Preparation of Water-soluble PEGylated Semiconductor Nanocrystals

Eui-Chul Kang, Atsuhiko Ogura, Kazunori Kataoka,[†] and Yukio Nagasaki*[††]
Tsukuba Research Laboratory, NOF Corporation, 5-10 Tokodai, Tsukuba 300-2635
[†]*Department of Materials Engineering, Graduate School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656*
[††]*Department of Materials Science & Technology, Tokyo University of Science, 2641 Yamazaki, Noda-shi, Chiba 278-8510*

(Received April 9, 2004; CL-040390)

We describe the synthesis of water-soluble PEGylated semiconductor nanocrystals and the characterization of their optical properties and colloidal stability by fluorescence and UV spectrometers. The fluorescence intensity of PEGylated CdSe/ZnS in PBS buffer was observed to be 75% of the initial values of conventional CdSe/ZnS in chloroform. The PEGylated CdSe/ZnS exhibited higher fluorescence intensity than PEGylated CdSe and CdSe/CdS in PBS buffer. The colloidal stability of PEGylated QDs in PBS buffer was estimated by transmittance as a function of time at 37 °C. The PEGylated CdSe/ZnS showed a colloidal stable dispersion in PBS buffer during 24 h.

During the past decade, many scientists have studied synthetic methods for and applications of semiconductor nanocrystals (quantum dots, QDs) because of their unique size-dependent chemical and physical properties.[1–7] Generally, the band gap of semiconductor nanocrystals increases with decreasing particle size owing to quantum size effects. In high-quality CdSe nanocrystals, for example, the emission colors of the band-edge photoluminescence (PL) shift from red (650 nm) to blue (450 nm) with decreasing particle size. Because the emission colors of semiconductor nanocrystals are strongly dependent on the size and shape distribution of the nanocrystals, these semiconductor nanocrystals can be used for many purposes, such as light-emitting diodes (LED),[8] lasers,[9] biological labels,[4,5] and so forth. Especially, for use in biological labels, the preparation of stable aqueous dispersions of semiconductor nanocrystals with a high PL yield and the reduction of nonspecific interaction with proteins and cells are highly desirable. In this paper, we report a new approach to achieve these purposes, which involve making water-soluble CdSe/CdS and CdSe/ZnS nanocrystals by overcoating with higher band gap semiconductor materials and PEGylation. Overcoating the CdSe core with a higher band gap semiconductor materials such as ZnS, CdS, and ZnSe reduces the leakage of excitons outside the core and hence increases the PL quantum yield without affecting the location or spectral bandwidth of emission.[3] PEGylation has become a major approach to reduce the nonspecific interaction of proteins and cells with biomedical devices.[10]

A CdSe QD of red emission color was synthesized following a method developed by Weller et al.[6] and Peng et al.,[7] with a high-temperature reaction in a trioctylphosphine oxide (TOPO) /hexadecylamine (HDA) mixture. The emission maximum and the full width at half maximum (fwhm) of the PL peak of the CdSe QD were 620 and 27 nm in chloroform, respectively.[11] The synthesis of a CdSe/ZnS Core/Shell QD was performed by the following method: At first, the excess Se and the coordinating solvent in CdSe QD were removed by centrifugation with chloroform/methanol. The CdSe/ZnS QD was synthesized with purified CdSe, zinc stearate/TOPO, and bis(trimethylsilyl) sulfide ($(TMS)_2S$)/ tributylphosphine (TBP) at 100 °C under argon atmosphere. The fluorescence spectrum of CdSe/ZnS QD retained the sharp peak with a small red shift (≈3 nm) as the ratio of Zn/Cd, but that fluorescence efficiency was enhanced about 4.5 times (Figures 1 and 2A). The CdSe/CdS QD was prepared using a method similar to that for CdSe/ZnS QD preparation. The fluorescence efficiency of CdSe/CdS QD was enhanced about 2.5 times that of CdSe QD (Figure 1). When these three QDs, CdSe, CdSe/CdS, and CdSe/ZnS, were dissolved in pyridine, the fluorescence intensities dramatically decreased. The fluorescence intensities of CdSe, CdSe/CdS, and CdSe/ZnS QDs in pyridine were observed to be 5, 20, and 26% of the initial values of each QD in chloroform, respectively. Pyridine displaces TOPO molecules on the QD surface and forms a weak bond to surface Cd or Zn atoms of CdSe, CdSe/CdS, and CdSe/ZnS QDs.[3] Consequently, the fluorescence intensities of QDs in pyridine largely decreased with an increase in the leakage of excitons outside the core.

