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Photoinduced Particle Size Change of Core–Shell Polymeric Micelle Containing Spirobenzopyran in Its Inner Core

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Amphiphilic diblock copolymers comprising poly(methyl methacrylate-*co*-spirobenzopyran methacrylate) and poly(ethylene glycol) were synthesized using atom transfer radical polymerization to develop a new photoresponsive drug carrier. The diblock copolymers formed core–shell polymeric micelles with photoresponsive inner cores. Spirobenzopyran in the core was isomerized to the merocyanine form by UV irradiation, which resulted in reduction of the micellar size without decrease in the association number. This inferred that the strongly intermolecular interactions between merocyanine residues caused the conformational change of the core segments, following reduction of the micellar size.

Environmentally responsive biomaterials have attracted a great deal of attention as functional materials for drug delivery systems[1] and cell-cultures.[2] Numerous biomaterial species have been so far presented due to the demand for changes in physicochemical properties, such as solubility, morphology, and polarity, in response to various external stimuli, such as pH[3], temperature,[4] light,[5] and specific chemical species.[6] Among external stimuli, light-irradiation has some advantages, such as local targeting, being contactless, and immediately effective. Research into photoresponsive materials has been extensive for various practical applications.[7] The strategy for the fabrication of photoresponsive materials generally involves the combination of photochromic compounds with biomaterials, whose structures and functions are controlled by light. The photochromic molecule spirobenzopyran exhibits unique isomerization by UV-light irradiation, changing from the colorless hydrophobic spiropyran form (Sp-form) to the colored hydrophilic merocyanine form (Mc-form). Reverse-conversion from the Mc-form to the Sp-form is induced by heat and accelerated by visible light irradiation. However, a practical and satisfactory drug carrier containing spirobenzopyran has not yet been developed. Therefore, we intended to fabricate a new photo-controlled drug carrier with polymeric micelle structure using spirobenzopyran.

The objective of the present study is to synthesize amphiphilic diblock copolymers [PEG-*b*-P(MMA-*co*-Sp)] comprising poly(methyl methacrylate-*co*-spirobenzopyran methacrylate) (P(MMA-*co*-Sp)) as photoresponsive hydrophobic segments and poly(ethylene glycol) (PEG) as hydrophilic segments. The change in the physicochemical properties of PEG-*b*-P(MMA-*co*-Sp) in response to light, assuming a micellar structure, were then scrutinized by means of UV–vis absorbance and dynamic light scattering (DLS) measurements.

PEG-*b*-P(MMA-*co*-Sp) was synthesized by atom transfer radical polymerization (ATRP) technique using PEG with a 2-bromopropionate group (PEG-Br) at one end as a macroinitiator. The number-averaged molecular weight (M_n) and polydispersity index (M_w/M_n) of PEG-Br were determined to be 5000 and 1.18 by ^{1}H nuclear magnetic resonance (NMR) spectroscopy and gel permeation chromatography measurements, respectively. Similarly, those of PEG-*b*-P(MMA-*co*-Sp) were determined to be 6300 and 1.24, respectively.

The numbers of MMA and Sp units in the synthesized PEG-*b*-P(MMA-*co*-Sp) molecule were estimated from ^{1}H NMR spectra to be 6 and 2, respectively. The polymeric micelles were formed by a dialysis method.[8] The molecular assembly formation for PEG-*b*-P(MMA-*co*-Sp) in aqueous solution was confirmed by ^{1}H NMR measurements, according to a conventional method,[8] in which $CDCl_3$ and D_2O were used as solvents in organic and aqueous systems, respectively. Furthermore, transmission electron microscopy showed that PEG-*b*-P(MMA-*co*-Sp) formed spherical nanoparticles with an average particle size of 110 nm. These results revealed that PEG-*b*-P(MMA-*co*-Sp)s formed core–shell polymeric micelles in water.

Figure 1 shows the UV–vis absorption spectrum of 0.1-mg mL^{-1} aqueous PEG-*b*-P(MMA-*co*-Sp) solution as a function of UV irradiation time in the wavelength range from 280 to 320 nm. The intensity of the PEG-*b*-P(MMA-*co*-Sp) absorption band at around 555 nm, assigned to the Mc-form of spirobenzopyran, increased with UV irradiation time up to 4 min, and then plateaued at the photostationary state, displaying a violet color. This was ascribed to the isomerization of spirobenzopyran in the micellar core from the Sp-form to the Mc-form by UV irradiation. The Mc-form molecule is known to have solvatochromism, in that the maximum absorption peak of the Mc-form is shifted to shorter wavelength with increasing solvent polarity.[9] In a high polar media, such as methanol or water, the Mc-form exhibits a red or reddish-orange color. However, in the PEG-*b*-P(MMA-*co*-Sp) micelle aqueous solution, the Mc-form of spirobenzopyran in the core had a violet color with a longer maximum absorption wavelength than that in methanol (λ_{max} =

