Delivery of Ferritin-encapsulated Gold Nanoparticles on Desired Surfaces

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ABSTRACT

In this work, we presented an encapsulation-delivery system of gold nanoparticles. A genetic modified horse L-ferritin mutant was used in this system, which has a gold binding peptide and a titanium binding peptide at the C-terminus and N-terminus, respectively. We found that this modified ferritin mutant can efficiently catches gold nanoparticles with diameter of 5nm or 15 nm during its pH-dependent dissociation and reassembly process, and deliver them to a silicon surface or titanium surface.

Keywords: ferritin, gold nanoparticles, size-varied, encapsulation, delivery

1 INTRODUCTION

Nanoparticles (NPs) play an important role in the modern nanotechnology, due to their superior characteristics which can achieve functions hard to be realized using conventional bulk materials. Among all NPs, gold NPs (GNPs) must be the most studied one, because of their fascinating application using their optical properties, catalytic properties, and possibility to be assembled to higher nanostructure.[1] It is known that the properties of GNPs mainly depend on their dimensions, shape, crystallinity and composition. Therefore, efforts have been exerted these years to control these factors during GNPs' synthesis, particularly size and shape.[2] Hitherto, several protocols have been established to control the size and shape of GNPs. These novel materials will be key materials to bring new innovations to nanotechnology and nanoscience.

However, when thinking about application, the issue that how to modify of GNPs' surface which can make GNPs aqueous-stable and enable GNPs to adhere to a desired site, should be solved. Polymer, silica, dendrimers were reported as encapsulating agents of GNPs. Here, we found a simpler and lower costing method to encapsulate GNPs by using biologic material, ferritin protein. Ferritin is an iron-storage protein, widely exists in the three kingdoms of life, in aerobic or anaerobic organisms. Their structure and function have been investigated in detail.[3] Ferritin is a spherical protein formed by twenty-four subunits, with an outer diameter of 12 nm and an inner diameter of 7 nm. The N-terminus is exposed to the outer solution and the C-terminus to the inner cavity. One important property of ferritin is its pH-dependent reversible dissociation-reassembly process, during which ferritin dissociates into subunit dimers in an acidic environment and the subunit dimers can reassemble into the 24-mer ferritins when the pH is increased to 7.[4] In this proceeding, we exactly take advantage of this property of a horse L-ferrtitin mutant TFG, which has a gold binding peptide (GBP) and a titanium/silicon binding peptide (TBP) in its N- and C-termini, to effectively encapsulate/deliver size-varied GNPs to the desired site.

2 EXPERIMENTAL

2.1 Preparation of TFG and GNPs

Ferritin mutant TFG was prepared as follow: *E.coli* expressing TFG was grown in LB medium for overnight at 37°C. Cells were recovered by centrifugation at 8,000 r.p.m. for 20 min, suspended in a proper volume of 50 mM Tris–HCl and pH 8.0, and lysed by ultrasonication. After centrifugation at 12,000 rpm for 20 min, the supernatant was subjected to a thermal denaturation at 60°C for 20 min in a shaking water bath. After centrifugation at 12,000 r.p.m. for 20 min, the supernatant was loaded onto a Q-Sepharose column pre-equilibrated by 50 mM Tris–HCl and pH 8.0 buffer and eluted with a 0–1 M NaCl linear gradient. Fractions containing TFG protein were collected and concentrated via centrifuge filtration, then loaded onto a Sephacryl S-300 column (Pharmacia) equilibrated in 50 mM Tris–HCl and pH 8.0 buffer, 150 mM NaCl. Protein purity was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie Brilliant Blue staining.

5nm-diameter GNPs (GNP5s) and 15 nm-diameter GNPs (GNP15s) were used. GNP5s were purchased from BBI, UK. GNP15s were synthesized following reported protocol.[6] Diameters of both were testified by transmission electron microscope (TEM) observation.

2.2 Construction of TFG-GNP Conjugates

Encapsulation of GNPs by TFG was reported recently.[5] Briefly, excessively dissociated TFG comparing to the GNPs was mixed with GNPs, and at the same time pH of mixture solution was adjusted to neutral. The mixture was

incubated at room temperature for overnight. Bio-conjugate i.e. a GNP (TFG/GNP5 or TFG/GNP15) surrounded by TFG subunit dimers, was purified. TFG/GNPs were purified in two steps. First, the non-encapsulated GNPs were removed using Sephacryl S-300 gel filtration with running buffer containing 50 mM Tris-HCl, 150 mM NaCl and pH 8.0. TFG/GNPs and excessive TFG protein were eluted and collected, while non-encapsulated GNPs aggregated and stuck to the column firmly. Secondary, TFG/GNP5s was further purified by sucrose density gradient centrifugation (20000 rpm, 1hr). In comparison, TFG/GNP15s were purified by directly centrifugation （16000 rpm, 40 min), after which excessive TFG protein in supernatant was removed. Both TFG/GNP5 and TFG/GNP15 were dissolved in 50 mM Tris-HCl, 150 mM NaCl and pH 8.0. Protein concentration was determined by Lowry method. Concentration of GNPs was calculated using the absorbance at 520 nm.

