Hydrogenation Catalysis

Size-Selective Olefin Hydrogenation by a Pd Nanocluster Provided in an Apo-Ferritin Cage**

Takafumi Ueno, Masako Suzuki, Toshiaki Goto, Tomoharu Matsumoto, Kuniaki Nagayama, and Yoshihito Watanabe**

There has been much interest lately in chemical transformations in single capsules of supramolecular assemblies.[1–4] Well-designed capsule structures could provide increased concentration of substrates in the cavities with high selectivity to allow transformation of the substrates. Proteins are very attractive building components for the construction of self-assembled cage structures.[1,5–12] For example, Mann and co-workers have reported in their pioneering works the preparation of size-restricted metal oxides and sulfides by using the biosupramolecular cage of ferritin.[5–7] In addition, protein assemblies of viruses and the chaperonin protein GroEL were also utilized for the encapsulation of metal nanoclusters.[10–12] However, difficulties still remain in controlling reactions catalyzed by metal clusters in protein cages.

Ferritin is known as an iron-storage protein and comprises 24 subunits that assemble to form a hollow cagelike structure of 12 nm in diameter as shown in Figure 1. Iron atoms are stored as a cluster of ferric oxyhydroxide within a cavity of diameter 8 nm formed by the protein subunits (Figure 1 b).[13,14] One of the most important consequences of this protein cage is the perforation of the protein shell by small channels that locate at the junctions of the subunits (Figure 1 c and d). The channels are required for the transport of several metal ions and other organic molecules.[15] In addition, penetration studies of organic molecules have

[*] Dr. T. Ueno
Research Center for Materials Science
Nagoya University
Nagoya 464-8602 (Japan)
Fax: (+81) 52-789-2953
E-mail: taka@mbox.chem.nagoya-u.ac.jp

M. Suzuki, Prof. Dr. Y. Watanabe
Department of Chemistry
Graduate School of Science
Nagoya University
Nagoya 464-8602 (Japan)
Fax: (+81) 52-789-2953
E-mail: yoshi@nucc.cc.nagoya-u.ac.jp

T. Goto
Department of Physics
Graduate School of Science
Nagoya University
Nagoya 464-8602 (Japan)

Dr. T. Matsumoto, Prof. Dr. K. Nagayama
National Institute for Physiological Science
Okazaki 444-8585 (Japan)

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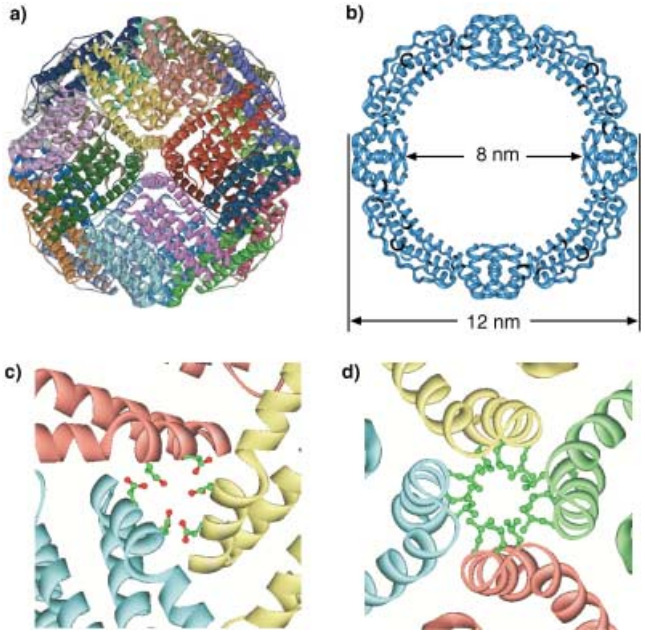
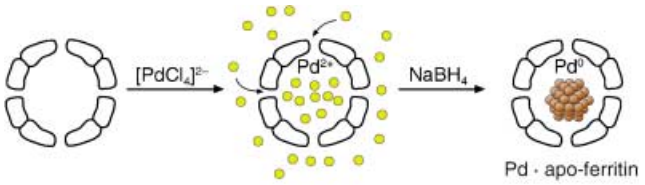


