Fabrication of Gold Sulfide Nanoparticles Using the Protein Cage of Apoferritin

Keiko Yoshizawa, Kenji Iwahori, Kenji Sugimoto, and Ichiro Yamashita*
CREST, Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi 332-0012
Graduate School of Material Science, Nara Institute of Science and Technology, 8916-5 Takayama-cho, Ikoma 630-0192

(Received July 4, 2006; CL-060755; E-mail: ichiro@ms.naist.jp)

Au_2S nanoparticles (NPs) were prepared in the cavity of the cage-shaped protein, apoferritin. Apoferritin has a cavity, 7 nm in diameter, and the diameter of fabricated Au_2S NPs is about the same size with the cavity and size dispersion was small. Although Au_2S is generally insoluble in water, our prepared Au_2S NPs can be dissolved over 100 mg/mL in water due to the water-soluble protein shell. This solubility will make it possible to place and construct nanostructures made of Au_2S NPs.

Semiconductor NPs exhibit intriguing properties and are a powerful tool for the development and fabrication of materials with novel functions. For examples, CdS, CdSe, and ZnS NPs are used as a fluorescent marker because of their dramatic size-dependent alteration in optical absorption and emission spectra.[1] Ag_2S NPs have been used as a photosensitizer for photographic purposes.[2] Au_2S, p-type semiconductor,[3] is also used to sensitize silver halide crystals to enhance the photographic process.[4] If these kinds of semiconductor NPs can be placed at designed positions in electric devices, they will be functioned as quantum dots with specific characteristics of each material.

We have been trying to prepare homogenous semiconductor NPs in the cage-shaped protein, apoferritin. Apoferritin is a spherical protein with a diameter of 12 nm and its cavity is 7 nm in diameter, which is surrounded by 24 polypeptide subunits.[5] There are narrow channels connecting outside and cavity. Several kinds of semiconductor NPs were already reported to be formed in the apoferritin cavity, such as CdS,[6] CdSe,[7] and ZnSe.[8] This novel method to use apoferritin cavity as a specially restricted chemical chamber can produce the water-soluble NPs with the same size. In this study we report the fabrication of Au_2S NPs in the apoferritin cavity by designing the chemical reaction system and optimizing preparation conditions.

There are a few publications concerning the preparation of Au_2S NPs and Au_2S NPs are strong tendency to aggregate in water.[9,10] Therefore, we first surveyed the proper chemical agents which are unstable but change slowly to provide ion source for Au_2S. We have selected $KAuCl_4$ as a gold source and thiourea as a sulfur source. Previous studies reported that mixing Au^{III} ion and thiourea under acidic condition produce a Au^{I}–thiourea complex ($Au[CS(NH_2)_2]_2^+$).[11] Au^{I}–thiourea complex change slowly into AuS^- or Au_2S depending on a pH value and redox potential.[12]

We mixed Au^{I}–thiourea complex and apoferritin with ratio of 3000:1 in pH from 6 to 9, and they were incubated at room temperature overnight. After incubation, the reaction solution colored a yellowish-brown. Aggregates of ferritins and Au_2S produced during the Au_2S formation were removed by centrifuging at 15,000 rpm for 10 min. Proteins in the supernatants were quantified by Bradford-assay to estimate the loss of proteins and the protein ratio, [protein in the supernatant]/[initial apoferritin], was calculated as Yield of Protein Ratio (YPR). Furthermore, Au_2S-incorporated ferritins in the supernatants were observed by TEM to measure Core Formation Ratio (CFR), which is represented as

$$\mathrm{CFR}\ (\%) = [\mathrm{Au_2S\text{-}fer}]/[\mathrm{fer}]_{\mathrm{total}}, \quad (1)$$

where, $[Au_2S\text{-fer}]$ is the number of Au_2S-incorporated ferritins and $[fer]_{total}$ is the total number of apoferritin and $[Au_2S\text{-fer}]$. Au_2S-incorporated ferritins were distinguished by negative staining with aurothioglucose, which does not stain the cavity.[13] The ferritin protein shell and the core were observed as white ring and black dot with diameters of 12 and 6 nm, respectively (Figure 1A). In all conditions, some apoferritins accommodated almost the same size core, and others remained empty. The CFR was the highest in TAPS buffer at pH 8.0, while it was lowest at pH 6 (Figure 1B). This result is consistent with the previous study, which showed that the high pH value is favorable for the formation of Au_2S.[12] However, the condition higher than pH 9 caused loss of ferritin proteins in the supernatant (Figure 1B). Because the Au_2S formation in the solution intensely occurred, a large amount of Au_2S formations outside apoferritin involve in precipitation of the aggregates between ferritin proteins and Au_2S, resulting in the loss of proteins in the supernatant. While the Au_2S formation can be repressed outside the apoferritin and accelerated inside the apoferritin by adjusting in mild pH value (pH = 8.0) with TAPS buffer; therefore, this condition was optimum for preparing the Au_2S incorporated ferritin.