2.3 Adsorption of TFG/GNPs on Si/Ti Substrate

The Ti patterns with the thickness of 2 nm were fabricated on a thermally oxidized Si substrate using electron beam lithography or photo lithography and lift-off process. GNP5, TFG/GNP5, GNP15 and TFG/GNP15 were used as adsorption agents in the adsorption experiment. GNP5, TFG/GNP5, GNP15 or TFG/GNP15 of similar concentration was applied to the substrate, which was pre-cleaned by UV/ozone treatment at 115 ℃ for 10 min with a UV ionizer (UV-300, SAMCO) to remove hydrocarbon contaminants and hydrophilize the surface, and left for 10 min at room temperature. After incubation, the substrate was washed with pure water and dried. Finally, UV/ozone treatment was carried out for 50 min. Scanning electron microscope (SEM) observation was carried out consequently.

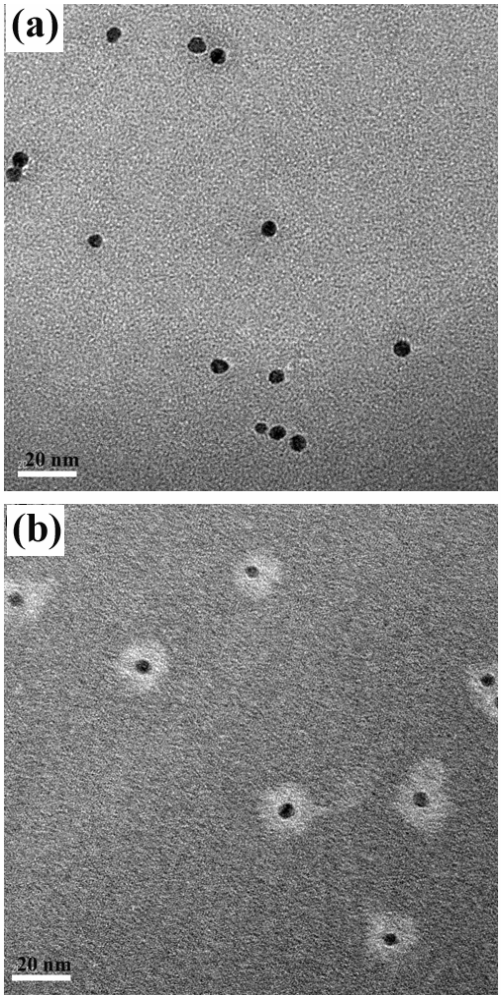

Figure 1: Ferritin-GNP5 conjugates. (a) TEM image of GNP5, (b) TFG/GNP5.

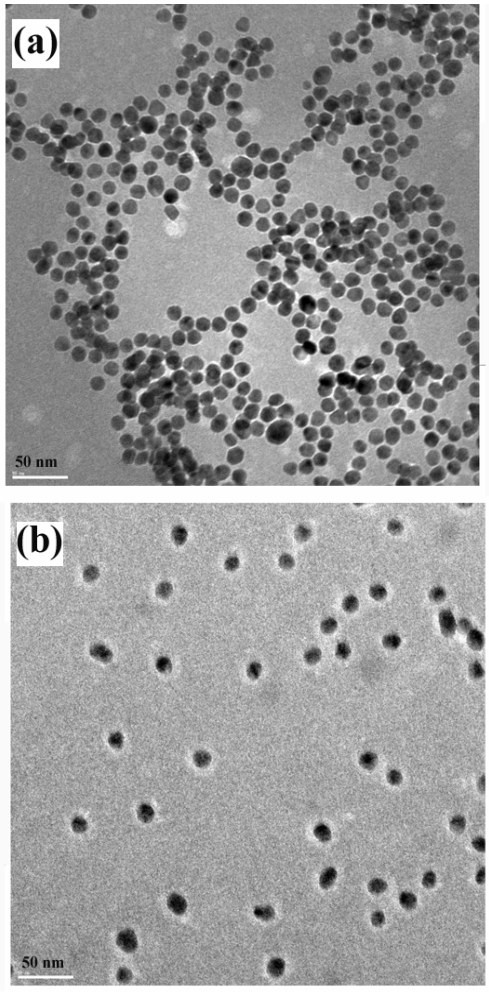

Figure 2: Ferritin-GNP15 conjugates. (a) TEM image of GNP15, (b) TFG/GNP15.

