CdSe Nanoparticles Modified with Water-soluble Terpolymers and their Application to Biomarkers

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ABSTRACT

We prepared water-soluble terpolymers containing *N*-isopropylacylamide (Nipam), oleic acid (Ole), and acrylic acid (Ac) to solubilize cadmium selenide zinc sulfide core-shell quantum dots (CdSe QDs) into water. The CdSe QDs modified with the terpolymers were soluble into water. The water-soluble CdSe QDs provided highly luminescent with sharp spectrum. The intensity of the luminescent was kept to about 70% level after 40 days of the modification treatments at room temperature. These QDs were further conjugated with antibodies and applied to cell staining as a biomarker.

Keywords: cadmium selenide, terpolymer, water-soluble, antibody, biomarker

1 INTRODUCTION

Fluorescent labeling reagents are used as diagnosis and research reagents that label specific protein, antigen, antibody, and DNA [1]. 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. Nanometer-sized semiconductor particles (for example, CdS or CdSe nanometer-sized particles) provide high intensity fluorescence with durability under long-time UV irradiation [2-8]. Thus, using the nanometer-sized CdS or CdSe particles for chromophore of fluorescent labeling reagents will overcome the problem. Nanometer-sized CdSe particles (CdSe) are prepared with various methods and the CdSe particles are usually stabilized by surface modification with hydrophobic reagents, trioctylphosphate (TOP) or trioctylphosphine oxide (TOPO). The CdSe particles are insoluble to water. Solubilization of the CdSe to water is one of the key processes of application the CdSe particles to fluorescent labeling reagents. We solubilized the CdSe particles to water by using water-soluble terpolymers containing acrylic acid (Ac), *N*-isopropylacrylamide (Nipam), and oleic acid (Ole). The polymers which consist of water-soluble and hydrophobic part, and functional group for antibody binding site (Fig. 1). Water-soluble part in the polymers is composed of *N*-isopropylacrylamide (Nipam). Hydrophobic part for anchoring to CdSe nanoparticles coated with hydrophobic stabilizing reagents (TOP) and oleic acid (Ole) is prepared by copolymerization of oleic acid (Ole) as a monomer.

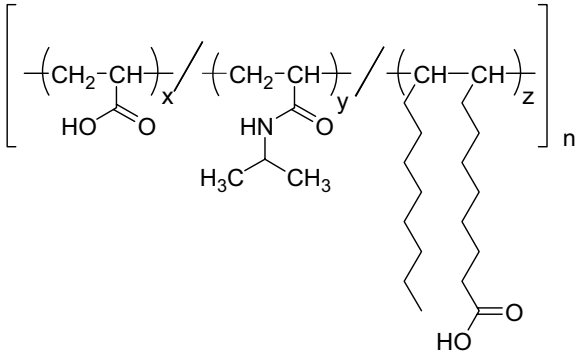

Figure 1: Structure of P[Ac(x)Nipam(y)Ole(z)] terpolymer.

2 EXPERIMANTAL

2.1 Materials

The stock chloroform solution with CdSe QDs covered with TOP and Ole was gifted from Dr. H. Maeda, AIST-Kyushu (Tosu, Japan). The solution was kept at 5°C in the dark. Monomers (Nipam and Ole) for preparation of terpolymers were purchased and used as received. Ac was purified by distillation before polymerization procedure.

2.2 Preparation of terpolymers

The terpolymer, P[Ac(x)Nipam(y)Ole(z)], was prepared by radical polymerization. Typical polymerization procedure is as follows. Ac (39.6 mg, 0.55 mmol), Nipam (1.13 g, 10 mmol), Ole (155.4 mg, 0.55 mmol), α, α'-azoisobisbutylonitrile (7 mg, 42 μmol), and 2 ml of THF were charged into a reaction glass tube. The reaction tube was degassed with the freeze-pump-thaw cycles. After the degas procedure, the reaction tube was set into the oil bath (70°C) for 20 h. The THF solution in the tube was poured into *n*-hexane and the precipitated polymer was collected. The resulted polymer is annotated as P[Ac(5)Nipam(90)Ole(5)]. P[Ac(5)Nipam(85)Ole(10)] and P[Ac(10)Nipam(85)Ole(5)] were also prepared with similar

procedure. The structures of the terpolymers were confirmed with FTIR and ^{1}H NMR spectroscopic measurements.