In order to characterize the Au_2S–ferritin in detail, we prepared several hundred mg of Au_2S–ferritin in TAPS buffer

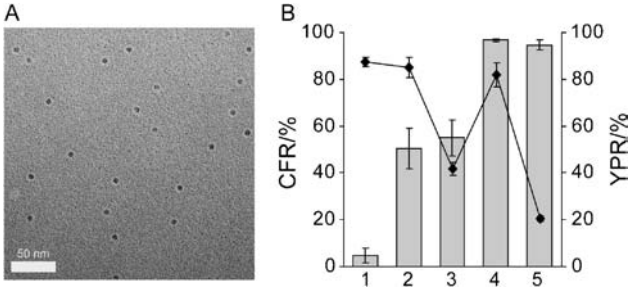

Figure 1. TEM image of Au_2S-incorporated ferritin prepared under 50 mM TAPS buffer (pH 8) and stained by aurothioglucose (A), and CFR (gray bar) and YPR (filled square) under various pH values (B). YPR × CFR means yield of Au_2S-incorporated ferritin. **1**. 50 mM phosphate buffer pH 6; **2**. 50 mM phosphate buffer pH 7; **3**. 50 mM phosphate buffer pH 8; **4**. 50 mM TAPS buffer pH 8; **5**. 50 mM TAPS buffer pH 9.

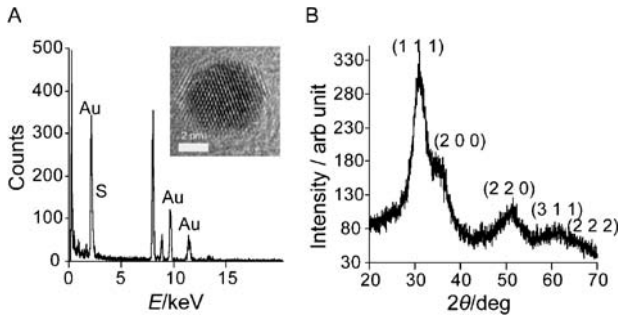

Figure 2. EDX and HRTEM measurement for nonstained Au_2S-incorporated ferritin (A) and XRD patterns of Au_2S-incorporated ferritin (B).

at pH 8.0 and then purified by gel filtration chromatography. Previous study reported that Au_2S is insoluble in water,[14] but our prepared Au_2S NPs were considerably soluble in water owing to the protection of Au_2S NPs by ferritin shells. It can be dissolved over 100 mg/mL in water. We performed high resolution TEM (HRTEM) and EDX measurement for nonstained sample (Figure 2A). The core size was 6.1 ± 0.4 nm in diameter, and the clear lattice fringe was visible (Figure 2A inset). The results of EDX spectrum showed gold peaks (2.1, 9.7, and 11.4 keV) and a sulfur peak of Kα (2.4 keV) (Figure 2A). The two peaks at 8.0 and 8.9 keV were assigned to copper from the supporting grid. To investigate further the lattice structure of the produced cores, X-ray diffraction (XRD) measurement was carried out. Peaks are broad because of the nanometric particle size but peaks are discernable (Figure 2B). All XRD peaks are attributed to Au_2S. No intense (111) diffraction peak from Au NP was observed. From these results, it can be concluded that the Au_2S NPs were produced in the apoferritin cavity.

The absorption spectra of the Au_2S-incorporated ferritin is shown in Figure 3. The broad peak (600–1000 nm) observed for the Au_2S-incorporated ferritin should be attributed to Au_2S NPs incorporated in the cavity of ferritin. The approximate absorption edge of the Au_2S NPs was measured, indicating occurrence of quantum size effect in the Au_2S NPs. The band gap can be estimated by following function.[15]

$$(\alpha h\nu)^2 = C(h\nu - E), \quad (2)$$

where α is the absorption coefficient of the materials, ν is the frequency of the light, and E is the band gap. Then, the absorption spectrum gave the direct band gap estimated to be 1.8 eV. Averitt et al. reported that a theoretical direct band gap of Au_2S is in the range from 1.3 to 2.6 eV,[16] and the middle-energy region of the calculation is consistent with our experimental band gap.

