Fabrication of CdS nanoparticles in the bio-template, apoferritin cavity by a slow chemical reaction system

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Abstract. Cadmium sulfide (CdS) nanoparticle (NP) was synthesized in the apoferritin cavity by slow chemical reaction system (SCRY) and two step synthesis protocol (TSSP). The survey showed thioacetic acid as the optimum sulfur source to form CdS NPs in the apoferritin cavity. Thioacetic acid degrades slowly in weak acidic solution and releases the sulfide ions continuously for long period. The synthesized NP was determined as CdS in cubic phase by energy dispersive X-ray spectroscopy (EDS) and X-ray diffraction measurement (XRD). Semiconductor CdS NP surrounded by protein shell can disperse in aqueous solution and will be able to be applied to nano-electronic devices or fluorescent biomarker.

1. Introduction

Nanoparticle (NP) is getting more and more important as the indispensable material for nanotechnology applications. Many methods to fabricate nanoparticles (NPs) have been reported so far. Among them, bio-template method, which employs the cavity of the cage shaped protein as a spatially restricted chemical NP synthesis chamber, is one of the most suitable methods for making size defined NPs, because the inner space of protein cage is exactly the same size. The characteristic of the same size is an ideal property as a component of nano-electric devices, because the electron energy levels of NP are greatly affected by their sizes. There were reports of artificial synthesis of metal oxide, metal alloy, or inorganic NPs using the ten-nanometer-size cage shaped protein, apoferritin, as bio-template [1-3]. Apoferritin has a spherical hollow structure. The inner and outer diameter of protein shell are 7 and 12 nm respectively, and apoferritin stores iron as a hydrated iron oxide core in the inner space in vivo. However, there is a few reports of the synthesis of semiconductor NPs using apoferritin. We reported in the previous work that the synthesis of cadmium selenide (CdSe) NP and zinc selenide (ZnSe) NP was successfully achieved by designing a new method composed of slow chemical reaction system (SCRY) and two step synthesis protocol (TSSP), which employs tetraamminecadmium ion or tetraamminezinc ion and selenourea. [4-6]. The important essences of this method are (1) stabilization of Cd or Zn ions by forming tetraammine-complex ion
with excess ammonium ions and (2) slow and long supply of Se ions by slow-decomposition of selenourea in the aqueous solution. In this study, we report the modification of the SCRY and TSSP, especially selection of the optimum sulfide ion source, to achieve the synthesis of CdS NP in the apoferritin cavity. The obtained conditions made it possible to synthesize CdS semiconductor NPs in the apoferritin cavity and control their sizes.

2. Experimental procedure
The horse spleen apoferritin (HsAFr) was purchased from Sigma. In the study of the sulfide ion concentration in reaction solutions, a 3 mL of solution containing 40 mM ammonium acetate, 7.5 mM ammonia water, 0.3 mg/mL HsAFr, 1 mM cadmium acetate, and one of thiourea, thiosulfate or thioacetic acid with the concentration up to 50 mM was left at room temperature for 24 h, sulfide ions in the solution were measured by the methylene blue method [7] after centrifugation at 12,000 rpm for 5 min.

For the effect of thioacetic acid on the core formation ratio (CFR), a 3 mL of solution containing 40 mM ammonium acetate, 7.5 mM ammonia water, 0.3 mg/mL HsAFr, 1 mM cadmium acetate, and thioacetic acid with the concentration up to 50 mM was left at room temperature for 24 h. CdS NPs were synthesized in the reaction solution (pH 6.5) containing 40 mM ammonium acetate, 7.5 mM ammonia water, 0.3 mg/mL HsAFr, 1 mM cadmium acetate, and 1 mM thioacetic acid. The reaction solution was centrifuged at 12000 rpm for 5 min and the supernatant was carefully retrieved. Several micro liters of the supernatant was put on the carbon film coated TEM mesh, and excessive solution was removed. The samples were stained by 1% aurothioglucose and observed by TEM (JEM-2200FS, JEOL, JAPAN). The EDS analysis of synthesized CdS NPs was performed by unstained sample using TEM (JEM-3100FEF, JEOL, JAPAN). The XRD analysis was performed as reported in the literature [6].