Figure 1. Schematic representation of horse-spleen ferritin: a) the complete 24-meric assembly; b) the inner cavity; c) threefold axis hydrophilic channel; d) fourfold axis hydrophobic channel taken from PDB code: 1IER.[14]

confirmed the size-selective insertion of them into the holo-ferritin interior through the channels.[16,17] These results could imply that holo-ferritin could accommodate organic foreign compounds in its cavity even in the presence of metal clusters. Herein we describe a novel strategy for the construction of a size-selective hydrogenation biocatalyst: a Pd nanocluster encapsulated in the apo-ferritin cavity. The Pd cluster is synthesized by in situ chemical reduction of Pd^{II} ions concentrated in the interior of apo-ferritin (Scheme 1) and the size selectivity of the olefin hydrogenation is given by the penetration of substrates through the channels.



Scheme 1. Preparation of Pd·apo-ferritin.

Apo-ferritin is treated with 500 equivalents of K_2PdCl_4 to afford a homogeneous pale yellow aqueous solution at pH 8.5 adjusted by 0.01N NaOH (aq). The Pd^{II} ions are reduced by 5 equivalents of $NaBH_4$ to yield zero-valent Pd clusters (Figure 2a). According to the reduction, the solution spectrum has been changed significantly; there is a broad intense absorption at a longer wavelength (Figure 2a). A similar spectroscopic change was observed for the reduction of Pd^{II} ions in dendrimers.[18] Under the same conditions, black precipitates were observed in a protein-free control experiment, whereas the solution containing apo-ferritin remained a

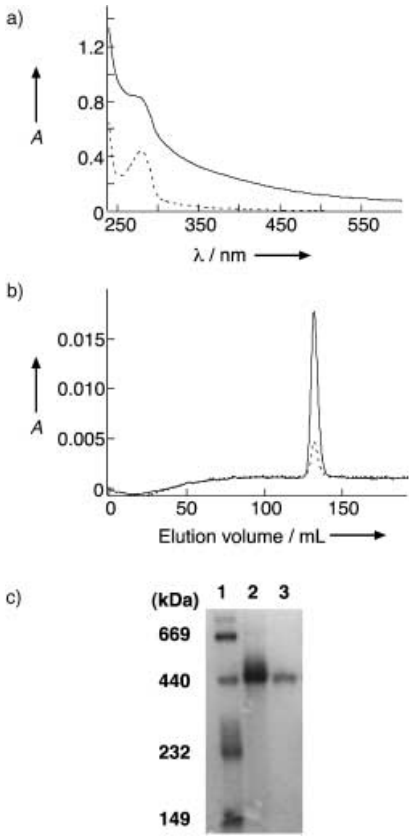


Figure 2. a) UV/Vis spectra of Pd·apo-ferritin (solid line), and apo-ferritin (dashed line); b) size-exclusion chromatography elution profile of Pd·apo-ferritin monitored at 280 nm (solid line) and 400 nm (broken line); c) native PAGE of marker (lane 1), Pd·apo-ferritin (lane 2), apo-ferritin (lane 3). The gel (7.5%) was stained by Coomassie brilliant blue.

clear brown solution. These results suggest specific formation of Pd clusters within the protein cavities. Finally, the Pd·apo-ferritin composite was isolated by size-exclusion chromatography (Superdex G-200). The product elution was monitored either at 280 nm (protein) or at 400 nm (zero-valent Pd cluster), and the coelution of the protein and metallic components through the column is the clear indication of the composite nature of the material (Figure 2b). In addition, a polyacrylamide gel electrophoresis (PAGE) experiment with the Pd·apo-ferritin and apo-ferritin under native conditions also indicates that the protein cage remains unchanged during the reduction as shown in Figure 2c.

The composite materials in aqueous solution were examined by transmission electron microscopy (TEM). The TEM images clearly show that 1) the metal clusters are almost monodispersed particles, 2) their shape is roughly spherical (Figure 3a), and 3) an intact protein shell surrounds the metallic core (Figure 3b). The diameter of the metal particles of Pd·apo-ferritin is 2.0 ± 0.3 nm as shown in the histogram in Figure 3a. We have also confirmed by electron-energy-loss spectroscopy that the metal core contains only palladium but not iron (data not shown).

