PEG-based Surface Modification on Upconversion Nanophosphors for Bio-imaging under IR Excitation

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One of the most serious problems in fluorescence bio-imaging is the limitation of observation time due to color-fading of the fluorescence probes and damages on the bio system, both of which are caused by the excitation ultraviolet irradiation. This problem can be solved by using an upconversion emission of rare-earth doped inorganic nanophosphors under infrared excitation. For the application of nanophosphors as bio-imaging probes, surface modification by biocompatible polymers is needed to obtain biological functionality in organic tissues. In this paper, authors demonstrate the formation of poly(acrylic acid) (PAAc) and α -acetal-poly (ethylene glycol)-*block*-[poly (2-(*N,N*-dimethylamino) ethyl methacrylate)] multilayers on the Er-doped Y_2O_3 nanoparticles as an upconversion nanophosphor. The existence of the layers was confirmed by using zeta electrometry and infrared vibrational spectroscopy measurements.

Keywords: upconversion, bio-imaging, inorganic-polymer composite, nanoparticles, PEG

1. Introduction

Bio-imaging is a technique to visualize biological phenomena both in vivo and vitro, and is one of the key technologies in the biomedical field of research [1]. Particularly, fluorescent microscopy of tissues has drawn much attention as an essential tool for medical prevention, diagnosis and cure through the description of biological phenomena. However, the current imaging by use of organic dyes or fluorescent protein as probes leaves serious problems. The duration for the observation is often limited since ultraviolet light used for excitation bleaches the fluorescent probes and damages the bio system. Quantum dots may solve this problem

but still the color fading in water and the cost for their manufacture are unsolved problems. Commercially available fluorescent microscopes are ordinarily equipped with a CCD camera which detects visible light. Thus, the microscopic bio-imaging technology would be drastically improved by fluorescent probes without color fade emitting visible light under infrared excitation. Another advantage of the infrared excitation is that infrared light penetrates deeper into tissue because of its low scattering nature due to its longer-wavelength.

It is well known that low-phonon inorganic materials containing rare earth ions exhibit upconversion (UPC) phenomenon, where the

rare earth emits visible light under infrared excitation by a stepwise excitation. For example, yttrium oxide (Y_2O_3) works as good host matrix for several atomic % of erbium (Er), which is known to show upconversion emission at 540 nm (green) and 650 nm (red) under excitation by 980 nm light of a laser diode [2].

In order to fabricate UPC bio-imaging probes emitting visible light under excitation by infrared light, the following two issues are required. The first requirement is to fabricate nanometer-sized inorganic phosphor that reveals the UPC emission. The second one is simultaneously achieving of a specific adsorption to the target tissue and the prevention of agglomeration and non-specific adsorption to any other part than the target in the organic tissue for the UPC nanophosphors.

One solution to fulfill the second requirement is the surface modification of nanophosphors with biocompatible polymer such as α -acetal-poly (ethylene glycol)-*block*-[poly(2-(*N,N*-dimethylamino) ethyl methacrylate)] (acetal-PEG-b-PAMA, Fig. 1) block copolymer [3,4]. Because the acetal group at the one end of the PEG chain in the block copolymer is easily converted into an aldehyde group, where various ligands can be anchored such as biotinyl groups by a reductive amination reaction. The PAMA segment at the other end of the PEG chain is positively charged polyelectrolyte which gives the copolymer a high ability to anchor on a negatively charged surface of substances by multipoint adsorption. The PEG chain has a function both to disperse the particles into water by steric repulsion and to prevent non-specific adsorption.

This paper is focused on the surface

Figure 1. Structure formula of acetal-PEG-b-PAMA block copolymer.

modification of Y_2O_3 nanoparticles, as model inorganic nanoparticle, with acetal-PEG-b-PAMA block copolymer. It is reported, however, that the isoelectric point of Y_2O_3 is at pH =8~9 [5,6]. In other words, because the surface of Y_2O_3 colloids has weakly positive or neutral charge at pH ~7.4 which corresponds to body fluid, the positively charged PAMA segment of the block copolymer cannot firmly be electro-statically adsorbed on it [7]. Authors will propose and demonstrate a new method to solve this problem by inserting an intermediate layer of negatively-charged polymer, poly(acrylic acid) (PAAc) poly-electrolyte as the binder between them (Scheme 1).