2.3 Surface modification procedure of QDs with terpolymer

The QDs were covered with TOP and Ole for stabilization in the stock solution. We modified the QDs with the terpolymers with two ways.

(1) Treatment A: The QDs/$CHCl_3$ solution (30μl) was poured into a glass tube. The chloroform in the QDs stock solution was removed and then 1 ml of THF added to the QDs. The QDs/THF (1 ml) solution and P[Ac(x)Nipam(y)Ole(z)] (1 mg)/THF (1 ml) solution were mixed each other. The mixed solution was sonicated with an ultrasonic cleaner. Then the THF was removed under reduced pressure and 1 ml of water was added to the QDs coated with the terpolymer. The QDs coated with P[Ac(x)Nipam(y)Ole(z)] by the Treatment A are annotated as CdSe- P[Ac(x)Nipam(y)Ole(z)](A).

(2) Treatment B: The QDs/THF solution was prepared with similar procedure of Treatment A. Then some amount of the THF was gradually removed from the QDs/THF solution and some amount of water was added to keep the total volume of the solution. Total volume of the added water was 1 ml. The QDs coated with P[Ac(x)Nipam(y)Ole(z)] by the Treatment B are annotated as CdSe- P[Ac(x)Nipam(y)Ole(z)](B).

2.4 Conjugation of water-soluble QDs with anti-tublin antibody

Conjugation of water-soluble QDs with anti-tublin antibody was performed by using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) as a coupling reagent [9]. Activation buffer (2-(*N*-morpholino)ethanesulfonic acid buffer, adjusted pH 7.31 with sodium hydroxide, 60 μl) was added to CdSe-P[Ac(10)Nipam(85)Ole(5)](A) /water solution (100μl) and then the solution was sonicated for 30 min. Then coupling solution (3-(*N*-morpholino)propanesulfonic acid buffer (pH 7.29) with EDC) was pored into the QDs solution and incubated at 37°C for 15 h. To terminate the reaction the termination solution (0.3 % glycine aqueous solution, 60μl) was added to the QDs solution. The solution was used for cell staining.

2.5 Cell staining with conjugated QDs

HeLa cells were fixed by treatment with 100% ethanol at -20°C and then dried for 1 h. The plate with fixed HeLa cells was dipped into PBS buffer for 5 min. Then the plate was dipped into the BSA (bovine serum albumin) solution (*ca.* 10 % in PBS buffer) for 15 min to prevent non-specific adsorption of conjugated QDs. The plate was dipped into the PBS buffer containing 1% (v/v) Triton X-100, 2% BSA for 5 min. The cells on the plate were incubated with the conjugated QDs solution for 2 h in dark place. Then the cells on the plate were incubated for 5 min and washed with PBS buffer in 3 times. The plate was observed with a standard fluorescence microscope.

3 RESULTS AND DISCUSSION

3.1 Characterization of terpolymer, P[Ac(x)Nipam(y)Ole(z)]

Structure of terpolymers, P[Ac(x)Nipam(y)Ole(z)] was confirmed by FTIR and NMR measurements. Three monomer units, Ac, Nipam, and Ole were introduced randomly. The molecular weight of the terpolymers was measured with a SEC chloromatograph technique. The average molecular weight was 37200 – 81600 based on the retention time with polystyrene as molecular weight standard.

