Preparation of Water Soluble CdS Quantum Dots Stabilized by Functional Poly(ethylene glycol) and Its Application for Bioassay

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1. Introduction

In the bioanalytical field, colloidal semiconductor quantum dots (QDs) such as CdS, CdSe, ZnS, CdS/CdSe, and CdSe/ZnS have attracted much attention as a biolabeling agent due to the advantages of there size dependent optical properties of i) tunable photoluminescence (PL) by size, ii) sharp and symmetrical luminescence peak, iii) strong and long life emission, and iv) wide excitation wavelength for the past several years. Many applications as a biolabeling have been reported.¹,²

Though the QDs are expected as one of the important nanomaterials in the bioanalytical field, their dispersion stability in aqueous media is not enough especially in a physiological environment. Actually, QDs stabilized by an ionic repulsion were easily coagulated with their decreasing size. Several types of water soluble polymers were reported to improve the dispersion stability of QDs in aqueous media.³,⁵

Recently, we have succeeded to prepare functionalized colloidal semiconductor CdS QD stabilized by our water soluble block copolymer, poly(ethylene glycol)/poly(V,N-(2-dimethylamino)ethyl methacrylate (PEG/PAMA) possessing an aldehyde group at the PEG chain end.⁶ The prepared CdS QD was remarkably stabilized in aqueous media even in a high salt concentration. The tert-amino groups in the PAMA segment of the block copolymer multivalently coordinate to the surface of the CdS QD. The other segment, PEG forms the hydrophilic shell layer of tethered chains on the CdS QD surface, increasing the solubility and dispersion stability of the QD in aqueous media via a steric repulsion effect. The aldehyde group at the distal end of the PEG/PAMA chain can be utilized as a ligand installing moiety. The CHO-PEG/PAMA block copolymer is, therefore, a promising material to achieve both stabilization and functionalization of the nanoparticles.

Thus prepared CdS QD had a wide PL and coated with the CHO-PEG/PAMA block copolymer was fairly stable in aqueous media even in a high salt concentration. The biotin moiety was installed at the PEG chain end as a ligand molecule, allowing it to specifically recognize streptavidin. By the use of streptavidin with a fluorescent probe, Texas Red in this study, an effective fluorescent resonance energy transfer (FRET) was observed, indicating the high utility of this method for a highly sensitive assay of biological components. This type of FRET system allows us to detect conventional fluorescent dyes at the same time with a variety of excitation wavelength due to the wide PL of the CdS QD. It may also enable a high throughput detection of specific proteins in solution. This paper communicates the proof

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of FRET via biotin-streptavidin interaction along with application of this system for a rapid molecular recognition system.

2. Experimental

2.1. PEG/PAMA block copolymer synthesis

CHO-PEG/PAMA was synthesized by a previously reported method. Potassium 3,3-dioxy-1-propanoate initiated the ring opening anionic polymerization of ethylene oxide was carried out in THF followed by the block copolymerization of 2-(N,N-dimethylamino)ethyl methacrylate (AMA) to form acetal-PEG/PAMA. These reactions were done under argon atmosphere at ambient temperature. The block copolymer was recovered by precipitation into a large excess of 2-propanol. The small amount of remaining PEG prepolymer was removed by Solvex extraction with THF after the protonation of the PAMA segment. The molecular weights of both segments in the resulting block copolymer, PEG and PAMA, were 4,200 and 15,800, respectively determined by a size exclusion chromatography (SEC) and $^1$H NMR. In order to convert the end $\alpha$-acetal group to an aldehyde group, the block copolymer was dissolved in an acetic acid / water mixture (10:1 v/v) and then stirred for 5 hours at 35 °C.

After the reaction, the mixture was neutralized by NaOH and dialyzed against water. In order to introduce the biotin molecule, biocytin hydrazide (Pierce, U.S.A.) biotin with hydrazyl group was added to the reaction mixture and reacted for 2 h followed by the addition of NaBH$_4$ to reduce the formed Schiff base. Then, the mixture was dialyzed against water.