2.Experimental

2.1. Materials

For Y_2O_3 nanoparticles, 99.5% grade of commercially available reagent (Kanto Chemical, Japan) was used as it is. The particle size ranged from 20 to 88 nm, and the surface area ranged from 14 to 60 m²/g. The PAAc (M_w =5000) was supplied from Wako Pure Chemical Industries (Japan) and poly(acrylic acid) (sodium salt) (PAAcNa) (M_w =5100) was supplied from Aldrich. Both of them were used without further purification. The acetal-PEG-b-PAMA was synthesized by ring opening anionic polymerization of ethylene oxide, and subsequent polymerization of 2-(*N,N*-dimethylamino) ethyl methacrylate according to the procedure described in literature [3]. The molecular weight and the structure of the obtained polymer were determined by gel permeation chromatography (GPC) recorded on HLC-8120GPC (Tosoh, Tokyo, Japan), and nuclear magnetic resonance (NMR) using EX-400 (JEOL, Tokyo, Japan). In this study, the molecular weights of the PEG and polyamine

Scheme 1. Process of surface modification of Y_2O_3 nanoparticles by PAAc and acetal-PEG-b-PAMA

segment were 4800 and 3600 respectively. For the other substances, reagent grade chemicals were used.

2.5. Measurement

Zeta potential of the Y_2O_3 nanoparticles was determined as a function of polymer content using electrophoretic light scattering spectrophotometer, ELS-600 (Otsuka Electronics, Osaka, Japan), equipped with a 10 mW He-Ne laser.

A series of samples was prepared by dispersing 100 mg of Y_2O_3 nanoparticles in PAAc (0–100000 molecules for one Y_2O_3 particle) aqueous solutions with various concentrations. The samples were equilibrated in aqueous solution by ultrasonication for 3 h. The solution was diluted to have a proper concentration for the measurements by adding 10 mM sodium phosphate buffer at a constant pH 7.4.

Another series of samples was prepared by adding various amount of acetal-PEG-b-PAMA (1000–10000 molecules for one Y_2O_3) into Y_2O_3 -dispersed PAAc aqueous solution (50000 molecules for one Y_2O_3). The pH of each solution was controlled to be 7.4 by adding NaOH in advance. The samples were equilibrated in aqueous solution by sonication for 3h, and rinsed in the above pH buffer by centrifugal purification process (15000 G, 15 min) for two times to remove excess polymer in the solvent.

Infrared spectra were recorded on a Shimadzu FTIR-8300 spectrometer. The

Y_2O_3 -dispersed solution was prepared with PAAcNa aqueous solution (50000 molecules for one Y_2O_3) and equilibrated by sonication for 3 h. Centrifugal separation and re-suspension in distilled water were repeated for five times to remove residual polymer dissolved in the solution. The precipitated particles were dried in vacuo over phosphorus pentoxide, and mixed with KBr so that the sample concentration will be 1 wt% to form a pellet sample. The used KBr had been previously dried at 150 °C for 12 h. A reference sample was also prepared from pure Y_2O_3 without any surface modification by a polymer.

3. Results and Discussion

To give negative charge on the surface of Y_2O_3 nanoparticles, we attempted to adsorb PAAc as the negatively charged first layer. As shown in Figure 2 the zeta potential of Y_2O_3 nanoparticles decreased with the increasing amount of PAAc addition. This implies that some of the added PAAc polyelectrolyte were adsorbed on the Y_2O_3 nanoparticle, giving negative charge with the carboxylate groups ($-COO^-$). The zeta potential reached a saturated value at 10000 molecules of PAAc for one Y_2O_3 particle. Also, when PAAc was added more than 10000 molecules for one Y_2O_3 nanoparticle, the colloids were kept suspended in the solution for at least 3 days. Without the PAAc modification, they started to agglomerate and settled down in a day. This stability of the suspension with Y_2O_3 nanoparticles was due to electrical repulsion by the negative charge of PAAc on the Y_2O_3 surface.

Figure 2. Zeta potential of Y_2O_3 nanoparticles as a function of the number PAAc in the solution. Dashed line is drawn as a guide to the eye.

