because it is important to effectively anchor, separate, and transport target proteins for research and applications in the fields of proteomics.

Materials and methods

Synthesis of 10 nm Fe_2O_3 nanoparticles. In a typical synthesis, a slurry of $Fe(acac)_3$ (5 mmol, Strem Chemicals, 99%), oleic acid (10 mmol, Aldrich, 90%), oleylamine (10 mmol, Aldrich, technical grade), hydrazine monohydrate (20 mmol, Aldrich, 99%), and trioctylamine (TOA, 50 mL, Aldrich, 90%) prepared in a 250 mL autoclave was heated from room temperature to 180 °C within 30 min and was kept at that temperature for 18 h with vigorous magnetic stirring. The resulting reaction mixture was cooled to room temperature to form a dark brown solution. Addition of toluene (50 mL), filtration, and precipitation via the addition of acetone/methanol (50:50 mL) produced a dark brown powder. This powder can be easily re-dispersed in various organic solvents such as hexane, toluene, and dichloromethane. The dark brown powder was kept under methanol/oleylamine/oleic acid (5:1:1 in volume) when not being used.

Surface modification of magnetic nanoparticles with phospholipids micelle. The synthesized iron oxide MNPs were washed with methanol three times and re-dispersed in chloroform solution before the surface modification step. 1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-methoxy(polyethylene glycol) 2000 (DSPE-mPEG 2000) and 1,2-dioleoyl-*sn*-glycero-3-{[*N*(5-amino-1-carboxypentyl)iminodiacetic acid]succinyl}(Nickel salt) (DOGS–NTA–Ni) were obtained from Avanti polar lipids (Alabaster, AL). Mixtures of DSPE-mPEG 2000 and DOGS–NTA–Ni were dissolved in 1.6 ml of chloroform (3:7 ratio of DSPE-mPEG 2000: DOGS–NTA–Ni), and iron oxide MNPs (400 μl of 3.7 mg/ml stock solution) were added to the mixture. This mixture was then dried under nitrogen gas and left in a vacuum desiccator for 48 h to remove all traces of organic solvents. The dried film was easily re-suspended in deionized water or PBS buffer with agitation. The solution obtained was filtered using 0.2 μm syringe filters.

Preparation of histidine-tagged enhanced yellow fluorescent protein [6×His-EYFP]. The EYFP protein bearing a 6×His segment on its C-terminus was prepared as follows. The gene of the EYFP derivative was created by PCR. The 717 bp fragment (679–1395) of pEYFP-N1 (Clontech, Palo Alto) containing the EYFP domain was amplified by primers possessing the sequences for *Nde*I and for the *Xho*I site. The amplified fragment was cleaved by *Nde*I and *Xho*I, and cloned into pET22b (+) (Novagen, Madison, WI). The 6×His-EYFP proteins were expressed in *Escherichia coli* strain BL21(DE3) in soluble form and purified using nickel–iminodiacetic acid (Ni–IDA) affinity chromatography (Bioprogen, Korea). For the large-scale purification of the mature and precursor forms under native conditions, expression was induced by exposure of the cells to 1 mM IPTG (Isopropylthiogalactoside). Cultured cells at 25 °C were harvested after 16 h of induction and lysed by sonication for 20 min on ice. The cell paste was resuspended in four volumes of PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 2 mM KH_2PO_4). The extract was then subjected to centrifugation at 15,000*g* for 20 min, and the aqueous fraction was poured into a Ni–IDA column, and washed with an additional 50 ml of PBS buffer containing 20 mM imidazole. Protein was eluted from the column using a PBS buffer containing 250 mM imidazole. The eluted protein was dialyzed immediately against a 100-fold excess of buffer containing 50 mM Tris–HCl (pH 8.0). Protein concentration was measured by a Bradford assay (Bio-Rad, Richmond, IL) and the protein purity was characterized by a SDS–PAGE.

Immobilization of His-EYFP proteins with phospholipids micelle coated MNPs. To immobilize the 6×His-EYFP to Fe_2O_3 nanoparticles, 1.5 mL of 6×His-EYFP (0.5 mg/ml) and 4 mL of Fe_2O_3–NTA-phospholipid (0.2 mg/ml) were mixed for 10 min. The complex MNPs were separated using a magnet. After washing away the residual protein solutions or physically absorbed proteins on functionalized Fe_2O_3 nanoparticles, we used 50 and 250 mM, respectively, imidazole elutions to sequentially wash the protein-bound nanoparticles.

Immunoblotting preparation of His-EYFP proteins. The purified histidine-tagged EYFP proteins using a commercial Ni–NTA column, the fraction of remaining solution after collection of MNPs binding, and the magnetically collected histidine-tagged EYFP proteins washed with the 50 or 250 mM imidazole solution, were separated by SDS–PAGE and immunoblotted on a nitrocellulose membrane (Bio-Rad). Membranes were blocked overnight at 4 °C in Tris-buffered saline (TBS) containing 5% skim milk plus 0.5% Tween 20 and then incubated at room temperature with primary (anti-Histidine mouse monoclonal antibodies; IG. THERAPY) and horseradish peroxidase-conjugated goat-antimouse IgG secondary antibodies (Bio-Rad, Richmond, IL). Detection was carried out by enhanced chemiluminescence according to the manufacturer's protocol (Amersham-Pharmacia Biotech, Cologno Monzese, Italy).

Results and discussion

Synthesis and surface modification of monodisperse iron oxide MNPs

Fig. 1 shows the synthetic route for making functionalized Fe_2O_3 nanoparticles with phospholipids micelle. The highly monodisperse MNPs were synthesized in toluene at elevated temperatures [6,7] and were insoluble in water prior to modification. After washing with methanol to eliminate residual surfactants and reactants used in the synthetic process, a chloroform solution containing phospholipids mixtures was added to the nanoparticles solution. After evaporation of the chloroform, the dried film could be re-dispersed into water for further biological conjugation. In this process, the hydrophobic part of the amphiphilic phospholipids interacted with the hydrophobic capping ligands on the MNPs, and the surface-exposed hydrophilic chains afforded higher solubility to the coated MNPs.

PEG-modified phospholipids are micelle-forming hydrophilic polymer-grafted phospholipids comparable to naturally occurring carriers such as lipoproteins and viruses [12,13]. They have been used for drug delivery and diagnostic imaging [12,13]. The main advantage of these