The catalytic hydrogenation of olefins by Pd·apo-ferritin was evaluated in aqueous medium. The turnover frequencies

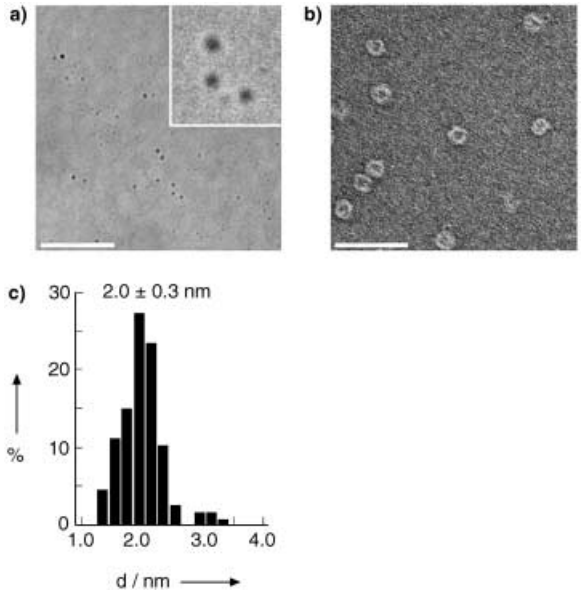


Figure 3. TEM images of the Pd·apo-ferritin: a) An ice-embedded unstained sample and 4× image magnification (inset); b) a sample negatively stained with uranyl acetate; c) histogram of the cluster size. The temperature of the specimen stage was 77 K. The electron microscopes were operated at acceleration potentials of 200 kV for (a) and 300 kV for (b). Scale bars are 50 nm.

(TOF = [product (mol)] per atom of Pd per hour) of hydrogenation for acrylamide derivatives are listed in Table 1. As estimated by atomic-absorption and protein quantitative analyses each apo-ferritin contains 460 atoms of Pd^0, thus, the TOF of 72 for **1** implies a TOF of 33000 per Pd·apo-ferritin molecule. The products of these hydrogenation reactions were confirmed by NMR spectroscopy in D_2O solutions. According to the reduction of each substrate by H_2 in the presence of Pd·apo-ferritin, the signals in the region of vinylic protons (5–6 ppm) decreased and concomitant appearance of new signals assignable to the hydrogenation of the C–C double bond in the aliphatic region (1–2 ppm) were observed. These results indicate that the hydrogenation by Pd·apo-ferritin proceeds without forming any other products. After the catalytic reactions, the solution of Pd·apo-ferritin remained clear and the native PAGE results showed no aggregation of the composites during the reactions. Thus, Pd·apo-ferritin is stable before, during, and after the catalytic reaction. Pd·apo-ferritin also catalyzes the hydrogenation at reasonable rates, for example, the TOF of Pd·apo-ferritin for the *N*-isopropyl acrylamide reduction is almost comparable to that of a dendrimer-encapsulated Pd catalyst in water.[18] On the other hand, the TOF for acrylic acid (**2**) catalyzed by Pd·apo-ferritin was eight times smaller than that of **3** though TOF of **2** in the absence of apo-ferritin is almost identical to that of **3** (Table 1). When the threefold channels in Pd·apo-ferritin (Figure 1c) were blocked with Tb^{III},[17,19,20] the TOF of **1** was decreased to 7% of the TOF of **1** by Pd·apo-ferritin without Tb^{III}. Previous diffusion studies have also shown that 4-amino-TEMPO (TEMPO is 2,2,6,6-tetramethyl piperidine-*N*-oxyl) could penetrate through the channels.[17] These results, therefore, suggestive that the anionic threefold hydrophilic channels (Figure 1c) are the pathway for substrates. In comparison with neutral substrates, the TOFs of **5** and **6** by Pd·apo-ferritin are smaller than those of **1**, **3**, and **4** although the electron-deficient alkenes **1**, **5**, and **6** are expected to be reduced faster than **3** and **4** if the electronic substituent effect on the hydrogenation is considered. Actually, the order of TOFs by Pd particle without apo-ferritin was **1** > **5** > **6** > **3** > **4** (Table 1). Molecular-modeling calculations of the substrates indicate that the volumes of the substrates are 54, 97, 110, 106, and 120 $Å^3$ for **1**, **3**, **4**, **5**, and **6** respectively.[21] The order of the substrate size roughly correlates to the experimental data for the hydrogenation by Pd·apo-ferritin (Table 1). Thus, the substrate size is discriminated by the threefold channels. While we have attempted chiral discrimination in the hydrogenation of racemic **6** at 15 °C, no enantioselectivity in the product was observed.

