CdSe nano-particles coated with thiol-terminated oligo(ethylene oxide) for protein targeting reagent

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ABSTRACT

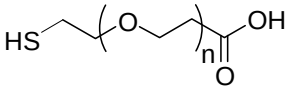
Fluorescent labeling reagents are used as medical and biochemical research reagents that label specific protein, antigen, antibody, and DNA. However, the chromophoric groups (organic compounds) usually are not stable under long-time ultra violet (UV) excitation. Nanometer-sized semiconductor particles (for example, cadmium sulfide (CdS) or cadmium selenide (CdSe) nanometer-sized particles) provide high intensity fluorescence with durability under long-time UV irradiation. CdSe nano-particles are usually stabilized by surface modification with hydrophobic reagents. Solubilization of the CdSe particles into water is one of the key processes of their application to fluorescent labeling reagents under aqueous condition. We prepared the CdSe nano-particles coated with thiol-terminated oligo(ethylene oxide), TPEG. The coated CdSe nano-particles are water-soluble and the solubilizing efficiency into water is 77 % estimated from UV-visible spectra. Coupling of the CdSe nano-particles coated with thiol-terminated oligo(ethylene oxide) and monoclonal antibody (anti-tubulin antibody was used.) was performed by using water-soluble carbodiimide. The fluorescence microscopic observation of the stained HeLa cells by the antibody conjugated CdSe nano-particles was performed. Clearly stained tubulin was observed in the cells.

Keywords: CdSe, nano-particle, oligo(ethylene oxide), antibody, targeting reagent

1 INTRODUCTION

Fluorescent labeling reagents are used as diagnosis and biochemical research reagents that label specific protein, antigen, antibody and DNA. However, the chromophoric groups are not stable under long-time Ultra Violet (UV) excitation measurement condition. This problem should be overcome to practical and wide-area use of fluorescent labeling reagents. Semicondoctor nano-particles (CdSe, CdS) provide high intensity fluorescence with durability under long-time UV irradiation [1, 2]. Thus, using CdSe nano-particles for chromophore of fluorescent labeling reagents will overcome the problem. CdSe nano-particles are prepared with various methods and the particles are usually stabilized by surface modification with hydrophobic reagents, trioctylphosphine oxide (TOPO) and oleic acid. The CdSe nanoparticles are insoluble into water. Solubilization of the CdSe to water is one of the key processes of their application to fluorescent labeling reagents.

In this work, we prepare water-soluble CdSe nano-particles by using thiol-terminated oligo(ethylene oxide) with carboxylic acid moiety (PEGn (n = 4 or 8), Figure 1). Stability and fluorescence characteristics of water-solubilized CdSe nano-particles are investigated. Furthermore, the conjugated CdSe nano-particles with antibody are prepared and label targeted protein molecules in HeLa cells.



TPEG4 n = 4, TPEG8 n = 8

Figure 1: Structure of TPEG4 and TPEG8.

2 EXPERIMENTAL

2.1 Materials

Cadmium selenide (CdSe) nano-particles were gifted from National Institute of Advanced Industrial Science and Technology Kyushu (AIST Kyushu) as chloroform solution. Commercially available CdSe nano-particles (Evident Technology) were also used as toluene solution. All chemicals were purchased and used as received. Thiol-terminated oligo(ethylene oxide) with terminal carboxylic acid moiety was purchased from Quanta BioDesign and used as received. The number of repeating unit was 4 to T-PEG4 and 8 to T-PEG8 (Figure 1).

2.2 Preparation of water-soluble CdSe nano-particles

Chloroform (1 mL) was added to the CdSe nano-particles chloroform solution (34.9 mg/mL) solution (2.5 μL) and then TPEG4 (2 μL) was also added into the chloroform solution. PBS (phosphate buffer solution) (1 mL) was added into the chloroform solution. The mixture was extracted for 48 h at room temperature under the dark condition. The aqueous solution was separated by pipeting. The crude aqueous solution was purified with Microcon®

YM-3 (Millipore, cutoff molecular weight 30,000) to remove excess T-PEG4 under centrifuged (14,000g) for 12 min and reverse centrifuged (1,000g) for 3 min. The water-soluble CdSe nano-particles are presented as CdSe-TPEG4. CdSe-TPEG8 particles were also prepared under the similar procedure.