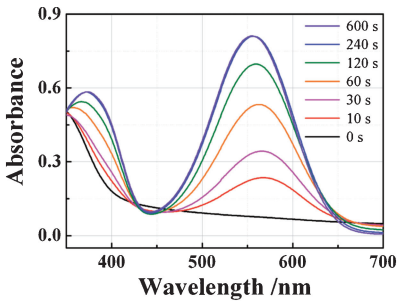

Figure 1. UV–vis absorption spectra of 0.1 mg mL^{-1} aqueous PEG-*b*-P(MMA-*co*-Sp) solution for various UV irradiation (280 to 320 nm) times at room temperature.

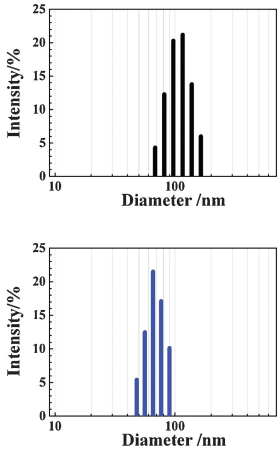

Figure 2. Particle size distribution of the PEG-*b*-P(MMA-*co*-Sp) micelle before (upper fig) and after (lower fig) UV irradiation for 10 min, determined by NNLS analysis of DLS measurements.

549 nm). This indicates that the spirobenzopyran moieties are located in the micellar hydrophobic core, where the microenvironment is less polar than water and methanol.

Figure 2 shows the particle size distributions of the PEG-*b*-P(MMA-*co*-Sp) micelles before and after UV irradiation for 10 min, which were obtained by a non-negatively constrained least squares (NNLS) analysis of the DLS measurements. UV irradiation caused a reduction in the particle size of the PEG-*b*-P(MMA-*co*-Sp) micelles from 110 to 90 nm. On the other hand, static light scattering measurements indicated that the degrees of association of the micelles remained unchanged by UV irradiation and were determined to be ca. 1240 (details to be reported elsewhere). These results imply that prior to UV irradiation, the PEG-*b*-P(MMA-*co*-Sp) forms a loose micelle structure due to the amorphous nature of the PMMA chain and the steric barrier of spirobenzopyran moieties, whereas after UV irradiation, the π–π interaction between Mc moieties including electrostatic interaction,[10] causes the dense packed core formation accompanied by micellar conformational changes. It should be noted that from preliminary experiments, it is found that the PEG-*b*-P(MMA-*co*-Sp) micelle plays an effective role as a drug carrier that exhibits slow release of an encapsulated drug by UV irradiation, accompanied by shrinkage of the micelle.

Figure 3 shows the solvent effects on the reverse-conversion from the Mc- to Sp-form of PEG-*b*-P(MMA-*co*-Sp) for tetrahydrofuran (THF), methanol, and water solvents under dark conditions. The value in the ordinate axis represents the degree of conversion from the Mc- to Sp-form of all Sp moieties in the solvent. The conversion rate of Mc residues in water was remarkably slow compared to those in THF and methanol. It took one week for the Mc-form to completely convert to the Sp-form in water. This behavior is ascribed to the structural stabilization of the Mc-form in the micellar core formed in water, due to the interaction between Mc moieties.

In conclusion, a novel polymeric micelle was prepared with a core–shell structure containing photoresponsive spirobenzopyran in the core segments. The particle size of the prepared micelles were reduced by UV irradiation, due to the intermolecular interaction between the Mc-form residues converted from the Sp-form. The PEG-*b*-P(MMA-*co*-Sp) micelle is expected to be used as a photoresponsive drug carrier for tumor therapy.

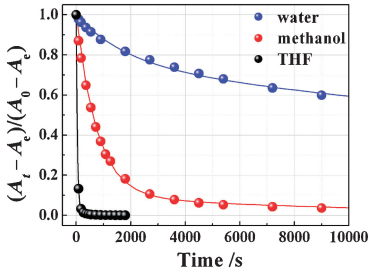

Figure 3. Mc-form to Sp-form isomerization behavior of PEG-*b*-P(MMA-*co*-Sp) in THF, methanol, and water as a function of time under dark conditions. A_t and A_0 denote the absorbance at λ_{max} at time t and the initiation of reverse conversion. A_e represents the absorbance at λ_{max} after reaching the photostationary state.

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