3. Results and discussions
Our previous experiments which employed the SCRY and TSSP methods showed that CdSe or ZnSe NPs synthesis in the apoferritin cavity took several hours [5,6]. Based on this result, we assumed that the CdS NPs synthesis in the apoferritin cavity also takes several hours. Therefore, we searched an optimum S source for the one-pod synthesis, which supplies sulfide ions for a long period. Solution containing 40 mM ammonium acetate, 0.3 mg/mL apoferritin, 7.5 mM ammonia water, 1 mM cadmium acetate, and one of thiourea, thiosulfate or thioacetic acid with the concentration up to 50 mM was prepared and after 24 h incubation, sulfide ion concentration was measured by methylene blue method [7]. If there are sulfide ions in the solution after 24 h, the source has the potential to supply sulfide ions for a long period. The difference from the solution of the previous CdSe synthesis

![Figure 1](image_url) Figure 1 Sulfide ions concentration and the pH of the reaction solution including different sulfide ion sources after 24 h from the solution preparation. The sulfide ions were detected by methylene blue method from thiourea (A), thiosulfate (B) and thioacetic acid (C). White circles show the reaction solution pH and black circles show the amount of sulfide ions in the reaction solution.
was the replacement of Se ion source by sulfide ion sources. The reaction solutions were mixed by magnetic stirrer bar and pH was not controlled at all. Figure 1 shows the sulfide ions concentration in the reaction solution containing thiourea (A) thiosulfate (B) and thioacetic acid (C), and pH of the solution after 24 h incubation. In the case of thiourea and thiosulfate, sulfide ions were not detected in any concentration. The pH range of the solutions was between 7 and 8. It is known that thiourea and thiosulfate are stable at this pH range and hardly decompose. In other word, thiourea and thiosulfate did not decompose and there had been no sulfide ions in the solution. This was consistent with the no color change of solution for 24 h incubation, which indicated that CdS NPs did not form in neither in the reaction solutions nor the apoferritin cavities because of the lack of sulfide ions. No CdS NPs synthesis in the apoferritin was also confirmed by TEM observation.

On the other hand, in the case of 1 mM to 20 mM thioacetic acid, there were 0.1 μM to 6 μM sulfide ions in the reaction solution even 24 h. This is explained as follows. Thioacetic acid was supposed to have decomposed slowly by the hydrolysis and supply H2S for 24 h following the equation (1).

\[ \text{CH}_3\text{COSH} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{COOH} + \text{H}_2\text{S} \]  

The produced hydrogen sulfide from the thioacetic acid further decompose into HS− and finally S− as shown in the equation (2,3).

\[ \text{H}_2\text{S} \rightarrow \text{HS}^- + \text{H}^+ \quad \text{pKa}_1 = 7.0 \]  
\[ \text{HS}^- \rightarrow \text{S}^- + \text{H}^+ \quad \text{pKa}_2 = 13.9 \]

From 0.2 mM to 1 mM, the pH of reaction solution was at neutral pH. Thioacetic acid was degraded slowly to H2S and then to sulfide ions. Therefore, sulfide ions increased in proportion to thioacetic acid concentration. From 1 to 10 mM, the same degradation behavior continued and sulfide ions increased. The solution pH started decreasing and the degradation of H2S was suppressed more in lower pH. Even in this condition, the thioacetic acid concentration was high enough to supply the sulfide ions. In the case of more than 20 mM concentration, however, the reaction solution pH decreased below 3 and thioacetic acid was degraded to H2S but was hard to be further degraded to sulfide ions. Since H2S volatilizes and vanishes easily, sulfide ions were not detected in these solutions at low pH.

**Figure 2.** The effect of the concentration of thioacetic acid on the CdS core formation ratio (CFR) in the pH 6.5 reaction solution. The CFR was calculated by dividing the number of core-containing apoferritin by the number of all apoferritins in TEM images.
As mentioned above, the release period of sulfide ions was affected greatly by the reaction solution pH. Therefore, we fixed the pH of the reaction solutions at 6.5, where S ions are supplied slowly for more than 24 h and moreover, the amount of S ions supply per time can be controlled by the initial thioacetic acid concentration. We synthesized CdS NPs in the 40 mM ammonium acetate, 0.3 mg/mL apoferritin, 7.5 mM ammonia water, 1 mM cadmium acetate and 0 - 50 mM thioacetic acid, pH of which was adjusted at pH 6.5 by the addition of acetic acid or sodium hydrogen oxide. The core formation ratio (CFR) was measured to study the efficient concentration of thioacetic acid for CdS NPs synthesis. The pH change throughout the 24 h reaction was experimentally shown less than 0.3 in all concentration.