2.3 Conjugation of water-soluble CdSe nano-particles with antibody

Water-soluble CdSe nano-particles were conjugated with antibody by using condensing reagent (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, EDC). EDC (12.8 mg) was dissolved into 1 mL of the PBS. Sulfo-NHS (1.82 mg) was dissolved into 1 mL of PBS. The mixture of CdSe-TPEGn (n = 4 or 8) (400 μL), IgG (8 μL), EDC (25 μL) and Sulfo-NHS (10 μL) solution was stirred for 2 h at room temperature. After the reaction procedure, the crude reaction mixture was purified with Microcon® YM-3 (Millipore, cutoff molecular weight 30,000) to remove EDC, Sulfo-NHS, and so on under centrifuged (14,000g) for 12 min and reverse centrifuged (1,000g) for 3 min. Conjugated CdSe nano-particles with goat-mouse antibody for indirect labeling method were also prepared under similar procedure.

2.4 Measurements

UV-visible spectra of samples were recorded with a UV-1800 spectrophotometer (Shimadzu). Fluorescence spectra were recorded with a FP-6300DS (JASCO).

Observation of stained cells with CdSe nano-particles were performed with a fluorescence microscope (BX51N-34-F1, Olympus). Excitation wavelength was 545 - 580 nm and observation wavelength was 610 nm. The cell images were photoed with a digital camera (DP70-SET-A, Olympus).

3 RESULTS AND DISCUSSION

3.1 Water- solubilization of CdSe nano-particles

Figure 2 shows appearance of CdSe nano-particle aqueous solutions under visible light and UV light irradiation (365 nm). As shown in Figure 2 (a), the color of the both aqueous orange solutions is clear. Under UV light irradiation (365 nm), the aqueous solutions show greenish fluorescence (Figure 2 (b)). Fluorescence intensity of the aqueous solutions maintained over 40 days at room temperature.

Figure 3 shows FTIR spectra of CdSe nano-particles removed the chloroform and CdSe-TPEGn (n = 4 or 8) particles removed the water. The peaks at 2924 cm^{-1} and 2852 cm^{-1} which are attributed to alkyl chain in the ligand around the CdSe nano-particles are observed in Figure 3 (a). As shown in Figure 3 (b) and (c), the peaks vanish away from the spectra. New peaks around 1700 cm^{-1} which are attributed to carboxylic acid appear in Figure 3 (b) and (c). This indicates that the ligand molecules around the CdSe nano-particles change from TOPO to T-PEGn (n = 4 or 8). Figure 4 shows the UV-visible spectra of CdSe/chloroform solution and water-solubilized CdSe nano-particles (CdSe-TPEG4). The solubilization efficiency was estimated from the change of absorbance at 544 nm. The efficiency with TPEG4 is 80 % and with TPEG8 is 72 %.

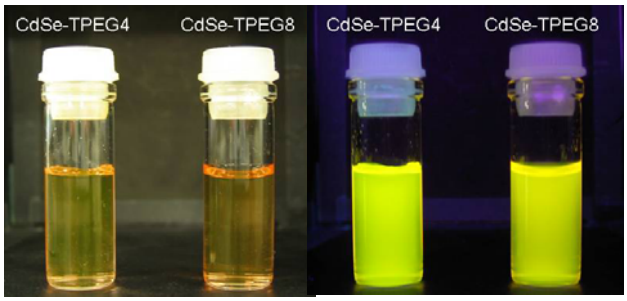


Figure 2: Appearance of CdSe-TPEGn (n = 4 or 8) aqueous solutions, (a) left: under visible light irradiation, (b) right: under UV light irradiation (365 nm).

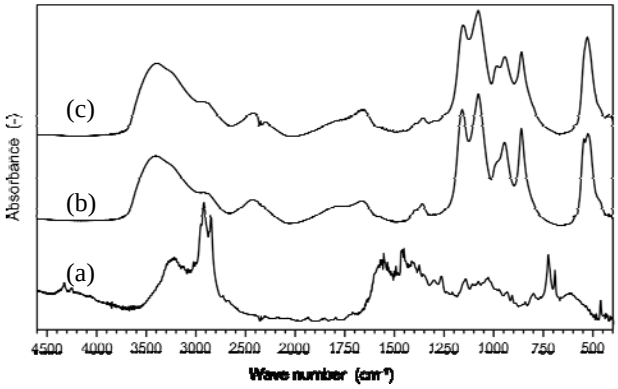


Figure 3: FTIR spectra of CdSe nano-particles (a), CdSe-TPEG4 (b), and CdSe-TPEG8 (c), all samples are removed their solvents, chloroform or water.

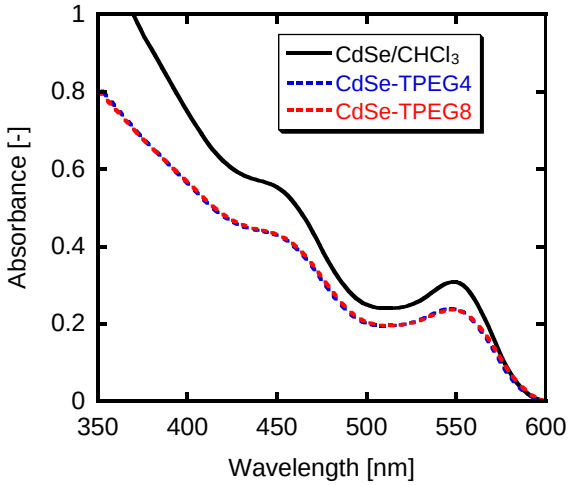


Figure 4: UV-visible spectra of CdSe/chloroform and CdSe-TPEGn (n = 4 or 8) aqueous solution.