3.2 Fluorescence spectra of water-solubilized CdSe nanoparticles coated with terpolymer, P[Ac(x)Nipam(y)Ole(z)]

Figure 2 shows the fluorescence spectra of CdSe-P[Ac(5)Nipam(90)Ole(5)] water solution (excitation wavelength 365 nm). Some peak shift was observed in the spectra. Original QDs stock chloroform solution had a fluorescence peak at 577 nm with exiting wavelength at 365 nm. The CdSe nanoparticles treated with P[Ac(5)Nipam(90)Ole(5)] provided the peak at 557 nm (Treatment A) and 534 nm (Treatment B).

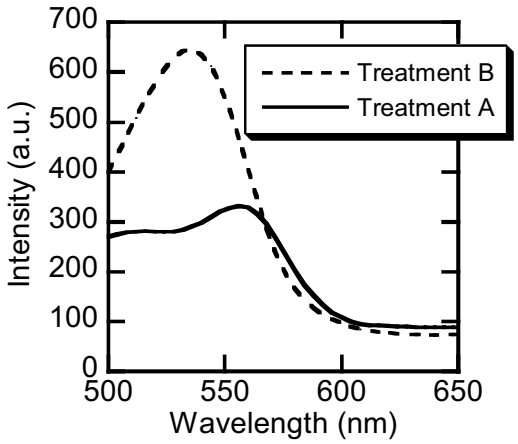

Figure 2: Fluorescence spectra of CdSe-P[Ac(5)Nipam(90)Ole(5)] water solution, excitation wavelength at 365 nm.

The peak shift indicates that the surface modification of CdSe nanoparticles with the terpolymer narrows the band gaps in them. Such peak shift is also observed in the CdSe nanoparticles treated with other P[Ac(10)Nipam(85)Ole(5)]. Figure 3 shows the fluorescence spectra of the CdSe-P[Ac(10)Nipam(85)Ole(5)]. Using the polymer for surface modification did not lower the intensity of the fluorescence. The intensity of fluorescence is very high as same as the CdSe stock solution. However, the peak shift was also observed.

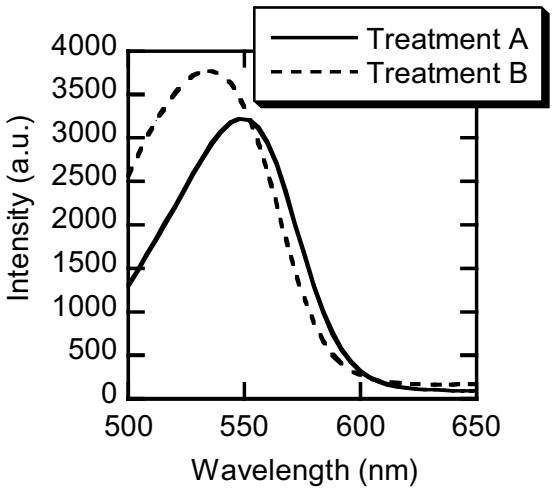

Figure 3: Fluorescence spectra of CdSe-P[Ac(10)Nipam(85)Ole(5)] water solution, excitation wavelength at 365 nm.

CdSe-	P[Ac(5)Nipam (90)Ole(5)]	P[Ac(10)Nipam (85)Ole(5)]	P[Ac(5)Nipam (85)Ole(10)]
(A)	557	549	529
(B)	534	533	505

Table 1: Peak wavelength of fluorescence spectra of water-solubilize QDs, excitation wavelength 365 nm.

The peak wavelength of the fluorescence spectra for CdSe-P[Ac(x)Nipam(y)Ole(z)] is listed in Table 1. Modification of CdSe nanoparticles with P[Ac(x)Nipam(y)Ole(z)] shortened the peak wavelength from 20 nm – 70 nm, higher energy side. This reason is not clear in our investigation.

The intensity of fluorescence is very high and stable over 40 days under the storage condition at room temperature. Peak wavelength and the intensity of the fluorescence after 40 days storage were listed in Table 2. In our investigation, CdSe-P[Ac(10)Nipam(85)Ole(5)](a) or (b) was very stable and their fluorescence intensity was very high. Therefore, we used CdSe-P[Ac(10)Nipam(85)Ole(5)] for conjugation with anti-Tublin monoclonal antibody.