Figure 2 shows the effect of the thioacetic acid concentration on CFR of CdS NPs. When the concentration is 0.2 mM, the CFR was 9%. This is because the concentration of S ions in the reaction solution was too low to supply S ions necessary for all apoferritins to form CdS core in 24 h. The reaction solution with 1 mM thioacetic acid showed the maximum CFR, 23%. When the thioacetic acid concentration was higher than 2 mM, CFR decreased in proportion to the thioacetic acid concentration and finally became 0 at 50 mM. This is because the supply speed of S ions per time was faster than the consumption speed for 0.3 mg/mL apoferritins for the formation of CdS cores. The excess S ions, which were not consumed by apoferritins, made CdS bulk precipitate even Cd ions were protected by forming tetraaminecadmium ions. As a result, the limited Cd ions, the concentration of which was initially 1mM, was consumed outside apoferritin shell much and CFR decreased. The higher thioacetic acid concentration, the more CdS formation outside, and the less CFR. This is consistent with the experimental result that yellow precipitate in the bottom of glass tube increased in proportion to the concentration of thioacetic acid. Therefore, we concluded that 1mM thioacetic acid concentration is the best.

Based on these experimental optimization, CdS NPs were synthesized in reaction solution containing 40 mM ammonium acetate, 0.3 mg/mL apoferritin, 7.5 mM ammonia water, 1 mM cadmium acetate, and 1 mM thioacetic acid which was added at final step. The color of the solution gradually changed from thin yellow to golden yellow and a little CdS bulk precipitates were observed after overnight synthesis. Figure 3A is the schematic drawing of apoferritin with core. Figure 3B shows a TEM image of apoferritin with CdS NPs by aurothioglucose stain. It was seen that cores were surrounded by protein shell that looked like white ring. The EDS and XRD measurement of NP cores was carried out to make sure that the formed core is CdS. EDS spectra showed Kα, Cd peak (3.1 keV) and Lα, S peak (2.2 keV) and the composition ratio of Cd and S was estimated to be 1 : 1 (Figure 3C). The titanium peak (4.5 and 4.9 keV) and carbon peak are contributed to the TEM grid and protein shell. The set of peak positions of the XRD spectra indicated obtained CdS NP cores were cubic crystal (data not shown). These data indicated that the synthesized NP in the apoferritin cavity by SCRY and TSSP is cubic CdS crystal.

Figure 3 The TEM image and EDS spectrum of CdS NPs synthesized in the apoferritin cavity. (A) shows the schematic drawing of apoferritin with core. (B) is TEM image of apoferritin with CdS NPs and the apoferritin without CdS NPs (white circler). The image was stained by the 1 % aurothioglucose. The scale bar is 50 nm. (C) shows the EDS analysis of obtained CdS NPs in the apoferritin cavity.
The essential points for one pod synthesis of semiconductor NPs in the apoferritin cavity are (1) positive charged materials are protected by complex ions such as tetraammine-complex avoiding bulk precipitate out of apoferritin cavity, (2) negative charged material degrade slowly in the reaction solution for few days. The result obtained in this study clearly demonstrated that satisfying these conditions, especially second condition, can fabricate compound NPs in the apoferritin cavity. Even this success, the CdS core formation mechanism remained unclear. Ferritin protein has many negative charged amino acids inner surface of cavity. This character is favorable to accumulate positive charged metal ions such as Ferrous ion in the living body. However, compound semiconductor NPs are synthesized from positive charged material (Cd$^{2+}$, Zn$^{2+}$) and negative charged ions (Se$^{2-}$, S$^{2-}$). It is a challenge to unravel the mechanism how negative charged materials such as S$^{2-}$ are introduced into the cavity. At this stage, we assumed the electrical charge transition in the cavity affects to it [6] but for the further understanding, more studies are necessary.

We have demonstrated that successful CdS NP synthesis in the apoferritin cavity by employing SCRY and TSSP. This simple NP fabrication method is proven to be applicable for not only CdSe and ZnSe NPs synthesis, but also the CdS NP synthesis in the apoferritin cavity. Our CdS NP surrounding hydrophilic protein shell will be useful to many kinds of nano-applications.

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