NSTI-Nanotech 2009, www.nsti.org, ISBN 978-1-4398-1783-4 Vol. 2, 2009

Figure 5 shows the fluorescence spectra of CdSe/chloroform, and water-solubilized CdSe nano-particles (CdSe-TPEG4 and CdSe-TPEG8). The peak wavelength of the spectrum of the CdSe chloroform solution is agreed with that of CdSe-TPEG4 or CdSe-PEG8. The half maximum full-width of the spectrum of $CdSe/CHCl_3$ is 46 nm, that of CdSe-TPEG4 aqueous solution is 50 nm, and that of CdSe-TPEG8 aqueous solution is 52 nm. Change of the ligand around the CdSe particles partially affects the peak wavelength of fluorescence spectra.

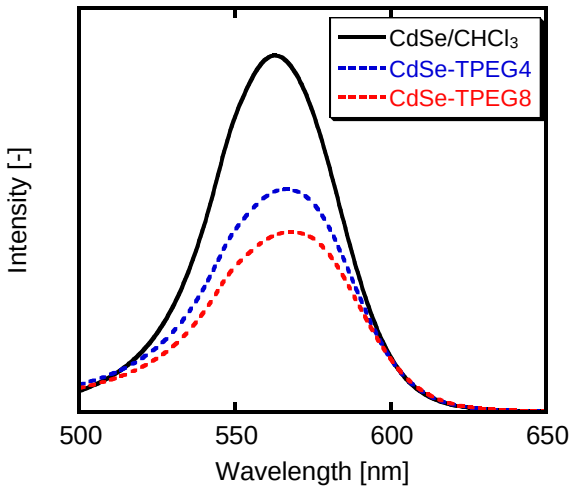


Figure 5: Florescence spectra of CdSe/chloroform and CdSe-TPEGn (n = 4 or 8) aqueous solution. Excitation wave length is 365 nm.

3.2 Conjugation of CdSe nano-particles with antibody

Conjugation of CdSe nano-particles with antibody was performed by using condensing reagent (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, EDC). Coupling between water-solubilized CdSe nano-particles and antibody was checked by SDS-PAGE electrophoresis (not see here). The results of the electrophoresis experiments suggest that water-soluble CdSe nano-particles are conjugated with antibody and the number of attached antibody molecules to a CdSe nano-particle is about one.

3.3 Cell staining with conjugated CdSe nano-particles

Figure 6 shows fluorescence microscopic images of HeLa cells which are stained with non-conjugated CdSe-TPEG4, conjugated CdSe-TPEG4 or CdSe-TPEG8 with anti-α-tubulin antibody. Figure 6 (a) shows the HeLa cells stained with non-conjugated CdSe-TPEG4. Non-specific adsorption of CdSe-TPEG4 to the proteins in HeLa cells is not observed as shown Figure 6 (a).

Figure 6 (b) is the stained cell images with CdSe-TPEG4 and Figure 6 (c) is that with CdSe-TPEG8, respectively. The tubulin molecules around the nuclei in the HeLa cells are stained with conjugated CdSe-TPEGn nano-particles clearly.