2.2. CdS quantum dot preparation

One of the representative procedures for the preparation of CdS QD stabilized by the CHO-PEG/PAMA block copolymer is described. To an 8 ml aqueous solution of the block copolymer (3.08 × 10$^{-4}$ mol/L as amine concentration in the block copolymer) in a glass vial, CdCl$_2$ (2.5 × 10$^{-3}$ mol/L) and Na$_3$S (2.5 × 10$^{-3}$ mol/L) were added in this order and stirred for 1 h at ambient temperature. The purification was carried out by dialysis against water. The biotin-installed CdS QD was prepared in a similar manner using the biotin-PEG/PAMA block copolymer.

3. Result and Discussion

We have already reported that the dispersion stability of CdS QD stabilized by CHO-PEG/PAMA block copolymer. For example, aqueous solution of the
obtained CdS was stabilized even under the ionic strength of 0.3M for several days. In addition, characteristic PL spectra were observed (typical absorption and PL spectra exited at 400 nm were shown in Figure 1). Note that, in the absence of PEG segment or PAMA segment or block copolymer, they could not maintain their dispersion stability even under low salt condition along with almost no PL intensity. The size of obtained CdS QD was estimated by UV-vis absorption spectra and transmission electron microscope (TEM). From the band gap theory reported by Henglein, the size of the CdS QD was estimated to be 4.8 nm, which was corresponding to the size measured from TEM images, indicating that the block copolymer effectively coordinates on the CdS surface to control the crystallization growth.

A biotin molecule was introduced at the distal end of the PEG chain on the QD can be utilized for protein binding assay. In order to utilize the FRET between the QD and fluorescent-labeled protein for molecular recognition, Texas Red-streptavidin was used as a model compound in this study. The mixture of biotinylated PEG/PAMA stabilized CdS QD and Texas Red-streptavidin showed specific FRET. The spectra peak at 620 nm is Texas Red luminescence, which was increasing with the increasing Texas Red-streptavidin concentration, indicating that the effective energy transfer occurred from the CdS emission to Texas Red on the streptavidin. Note that Texas Red has almost no absorption at 400 nm.

It is interesting to note that the strong FRET was observed when Texas Red BSA was added to biotinylated CdS QD solution under the very low ionic strength, which indicates a possibility of electrostatic interaction between BDA and CdS QD because the biotin-PEGylated CdS QD showed slightly positive surface charge under the neutral pH. In order to estimate the electrostatic interaction, an effect of ionic strength effect on the FRET intensity was investigated. As can be seen in Figure 3, with increasing ionic strength, the FRET intensity significantly decreased in the case of Texas Red BSA coupled with biotin-PEG CdS QD. On the contrary, almost no change of the FRET intensity was observed when Texas Red-streptavidin was used instead of BSA. On the basis of these results, it is confirmed the FRET was observed via specific interaction between biotin at PEG end of CdS QD and streptavidin under high salt concentration.

Since nano-sized colloids have high Brownian motion in liquid, it often requires long time for the interaction with protein molecules.

In the present system, however, the CdS QD possesses slightly positive charge along with PEG tethered chains on the surface. This structure may solve the above issue, viz., after the coagulation between biotin-PEG CdS QD and negatively charged proteins is formed by the electrostatic interaction, the salt is added to increase the ionic strength. By this procedure, the electrostatic interaction can be decreased. Finally, only the specific interaction retains. It should be noted that
PEG tethered chain may play an important role for the reversible coagulation – dispersion cycle.

Figure 4 shows a time dependency of the ionic strength effect. After Texas Red-proteins were mixed with the biotin-PEG CdS QD, NaCl was added. When Texas Red streptavidin was added, the FRET intensity did not change at all, while the FRET intensity decreased rapidly when Texas Red-BSA was added. On the bases of these results, specific molecular recognition can be achieved rapidly.

In conclusion, noble stabilized CdS QD was prepared by the simple coprecipitation method by using CHO-PEG/PAMA block copolymer. The obtained CdS QD had solubility and dispersion stability in aqueous media due to the tethered PEG segment of the PEG/PAMA on the CdS surface, which were tethered by the coordination of PAMA segment. The CdS QD stabilized by biotinylated PEG/PAMA showed an effective molecular recognition to Texas Red-streptavidin, which caused the FRET between CdS QD and Texas Red adjacent to the streptavidin molecule. The FRET via a nonspecific electrostatic interaction could be resolved by increasing the ionic strength up to such a physiological condition. The observed FRET system between CdS QD and the fluorescent-labeled protein can be utilized as one of the new molecular recognition and a high throughput detection system.

4. References
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