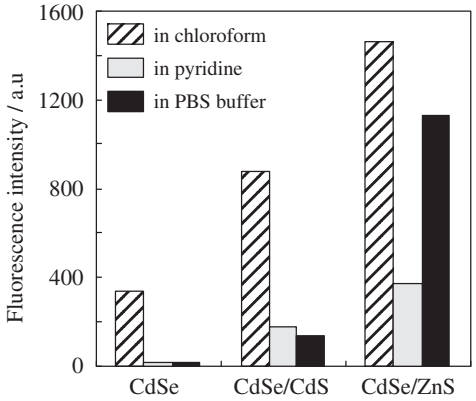

Figure 1. Fluorescence intensities of CdSe, CdSe/CdS, and CdSe/ZnS in chloroform, pyridine and PBS buffer (pH 7.4) before and after PEGylation.

We carried out the PEGylation of QDs for use in biological labels. The PEGylated QDs were prepared by the following method using α-methoxy-ω-mercapto-PEG (MPEG-SH, Mn 5000): CdSe and MPEG-SH are dissolved in a small amount of chloroform at room temperature for 1 h. Hexane and PBS buffer (pH 7.4) were added to CdSe and the MPEG-SH mixture chloroform solution, and the resulting mixture was stirred for 5 min. After reaching equilibrium, 1.0 mL of the sample solution

was pipetted from both the hexane top phase and the PBS bottom phase, and the fluorescence intensity of each solution was measured to determine the partitioning of the CdSe QD. The conventional CdSe QD without any PEGylation partitioned to the hexane top phase. On the other hand, the PEGylated CdSe QD was significantly partitioned to the PBS bottom phase. When the molar ratio of MPEG-SH to Cd of the CdSe QD was above 1.0, the PEGylated CdSe QD partitioned mostly at the PBS bottom phase. However, the fluorescence intensities of the PEGylated CdSe and CdSe/CdS QDs were largely decreased in PBS buffer compared to those of conventional CdSe and CdSe/CdS QDs in chloroform. It is considered that the large decrease in fluorescence intensity of the PEGylated QDs in PBS buffer occurred by a mechanism similar to those of conventional QDs in pyridine. On the contrary, effective solubilization of CdSe/ZnS in aqueous media was not observed by the same preparation method of PEGylated CdSe and CdSe/CdS QDs. The weaker interaction between the Zn atom and S at the end of the PEG chain compared to that between Cd and S may be the reason for the low efficacy. In order to improve the solubilization efficacy of CdSe/ZnS QD in aqueous media, the following new method was developed. Prior to the addition of MPEG-SH to CdSe/ZnS, MPEG-SH and $CdCl_2$ were mixed in PBS buffer to prepare a MPEG-S–Cd complex. After the obtained MPEG-S–Cd complex and CdSe/ZnS QD were mixed in a small amount of chloroform, PBS buffer and hexane added to extract the PEGylated QD. The PEGylated CdSe/ZnS was successfully prepared under these conditions. The fluorescence intensity of PEGylated CdSe/ZnS in PBS buffer was observed to be 75% of the initial values of conventional CdSe/ZnS in chloroform (Figures 1 and 2A). The PEGylated CdSe/ZnS exhibited higher fluorescence intensity than PEGylated CdSe and CdSe/CdS in PBS buffer.

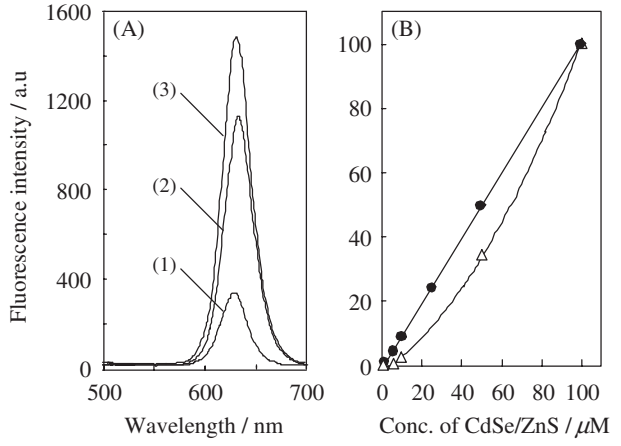

Figure 2. (A) Fluorescence intensities of (1) CdSe and (3) CdSe/ZnS in chloroform and (2) PEGylated CdSe/ZnS in PBS buffer (pH 7.4); (B) Relation between fluorescence intensity and concentration of PEGylated CdSe/ZnS with TOPO (open triangle) and mercaptoundecane (filled circle) as surface capping agents.