3 RESULTS AND DISCUSSIONS

During the dissociation-reassembly process, TFG protein subunits firmly surround each GNP, no matter that the NP's diameter is 5nm or 15 nm, and form a protein layer (Fig. 1 and Fig.2). TEM images showed that Naked GNP5 purchased from BBI remained mono-dispersed in solution, due to the capping regent on the GNP5's surface. Naked GNP15 aggregated heavily in the TEM sample grid. The TFG encapsulation enabled GNPs remain mono-dispersed in the solution. We discussed the encapsulation mechanism between TFG and GNP5 by determine the ration of GNP5 and TFG.[5] We concluded that encapsulation process takes place in two steps. During the dissociation and reassembly process, TFG subunits adsorb to the GNP surface mainly according to the interaction between GBP and GNP5 to form the first protein layer around GNP. The interaction is strong enough to cover the GNP surface almost fully. The whole GNP5 surface can be enclosed by the secondary encapsulation process, during which TFG subunits insert their E-helixes into the apertures of the first protein layer to form the secondary protein layer. Therefore, we anticipated that TFG proteins should be able to encapsulate GNPs of various sizes, which is proved by the success of encapsulation of GNP15 by TFG.

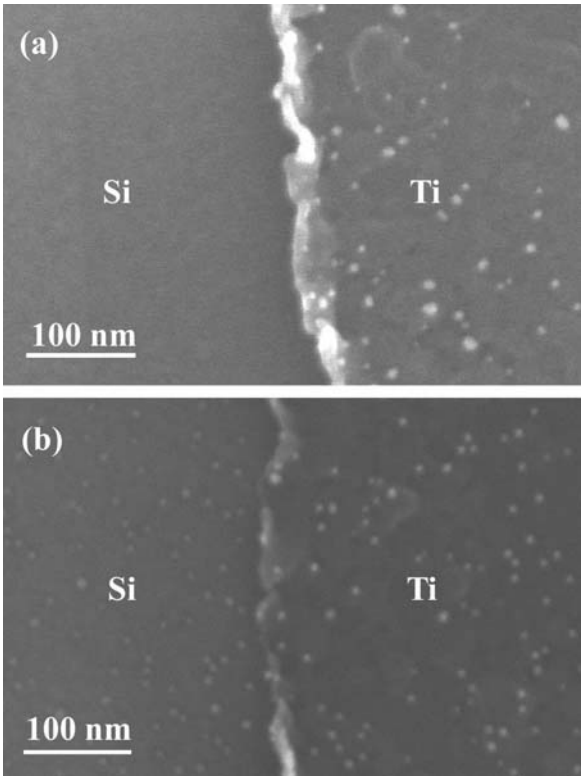

Figure 3: (a) Adsorption of GNP5 to Si/Ti surface. (b) Adsorption of TFG/GNP5 to Si/Ti surface.

It is known TBP is a peptide with affinity to Ti, Si and Ag but not to Au, Cr, Pt, Sn, Zn, Cu and Fe, and Tween-20 can enhance the affinity specificity only to Ti.[7-8] Genetic attaching TBP to ferritin endow the protein similar adsorptive property, which enable selective nanoscale positioning of ferritin and nanoparticles.[9-10] Hayashi and coworkers revealed that the sequence (RKLPDA) is strongly bound to charges originating from the protonation and deprotonation of the surface groups of a Ti substrate. On the other hand, it is said that the hydrophilicity or hydrophobicity is an important factor to govern the adhesion force between TBP attached ferritin (TBF) and the substrate. Against a hydrophobic surface, the strength of the adhesion exceeds the strength of the specific binding between TBF and Ti, indicating that might not distinguish the target when the target is mixed with hydrophobic objects. Therefore, a surfactant such as Tween 20 was reported to effective to enhance the selectivity and specificity as a restraining factor suppressing the nonspecific binding.

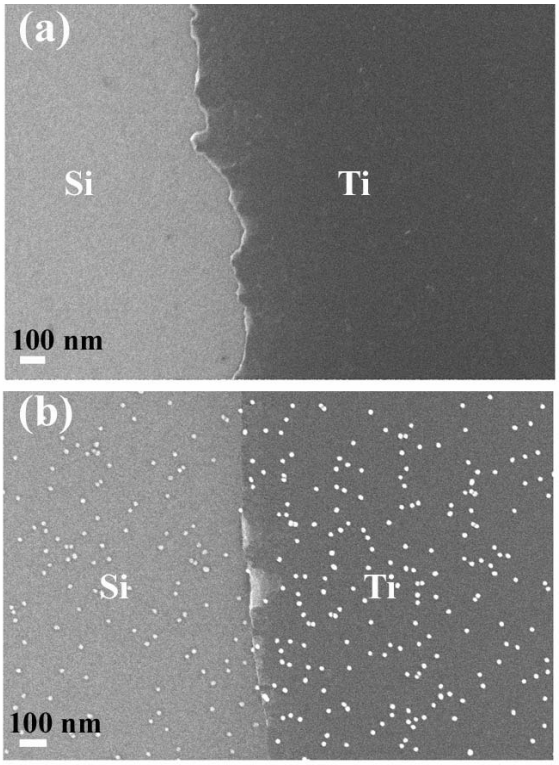

Figure 4: (a) Adsorption of GNP15 to Si/Ti surface. (b) Adsorption of TFG/GNP15 to Si/Ti surface.