The cause of preferable accumulation of Au_2S inside the cavity is unclear. The electrostatic gradients on the ferritin molecules are so designed to attract positive ions to the hydrophilic channels, which is considered to introduce ferric ions into the cavity efficiently in vivo.[17] $Au[CS(NH_2)_2]_2^+$ ions may be attracted in the vicinity of the entrance of the channels. $Au[CS(NH_2)_2]_2^+$ ions, then, change into AuS^- or Au_2S[12] and Au_2S somehow go through the channel and form Au_2S core in the cavity. However, the data so far obtained are limited and further study is indispensably necessary to understand the mechanism fully.

In conclusion, we have succeeded in making the Au_2S NPs

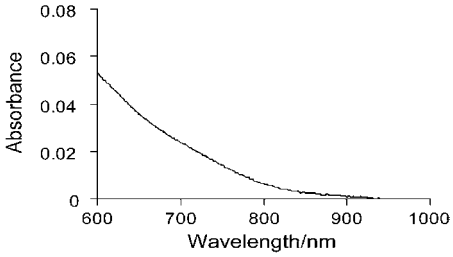

Figure 3. Absorption spectrum of Au_2S-incorporated ferritin measured at room temperature.

using cage-shaped protein, apoferritin. Although Au_2S is generally insoluble in water, our prepared Au_2S NP is considerably soluble in water owing to the use of water-soluble protein shell. As the application of the Au_2S-incorporated ferritin, the construction of nanoelectronic device keycomponents is expected. Our group has been investigating the fabrication of floating nanodot gate memory equipped with nanodots array made by ferritin protein.[18,19] Charge storage nodes of floating gate memory were fabricated by utilizing ferritin with cobalt oxide and iron oxide core. The successful formation of Au_2S NP in the apoferritin cavity will make it possible to produce a multivalue-memory by combining the Au_2S-incorporated ferritin with the other semiconductor or metal-incorporated ferritin and, furthermore, extends the potential to produce more complex, sophisticated functional structures.

We thank H. Furusho for the HRTEM observation and EDX measurement and S. Katao for the XRD measurement.

References and Notes

1 X. Peng, J. Wickman, A. P. Alvisatos, *J. Am. Chem. Soc.* **1998**, *120*, 5343.
2 G. L. De Rycke, F. Henderickx, Eur. Pat. Appl. EP 892026 13.9, **1990**.
3 K. Ishikawa, T. Isonaga, S. Wakita, Y. Suzuki, *Solid State Ionics* **1995**, *79*, 60.
4 T. Tani, *Photographic Sensitivity*, Oxford University Press, Oxford, U.K., **1995**.
5 P. M. Harrison, P. Arosio, *Biochim. Biophys. Acta* **1996**, *1275*, 161.
6 K. Kim, W. Wong, S. Mann, *Adv. Mater.* **1996**, *8*, 928.
7 I. Yamashita, J. Hayashi, M. Hara, *Chem. Lett.* **2004**, *33*, 1158.
8 K. Iwahori, K. Yoshizawa, M. Muraoka, I. Yamashita, *Inorg. Chem.* **2005**, *44*, 6393.
9 T. Morris, H. Copeland, G. Szulczewski, *Langmuir* **2002**, *18*, 535.
10 H. S. Zhou, I. Honma, H. komiyama, *Phys. Rev. B* **1994**, *50*, 12052.
11 Z. Wang, C. J. Medforth, J. A. Shelnutt, *J. Am. Chem. Soc.* **2004**, *126*, 16720.
12 V. Gasper, A. S. Mejerovich, M. A. Meretukov, J. Schmiedl, *Hydrometallurgy* **1994**, *34*, 369.
13 M. Okuda, K. Iwahori, I. Yamashita, H. Yoshimura, *Biotechnol. Bioeng.* **2003**, *84*, 187.
14 S. Licht, *J. Electrochem. Soc.* **1988**, *135*, 2971.
15 W. W. Scanlon, *Phys. Rev.* **1958**, *109*, 47.
16 R. D. Averitt, D. Sarkar, N. J. Halas, *Phys. Rev. Lett.* **1997**, *78*, 4217.
17 B. Wiedenheft, J. Mosolf, D. Willits, M. Yeager, K. A. Dryden, M. Young, T. Douglas, *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 10551.
18 T. Hikono, T. Matsumura, A. Miura, Y. Uraoaka, T. Fuyuki, M. Takeguchi, S. Yoshii, I. Yamashita, *Appl. Phys. Lett.* **2006**, *88*, 023108.
19 A. Miura, T. Hikono, T. Matsumura, H. Yano, T. Hatayama, Y. Uraoka, T. Fuyuki, S. Yoshii, I. Yamashita, *Jpn. J. Appl. Phys.* **2006**, *45*, L1.

Published on the web (Advance View) October 5, 2006; doi:10.1246/cl.2006.1192