CdSe-	P[Ac(5)Nipam (90)Ole(5)]	P[Ac(10)Nipam (85)Ole(5)]	P[Ac(5)Nipam (85)Ole(10)]
(A)	333/740 [a)]	3224/1620	1706/1240
(B)	648/1008	3780/3658	1712/1277

a) Fluorescence intensity: just prepared / after 40 days

Table 2: Time dependence of intensity of fluorescence of water-solubilized QDs.

3.3 Cell staining with conjugated CdSe nanoparticles coated with terpolymer, P[Ac(x)Nipam(y)Ole(z)]

Figure 4 shows the fluorescence microscope image of the stained HeLa cells with CdSe-P[Ac(10)Nipam(85)Ole(5)]. The Blue dots in Figure 4 are the stained nucleus with nuclear stain reagent (Hoechst). Around the blue stained nucleus, green fibrous texture is observed. Tubulin in the HeLa cells is stained with the conjugated CdSe nanoparticles coated with the terpolymer, P[Ac(x)Nipam(y)Ole(z)].

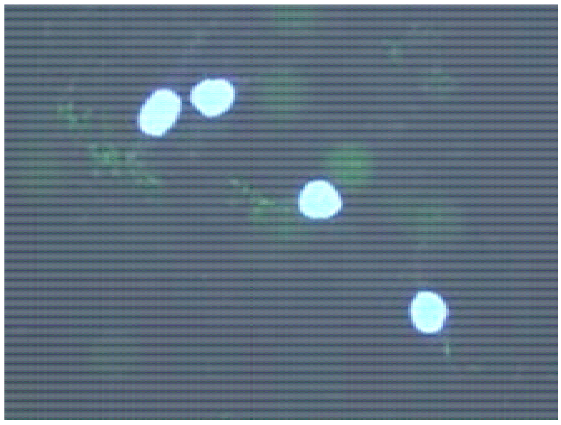

Figure 4: Fluorescence microscopic image of the HeLa cells stained with the CdSe-P[Ac(10)Nipam(85)Ole(5)] conjugated with anti-Tubulin antibody (green fibrous part) and with nuclear stain reagent (Hoechst) (blue cyclic part).

Figure 4 shows the application of the CdSe-P[Ac(10)Nipam(85)Ole(5)] conjugated with anti-Tubulin antibody prepared as described above to highly specific analysis of Tubulin in HeLa cells. Figure 4 also demonstrates that immunolabeling of Tublin is highly specific: the background nonspecific fluorescent signal was not detected. This shows the availability and usefulness of our conjugated CdSe-P[Ac(10)Nipam(85)Ole(5)] with monoclonal antibody.

4 CONCLUSIONS

In this paper, we describe a simple modification technique for preparation of water-soluble CdSe nanoparticles coated with terpolymers, prepared by radical polymerization of containing *N*-isopropylacylamide (Nipam), oleic acid (Ole), and acrylic acid (Ac). Prepared terpolymer, P[Ac(10)Nipam(85)Ole(5)], can solubilize the CdSe nanoparticles into water and the coated CdSe (CdSe-P[Ac(10)Nipam(85)Ole(5)]) aqueous solution is stable over 40 days at room temperature. We also can conjugate CdSe - P[Ac(10)Nipam(85)Ole(5)] with anti-Tubulin antibody by usual coupling procedure. The conjugated CdSe-P[Ac(10)Nipam(85)Ole(5)] demonstrates that immunolabeling of Tublin is highly specific: the background nonspecific fluorescent signal was not detected. This shows the availability and usefulness of our conjugated CdSe-P[Ac(10)Nipam(85)Ole(5)] with monoclonal antibody.

5 ACKNOWLEDGEMENTS

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