Figure 3. FTIR spectra of pure Y_2O_3 and Y_2O_3 nanoparticles dispersed in PAAc aqueous solution.

Surface modification of Y_2O_3 nanoparticles was also estimated by an infrared vibration spectroscopy. Y_2O_3 nanoparticles were dispersed in PAAcNa aqueous solution, washed by centrifugal purification process for 5 times, and dried for the measurement of FTIR spectrum. As shown in Figure 3 Y_2O_3 nanoparticle treated with PAAcNa exhibited two absorption bands which are attributed to alkyl groups of PAAc, whereas untreated Y_2O_3 did not exhibit such absorption. The absorption by carboxylate asymmetric and symmetric stretch modes at 1567 and 1402 cm^{-1} , respectively [8], were not separated from the Y_2O_3 absorption bands. This result shows that PAAc firmly adsorbed on the surface of Y_2O_3 nanoparticle after 5 times of ultrasonic and centrifugal washing processes.

Figure 4. Zeta potential of Y_2O_3 nanoparticles as a function of the number of acetal-PEG-b-PAMA addition to the colloidal solution. Dashed line is drawn as a guide to the eye.

Following the above process, we attempted to adsorb acetal-PEG-b-PAMA on PAAc-modified Y_2O_3 nanoparticle as the second layer. Y_2O_3 nanoparticles were dispersed in PAAc pH buffer solution with a concentration of 50000 molecules for one Y_2O_3 particle. The acetal-PEG-b-PAMA was subsequently added by 1000, 5000 or 10000 molecules for one Y_2O_3 particle. We washed Y_2O_3 nanoparticle by ultrasonic dispersion for 3 h and centrifugal separation process for 2 times to remove excess polymers from the pH buffer. The colloidal solution was diluted for the measurement of the zeta potential of the PAAc-modified Y_2O_3 nanoparticles. Figure 4 shows the zeta potential of the Y_2O_3 nanoparticles as a function of the number of the added acetal-PEG-b-PAMA molecules for one Y_2O_3 nanoparticle. Negative charge on the PAAc-modified Y_2O_3 nanoparticles was shielded

by at most 1000 molecules of the acetal-PEG-b-PAMA. This result implies that the PAAc-modified Y_2O_3 nanoparticles electrostatically overcoated with an acetal-PEG-b-PAMA layer. The zeta potential was almost unchanged by adding more than 1000 molecules of the acetal-PEG-b-PAMA for one Y_2O_3 particle. Generally, colloidal particles are rapidly coagulated due to lack of an electrostatic repulsion effect when the surface charge is neutralized. In the case of doubly polymer-modified particles, however, the particles coagulated very slowly in spite of the charge cancellation by the addition of acetal-PEG-b-PAMA. This is owing to steric repulsion by the acetal-PEG-b-PAMA molecules arranged on the surface of Y_2O_3 nanoparticles like a brush. Because the agglomeration rate of the colloids becomes smaller with the greater amount of acetal-PEG-b-PAMA addition, the net amount of adsorption is not saturated at this range of addition number even though zeta potential is almost constant in the vicinity of 0 mV.

We reported previously that Y_2O_3 nanoparticles modified by only a PEG-PAMA copolymer are easily agglomerated and result in sedimentation because of the weak adsorption of the copolymer on the surface of the particles. In the case of the present study, rigidly surface-modified Y_2O_3 nanoparticles were obtained by the insertion of an intermediate PAAc polyelectrolyte layer with negative charge.

4. Conclusion

For the application to bio-imaging photo-probes a new method to modify the surface of Y_2O_3 nanoparticles was demonstrated. A negative charge was introduced on the surface of Y_2O_3 nanoparticles by adsorption of PAAc polyelectrolyte as the first layer. Acetal-PEG-b-PAMA copolymer with a positive charge was consequently adsorbed on the Y_2O_3 nanoparticles as the second layer. This doubly modified structure formed rigidly and did not collapse after 3 h of ultrasonic dispersion and two times of centrifugal purification processes. It was also found that Y_2O_3 nanoparticle itself are chemically stable throughout the above process at pH 7.5 which is comparable to those of body fluid.

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