Bio-template Synthesis of Uniform CdSe Nanoparticles Using Cage-shaped Protein, Apoferritin

Ichiro Yamashita,*[†,††] Junko Hayashi,[†††] and Masahiko Hara[†††]
[†]*Advanced Technology Research Laboratories, Matsushita Electric Industrial Co., Ltd., Hikaridai, Seika, Kyoto 619-0237*
[††]*CREST, Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi 332-0012*
[†††]*Local Spatio-Temporal Functions Laboratory, Frontier Research System, RIKEN (The Institute of Physical and Chemical Research), Wako, 351-0198*

(Received May 10, 2004; CL-040519)

CdSe nanoparticles were synthesized in the cavity of the cage-shaped protein, apoferritin, by designing the slow chemical reaction system of Cd^{2+} and Se^{2-}. The cavity size of apoferritin molecules is around 7 nm and the diameter of synthesized CdSe nanoparticles is about 6 nm with small size dispersion. CdSe nanoparticles as prepared are multi-crystalline and can be made into a single crystal by the heat-treatment under nitrogen gas. This bio-template synthesis provides a new method to synthesize uniform CdSe nanoparticles.

Miniaturization of electronic device components to nano-scale dimensions is one of the most intensively studied research areas in nanotechnology. When the size of electronic device components becomes several nanometers, classical operation principles no longer apply and devices will be operated based on quantum effects. The quantum dot is expected to be a key component of such nano-scale electronic devices. Since the electronic energy levels of quantum dots are radically affected by their sizes, there is a large demand for reproducible methods for making and handling[1] the same size quantum dots. To achieve this challenging target, we employ the cage-shaped protein, apoferritin, as a size-restraining chemical reaction chamber for synthesizing uniform semiconductor nanoparticles (NPs). There is a literary reporting cadmium sulfide (CdS) NPs synthesis in the apoferritin cavity by incremental addition of Cd^{2+} and S^{2-}. Those CdS NPs did not fully develop in the cavity and the obtained CdS NPs had irregular morphologies.[2] In this letter, we demonstrate that another II–VI compound semiconductor, cadmium selenide (CdSe) NPs can be synthesized in the apoferritin cavity by applying a newly designed chemical reaction system and that the CdSe NPs developed fully in the apoferritin cavity.

Apoferritin, the major cellular iron-storage protein, composed of 24 polypeptide subunits, has a spherical hollow shell and ferro-oxidase centre in its subunits.[3–5] The inner and outer diameters of the protein shell are about 7 and 12 nm, respectively and there are atomically small channels along the three fold axis through which ions can enter the cavity. Native apoferritin sequesters and stores iron as hydrated iron oxide in the internal cavity, in vivo, which can easily be reproduced in vitro. Several kinds of metal complex cores were reported to be formed in the apoferritin cavity by selecting the appropriate buffer conditions.[6–13] It is predicted that the negative electrostatic potential inside the protein shell produced by acidic amino-acid residues at the cavity surface condenses the positively charged metal ion in the cavity and thus makes the chemical reaction proceed faster. However, the mechanism of core formation in the cavity and biomineralization is not well understood.

The reaction between Cd^{2+} and Se^{2-} is very fast and induces CdSe aggregation quickly in aqueous solution. Therefore, we first designed the slow chemical reaction so that these ions can penetrate into the apoferritin cavity through the narrow channels. Cd^{2+} was stabilized by ammonia solution in order to form positively charged tetraaminecadmium ions ($Cd(NH_3)_4^{2+}$). Se^{2-} was designed to be supplied from selenourea. Selenourea is comparatively unstable in aqueous solution and slowly degrades to release Se^{2-} into the reaction solution. The reaction mixture solution of 1 mM cadmium acetate and 5 mM selenourea was prepared and its pH was adjusted to be around 8.0 by adding ammonia solution. To this reaction mixture solution, apoferritin molecules were added with the final concentration of 0.5 mg/mL. This reaction mixture produced CdSe very slowly as designed. The solution color changed from thin yellow to clear red gradually, which made it easy to trace the reaction progress visibly. The reaction mixture was left overnight and all processes were carried out at room temperature. There was little precipitation, which indicated CdSe NPs were synthesized in the apoferritin cavity and the protein shells made them dispersed. This core-shell structure makes it easy to handle NPs in aqueous solution.