Our results coincide to hayashi's theory well. Naked GNP15s showed no adsorption to Si or Ti surface, while TFG/GNP15s have a property to deliver themselves to Si or Ti surfaces (Fig. 4). Obviously, encapsulation of TFG endows GNP15 the adsorptive property. Similar result was obtained in the case of TFG/GNP5. The only difference is naked GNP5 adsorbs to Ti surface while GNP15 does not (Figure 3). Capping agent on GNP5's surface should be responsible in this adsorption. Since TFG/GNP5 adsorbed

to Si substrate while GNP5 can not, we suggested that it is TFG which endow GNP5 the affinity ability to make TFG/GNP5 adsorb to Si or Ti surface.

4 CONCLUSION

Since there are several kinds of size/shape-varied GNPs have been successfully synthesized, effective encapsulation -delivery of them is required.[11-14]In this proceeding work, we demonstrated that TFG subunits can encapsulate/deliver GNPs of various sizes to desired site. This is a promising property for applications involving surface plasmon resonance, which is strongly dependent on GNP's size and shape.

On the other hand, study of the selectivity of adsorption basing on Hayashi's theory is our next plan, because high selective adsorption to a desired site makes it possible to immobilize a single GNP, or to construct a large-dimensioned GNPs embedded substrate, which should contribute to the next-generation bio-sensor construction.

REFERENCES

[1] Daniel MC, Astruc D, "Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology," Chem Rev., 104,293-346, 2004.

[2] Goy-López S, Taboada P, Cambón A, Juárez J, Alvarez-Lorenzo C, Concheiro A, Mosquera V. J, "Modulation of size and shape of Au nanoparticles using amino-X-shaped poly(ethylene oxide)-poly(propylene oxide) block copolymers," Phys Chem B., 114, 66-76, 2010.

[3] MassoverW H, "Ultrastructure of ferritin and apoferritin," Micron, 24, 389–437, 1993.

[4] Yoshizawa K, Mishima Y, Park S Y, Heddle J G, Tame R H J, Iwahori K, Kobayashi M and Yamashita I, "Effect of N-terminal residues on the structural stability of recombinant horse L-chain apoferritin in an acidic environment," J. Biochem., 142, 707–13, 2007.

[5] Zheng B, Yamashita I, Uenuma M, Iwahori K, Kobayashi M, Uraoka Y, "Site-directed delivery of ferritin-encapsulated gold nanoparticles," Nanotechnology, 21, 045305, 2010.

[6] Frens, G, "Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions," Nat. Phys. Sci., 241, 20, 1973.

[7] Sano K, Shiba K, "hexapeptide motif that electrostatically binds to the surface of titanium," J Am Chem Soc., 125, 14234-5, 2003.

[8] Sano K, Sasaki H, Shiba K, "Specificity and biomineralization activities of Ti-binding peptide-1 (TBP-1)," Langmuir, 21, 3090-5, 2005.

[9] Hayashi T, Sano K, Shiba K, Kumashiro Y, Iwahori K, Yamashita I, Hara M, "Mechanism underlying specificity of proteins targeting inorganic materials," Nano Lett., 6, 515-9, 2006.

[10] Yamashita I, Kirimura H, Okuda M, Nishio K, Sano K, Shiba K, Hayashi T, Hara M, Mishima Y, "Selective nanoscale positioning of ferritin and nanoparticles by means of target-specific peptides," Small, 2, 1148-52, 2006.

[11] Sun Y, Xia Y, "Shape-controlled synthesis of gold and silver nanoparticles," Science, 298, 2176-9, 2002.

[12] Millstone JE, Wei W, Jones MR, Yoo H, Mirkin CA, "Iodide ions control seed-mediated growth of anisotropic gold nanoparticles," Nano Lett., 8, 2526-9, 2008.

[13] Wei Y, Klajn R, Pinchuk AO, Grzybowski BA, "Synthesis, shape control, and optical properties of hybrid Au/Fe3O(4) "nanoflowers"," Small, 4, 1635-9, 2008.

[14] Chen HM, Peng HC, Liu RS, Asakura K, Lee CL, Lee JF, Hu SF, "Controlling the length and shape of gold nanorods," J Phys Chem B. 109,19553-5, 2005.