Figure 2B shows the dependence of fluorescence intensity upon the PEGylated CdSe/ZnS concentration in PBS buffer. When the PEGylated CdSe/ZnS with TOPO as a surface capping agent was diluted, the fluorescence intensity did not decease proportionally, indicating that the TOPO molecule was liberated from the surface of the PEGylated QD by dilution. In order to improve the stability of the surface capping agent, surface TOPO was exchanged with mercaptoundecane. The PEGylated CdSe/ZnS with mercaptoundecane as a surface capping agent yields a straight line on dilution as can be seen in the Figure.

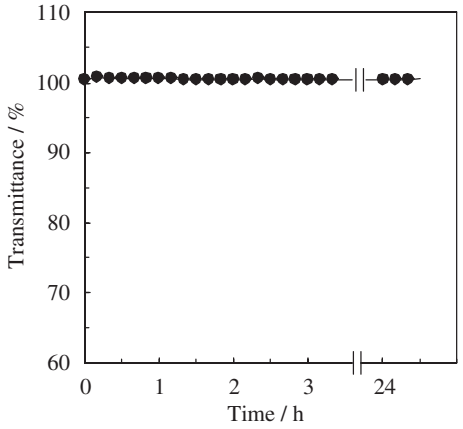

Figure 3. Transmittance change in PEGylated CdSe/ZnS in PBS buffer (pH 7.4) as a function of time at 37 °C.

The colloidal stability of the PEGylated QDs in PBS buffer was estimated by transmittance at 700 nm as a function of time at 37 °C as shown in Figure 3. The PEGylated CdSe/ZnS showed the colloidal stable dispersion in PBS buffer during 24 h. The shape and intensity of the fluorescence spectrum of PEGylated CdSe/ZnS after 24 h did not change at all.

References and Notes

1 C. B. Murray, D. J. Norris, and M. G. Bawendi, *J. Am. Chem. Soc.*, **115**, 8706 (1993).
2 A. P. Alivisatos, *Science*, **271**, 933 (1996).
3 X. Peng, M. C. Schlamp, A. V. Kadavanich, and A. P. Alivisatos, *J. Am. Chem. Soc.*, **119**, 7019 (1997).
4 M. Bruchez, M. Moronne, P. Gin, S. Weiss, and A. P. Alivisatos, *Science*, **281**, 2013 (1998).
5 W. C. W. Chan and S. Nie, *Science*, **281**, 2016 (1998).
6 D. V. Talapin, A. L. Rogach, A. Kornowski, M. Haase, and H. Weller, *Nano Lett.*, **1**, 207 (2001).
7 L. Qu and X. Peng, *J. Am. Chem. Soc.*, **124**, 2049 (2002).
8 H. Mattoussi, L. H. Radzilowski, B. O. Dabbousi, E. L. Thomas, M. G. Bawendi, and M. F. Rubner, *J. Appl. Phys.*, **83**, 7965 (1998).
9 V. I. Klimov, A. A. Mikhailovsky, S. Xu, A. Malko, J. A. Hollingsworth, C. A. Leatherdale, H. J. Eisler, and M. G. Bawendi, *Science*, **314**, 290 (2000).
10 M. Mrksich, G. M. Whitesides, in "Poly(ethylene glycol): Chemistry and Biological Applications," ed. by M. J. Harris and S. Zalipsky, American Chemical Society, Washington, DC (1997), p 361.
11 Fluorescence spectra were recorded on a Hitachi F-2500 fluorescence spectrometer. The excitation wavelength was 400 nm, and the slit widths were set at 2.5 nm (ex.) and 2.5 nm (em.).

Published on the web (Advance View) June 7, 2004; DOI 10.1246/cl.2004.840