In order to confirm the existence of Cd and Se atoms in the core, X-ray photoemission spectroscopy (XPS) measurements of the synthesized CdSe-ferritin molecules were carried out. Two main peaks at 413.5 eV for Cd 3d and 55.4 eV for Se 3d were observed, while the C 1s and O 1s peaks from the ferritin shell are additionally observed at 286.8 and 532.6 eV, respectively. These XP spectra revealed that the cores are composed of Cd and Se composite.

To investigate the structure of the synthesized cores, X-ray diffraction (XRD) measurement was carried out. After the shell proteins were stripped off by alkaline solution, the obtained cores were intensely washed with distilled water and dried. As XRD peaks were supposed to be broad because of the nanometer order core size, some cores were heat-treated under nitrogen gas expecting the sintering of cores and sharp XRD peaks. Figure 1 shows the typical XRD peaks before and after the heat-treatments. After heat-treatment at 500 °C, a set of fairly sharp XRD peaks was observed and the core structure was determined to be a cubic phase (zinc blend) with a very little hexagonal phase (wurtzite).[14] XRD peaks from as-prepared cores were very broad but the peak positions could be estimated, which were different from those of heat-treated cores. Considering together with the XRD result from the heat-treated cores at 150 °C, the mixture of a hexagonal and a cubic phase is the reasonable estimation for the structure of the cores as prepared. The CdSe crys-

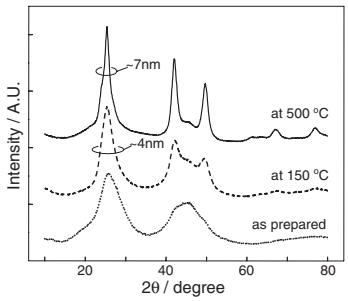

Figure 1. X-ray diffraction from the prepared cores before and after heat-treatment.

tal domain sizes of the cores after heat-treatments at 150 and 500 °C were calculated based on the XRD peak width and they were 4 and 7 nm, respectively.[14] These results indicate that the CdSe crystal domain grew nearly the same size with the core after heat-treatment at 500 °C and that cores became single crystals. The reason why the sintering did not occur is not clear. One possibility is that the outermost surface of the core is covered by a very thin oxide-layer and this crust may hinder fusion of cores.

To further characterize the CdSe cores, the synthesized CdSe-ferritin molecule was observed by transmission electron microscopy (TEM) with and without aurothioglucose staining. It has been demonstrated that aurothioglucose does not pass through the channels and does not stain the cavity.[12] Typical TEM images are shown in Figure 2. The negatively stained TEM image shows the synthesized cores surrounded by ferritin protein shells, while the TEM image without staining shows only spherically shaped cores on a smoother background. These images clearly show that CdSe were synthesized only in the apoferritin cavity, which explains the very small bulk precipitation of reaction mixture after overnight. Almost all apoferritin accommodated CdSe NPs. As shown in the inset of Figure 2b, the single-crystal lattice structures can be observed in some CdSe NPs, however, cores showed usually twin- or multi-crystal structures at higher magnification. This is consistent with the XRD measurement result.

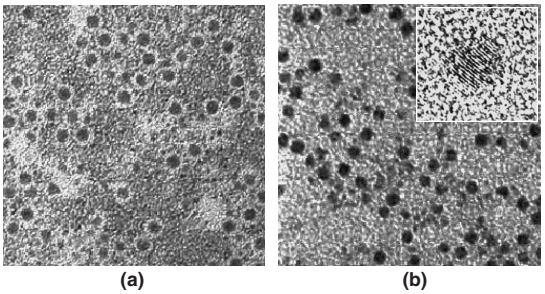

Figure 2. TEM images of synthesized CdSe-ferritin observed with aurothioglucose staining (a) and without staining (b).