Table 1: Hydrogenation activity of Pd·apo-ferritin in water.

Olefins	TOF for Pd·apo-ferritin[a],[b]	TOF for Pd particles[b],[c]
CH_2=CHCONH$_2$ (**1**)	72 ± 0.7	58 ± 5.9
CH_2=CHCOOH (**2**)	6.3 ± 1.1	12 ± 2.6
CH_2=CHCONH-*i*Pr (**3**)	51 ± 6.5	15 ± 0.3
CH_2=CHCONH-*t*Bu (**4**)	31 ± 5.9	6.1 ± 0.6
CH_2=CHCO-Gly-OMe (**5**)	6.3 ± 3.8	28 ± 2.6
CH_2=CHCO-D,L-Ala-OMe (**6**)	Not detected	23 ± 0.3

[a] Hydrogenation reactions catalyzed by Pd·apo-ferritin were carried out at 7 °C (pH 7.5) with 30 μM of Pd. [b] TOFs were calculated on the basis of the ratio of the product to substrate by analyzing the ^{1}H NMR spectra. [c] The same conditions were used except without apo-ferritin.

In conclusion, we have demonstrated in situ synthesis of a Pd nanocluster in the protein cage and size-selective olefin hydrogenation by the Pd·apo-ferritin. The results provide us with a method for the preparation of metal nanoclusters in restricted interiors of a protein assembly. Further design of the ferritin cage and channels to improve the reactivity is currently under progress.

Experimental Section

Horse spleen ferritin was obtained as an 85 mg mL^{-1} 0.15 M NaCl solution (Sigma). Substrates **4** and **5** were synthesized as reported.[22]

Preparation of Pd·apo-ferritin**:** Horse spleen ferritin was demineralized with thioglycolic acid to prepare apo-ferritin by a reported procedure.[23] The protein concentration was determined by using the reported extinction coefficient at 280 nm.[24] An apo-ferritin solution (9.3 mg, 0.02 μmol in 0.15 M NaCl/H_2O) was adjusted to pH 8.5 with 0.01 N NaOH, and aliquots of K_2PdCl_4 (40 mM, 250 μL in H_2O) were added and this solution was stirred for 30 min at room temperature. To the resulting solution, a solution of $NaBH_4$ (50 μmol in H_2O) was added and the mixture was stirred for 30 min at room temperature. The reaction mixture was dialyzed against 0.15 M NaCl/H_2O for 12 h. The protein was isolated by gel chromatography (Superdex 200, eluent: 0.15 M NaCl aq) and the purity was checked by native PAGE. The proportion of Pd atoms to protein was determined by atomic absorption analysis and Bicichonianate (BCA) method.

Hydrogenation reactions: Hydrogenation reactions were carried out in a 10 mL reaction tube sealed with a septam. A D_2O solution

containing the substrate (6 μmol, 1 mL) was added to the reaction tube and purged with H_2 gas for 15 min. The catalytic reaction was initiated by adding a Pd·apo-ferritin solution (1×10^{-4} μmol, 1 mL) and stirred for 1 h under an H_2 atmosphere with a flow rate of 50 mL min^{-1} at 7 °C. After the removal of Pd·apo-ferritin from the resulting solution, the products were identified by ^{1}H NMR spectroscopy. Pd particles for the blank experiments (Table 1) were prepared by the reaction of K_2PdCl_4 and $NaBH_4$ in D_2O. The mixtures were used for the hydrogenation reactions without further purification. The enantiomeric excess for the reaction of **6** at 15 °C was determined by HPLC analysis by using a DAICEL CHIRALCEL OD column (0.46 cm × 25 cm) at a flow rate of 0.5 mL min^{-1} (isopropanol/hexane = 5:95).

Transmission electron microscopy: A solution of Pd·apo-ferritin (2 μL) was applied to a copper grid covered with a thin amorphous carbon film, and the excess solution was removed with a filter paper. For the cryo observation, a sample grid was rapidly plunged into liquid propane (−190 °C). The images of the ice-embedded and the negatively stained samples were recorded on a JEM-2010HC microscope (200 kV) equipped with a CT-3500 cryoholder and a JEM-4010N microscope (300 kV), respectively.

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