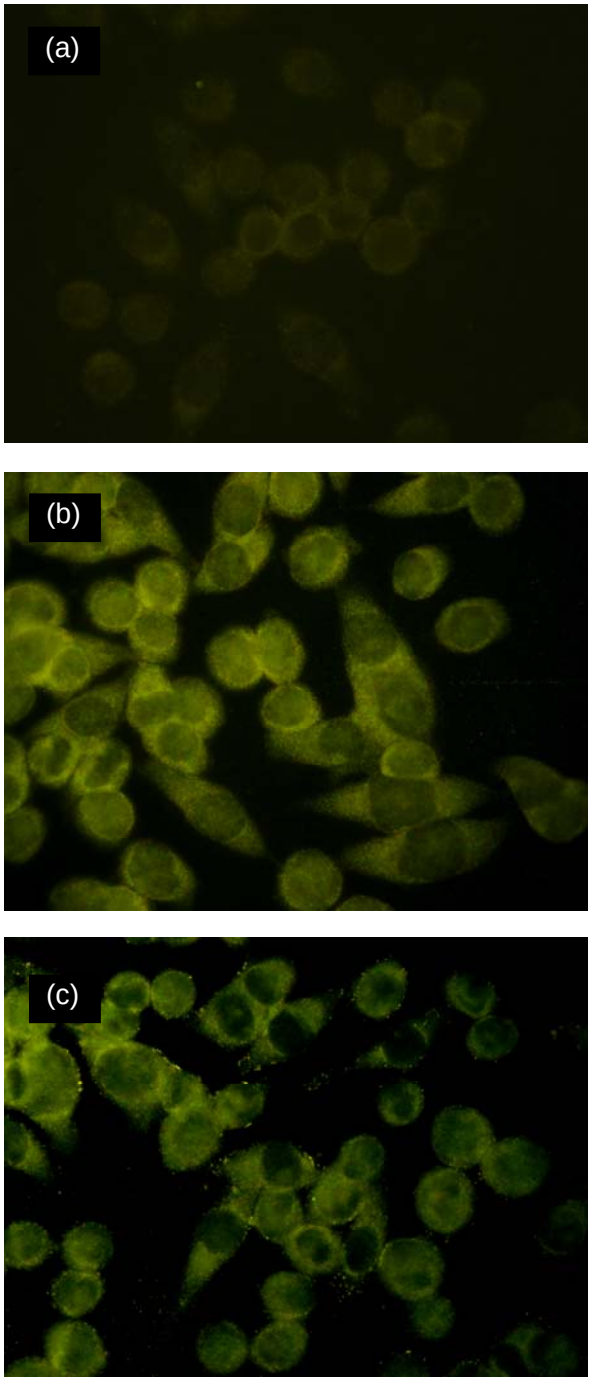


Figure 6: Fluorescence microscopic images of HeLa cells stained under various conditions, (a) stained with non-conjugated CdSe-TPEG4 (control sample), (b) with conjugated CdSe-TPEG4 nano-particles, and (c) with conjugated CdSe-TPEG8 nano-particles.

Figure 7 shows the images of indirect labeling of HeLa cells. Anti-α-tubulin antibody was used as a primary antibody and goat-mouse antibody was used as a secondary antibody. The images of stained tubulin proteins in the HeLa cells are also clear as the images in Figure 6 (b) and (c).

We conclude that the CdSe nano-particles modified with thiol-terminated oligo(ethylene oxide) (TPEG) are simple and useful tool for specific labeling of protein in cells.

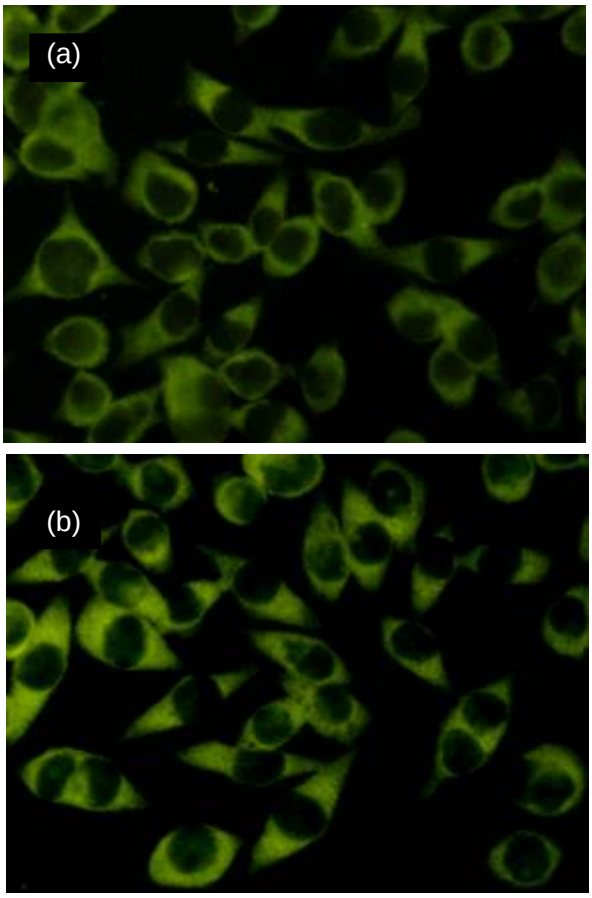


Figure 7: Fluorescence microscopic images of HeLa cells stained with indirect method, (a) stained with conjugated CdSe-TPEG4 nano-particles, and (b) with conjugated CdSe-TPEG8 nano-particles.

ACKNOWLEDGEMENTS

We thank Prof. Maeda, AIST Kyushu for gift of CdSe nano-particles. The authors also thank Prof. Sasaki and Dr. Furuya, Applied Molecular Bioscience, Graduate School of Medicine, Yamaguchi University for HeLa cell stain and capture of fluorescence microscope images.

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