The diameters of 509 CdSe cores were determined from the unstained TEM images of Figure 2b to investigate the core size distribution. Figure 3 shows the distribution of their diameters. The distribution is quite narrow with a standard deviation of 0.59 nm and an average diameter of about 6 nm. As the average diameter is nearly the same as the inner diameter of apoferritin, it can be concluded that CdSe synthesis continued until the cavity

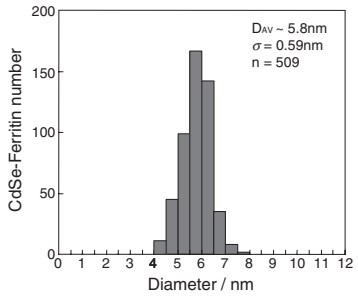

Figure 3. Diameter distribution of synthesized CdSe nanoparticles.

was completely filled. This result demonstrates that CdSe NPs with small-disperse diameter can be synthesized using apoferritn molecules as bio-templates.

In conclusion, uniform CdSe NPs were synthesized in the apoferritin cavity by designing the slow chemical reaction system of Cd^{2+} and Se^{2-}. This chemical reaction system can be applicable for synthesis of another II–VI compound semiconductor such as ZnSe NPs in the apoferritn cavity. This bio-template synthesis will open a new path to the production of uniform II–IV semiconductor nanoparticles in aqueous solution, which are applicable to biology and nanoelectronics devices.

We thank members of our laboratory, particularly Dr. K. Iwahori for experimental support, Dr. M. Hashimoto for valuable discussion about CdSe chemical reaction system design, Ms. R. Tsukamoto for technical assistance, and Dr. M. Muraoka for the theoretical discussion on biomineralization mechanism.

References and Notes

1 D. Ishii, K. Kinbara, Y. Ishida, N. Ishii, M. Okochi, M. Yohda, and T. Aida, *Nature*, **423**, 628 (2003).
2 K. K. W. Wong and S. Mann, *Adv. Mater.*, **8**, 928 (1996).
3 W. H. Massover, *Micron*, **24**, 389 (1993).
4 P. M. Harrison, S. C. Andrews, P. J. Artymuik, G. C. Ford, J. R. Guest, J. Hirzmann, D. M. Lawson, J. C. Livingstone, J. M. A. Smith, A. Treffry, and S. J. Yewdall, *Adv. Inorg. Chem.*, **36**, 449 (1991).
5 D. M. Lawson, P. J. Artymiuk, S. J. Yewdall, J. M. A. Smith, J. C. Livingstone, A. Treffry, A. Luzzago, S. Levi, P. Arosio, G. Cesareni, C. D. Thomas, W. V. Shaw, and P. M. Harrison, *Nature*, **349**, 541 (1991).
6 S. Mann and F. C. Meldrum, *Adv. Mater.*, **3**, 316 (1991).
7 F. C. Meldrum, V. J. Wade, D. L. Nimmo, B. R. Heywood, and S. Mann, *Nature*, **349**, 684 (1991).
8 F. C. Meldrum, B. R. Heywood, and S. Mann, *Science*, **257**, 522 (1992).
9 F. C. Meldrum, T. Douglas, S. Lei, P. Arosio, and S. Mann, *J. Inorg. Biochem.*, **58**, 59 (1995).
10 T. Douglas, D. P. E. Dickson, S. Betteridge, J. Charnock, C. D. Garner, and S. Mann, *Science*, **269**, 54 (1995).
11 T. Douglas and V. T. Stark, *Inorg. Chem.*, **39**, 1828 (2000).
12 M. Okuda, K. Iwahori, I. Yamashita, and H. Yoshimura, *Biotechnol. Bioeng.*, **84**, 187 (2003).
13 T. Ueno, M. Suzuki, T. Goto, T. Matsumoto, K. Nagayama, and Y. Watanabe, *Angew. Chem., Int. Ed.*, **43**, 2527 (2004).
14 R. J. Bandaranayake, G. W. Wen, J. Y. Lin, H. X. Jiang, and C. M. Sorensen, *Appl. Phys. Lett.*, **67**, 831 (1995).

Published on the web (Advance View) August 7, 2004; DOI 10.1246/cl.2004.1158