Synthesis and Characterization of a Temperature-responsive Amphiphilic Block Copolymer Containing a Liquid Crystalline Unit

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To create a stimuli-responsive micelle, we synthesized a novel temperature-responsive amphiphilic block copolymer, poly(ethylene glycol)-*block*-poly{6-[4-(4-pyridylazo)phenoxy]-hexyl methacrylate} [PEG-*b*-P(AzoPyl)], by undertaking the RAFT polymerization of an AzoPyl monomer with a PEG macro-RAFT reagent. This block copolymer possesses a liquid crystalline unit derived from a hydrophobic AzoPyl/carboxylic acid complex. The PEG-*b*-P(AzoPyl/decanoic acid (C_9COOH)) complex formed a micelle structure (weight-average diameter = 68 nm). DSC results confirmed that the PEG-*b*-P(AzoPyl/C_9COOH) micellar solution exhibited thermodynamic phase-transition behavior.

Stimuli-responsive polymeric materials such as polymeric micelles[1] and liposomes[2] can serve as drug carriers for drug-delivery systems, which release loaded drugs by means of external stimuli. The drug release triggered by the external stimuli at tumor sites is a very important technology for targeted cancer chemotherapy, resulting in both a high local drug concentration at the tumor sites and a suppression of toxic side effects at the normal organs and tissues. In this study, we applied liquid crystalline polymers (LCPs)—functioning as a temperature-responsive moiety—to the interior of polymeric micelles. This study uses as its liquid crystalline (LC) moiety an azopyridyl group that exhibits the ability to control the LC phase-transition characteristics by means of a simple complexation of carboxylic acids without any synthetic modifications. Introduction of the LC unit to the interior of the polymeric micelle allows for the phase transition of the micelle inner core between the LC phase and the solid crystalline phase. Compared to the rigid solid state of the core, the highly fluid character of the LC phase may enhance incorporation of hydrophobic drugs into the core. Moreover, it is possible to stably retain the incorporated drugs by lowering the temperature below the phase-transition temperature, since the solid inner core is expected to inhibit the drug release from the inner core. Therefore, highly efficient and stable drug incorporation in polymeric micelles is feasible by the use of LCPs.

For this purpose, we synthesized poly(ethylene glycol)-*block*-poly{6-[4-(4-pyridylazo)phenoxy]hexyl methacrylate} (PEG-*b*-P(AzoPyl)) by undertaking the RAFT polymerization of 6-[4-(4-pyridylazo)phenoxy]hexyl methacrylate (AzoPyl) (Figure 1). AzoPyl served as a monomer with poly(ethylene glycol)-4-cyano-4-[(thiobenzoyl)sulfanyl]pentanoate (PEG dithiobenzoate) serving as a marco-RAFT reagent. AzoPyl[3] and PEG dithiobenzoate[4] were prepared according to a previously reported method. AzoPyl and PEG dithiobenzoate were dissolved in a mixture of anhydrous THF and DMSO, to which an AIBN

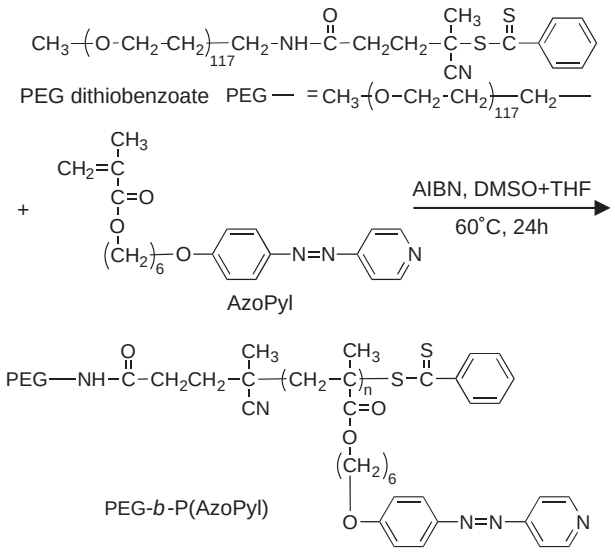

Figure 1. RAFT polymerization of AzoPyl with PEG dithiobenzoate.

DMSO solution was added. After five freeze–pump–thaw cycles, the solution was sealed in a vacuum, and the mixture was stirred at 60 °C for 24 h. The reaction mixture was poured into diethyl ether, and the precipitated polymer was dried in vacuo. Polymerization conditions are summarized in Table S1.[7]

RAFT polymerization was first tried in a dry DMSO solution.[5] However, the solubility of PEG dithiobenzoate in DMSO was low. To dissolve PEG dithiobenzoate well, we selected THF, which is a common solvent for a normal radical polymerization of AzoPyl homopolymers.[3] However, the monomer conversion in THF after 24 h was only 10% from ^{1}H NMR. In order to solve this low-conversion problem and this solubility problem, we used a mixture of DMSO and THF. As expected, the monomer conversion of the polymerization in the DMSO/THF mixture increased up to 53%. The molecular weight (MW) of the obtained polymer 5-16 was 11300 (MW of PEG dithiobenzoate: 5500; degree of polymerization: 16). These findings indicate that the DMSO/THF mixture was a proper solvent system for the preparation of PEG-*b*-P(AzoPyl) by RAFT polymerization.

As shown in Figure S2[7] the GPC measurement of 5-16 shows that the peak not only completely shifted to an area of high molecular weight but also remained narrow (PDI = 1.09), indicating that this RAFT polymerization proceeded well.

We carried out an ATRP of the AzoPyl as well. Reaction conditions are described in the Supporting Information section.[7]

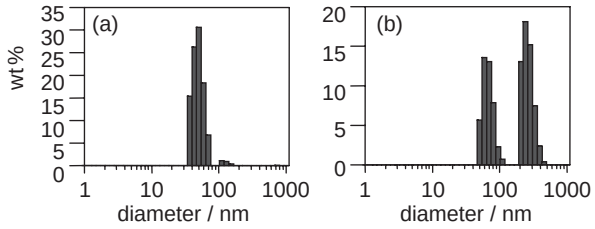

Figure 2. Size distributions of (a) the PEG-*b*-P(AzoPyl) (5-11) micelle and (b) the PEG-*b*-P(AzoPyl/C_9COOH) (5-11) micelle as determined by DLS.

However, no peak of the AzoPyl unit in the ^{1}H NMR spectrum was observed, whereas peaks of the PEG initiator were found. A basic pyridyl group in the AzoPyl unit was expected to disturb the activity of the ATRP catalyst.

The capability of micelle formation is an important function for drug carriers. To confirm the existence of micelle formation, we used PEG-*b*-P(AzoPyl) (5-11, MW: 9400; degree of polymerization: 11), which had a composition similar to that of 5-16. Hydration of 5-11 was carried out by means of sonication.[6] To introduce the LC phase-transition function into the micellar core, PEG-*b*-P(AzoPyl) was complexed with decanoic acid (C_9COOH). DLS results of the PEG-*b*-P(AzoPyl) micellar solution indicate that two different-size types of particles, a 51 nm micelle and a 110 nm micelle, were distributed in the solution (Figure 2a). We considered that the smaller diameter was derived from a spherical micelle structure, and that the larger diameter was derived from a secondary associate of the spherical micelles. The PEG-*b*-P(AzoPyl/C_9COOH) micelle solution exhibited a similar distribution, 68 and 270 nm (Figure 2b). These results confirm that PEG-*b*-P(AzoPyl/C_9COOH) exhibited micelle-forming behavior similar to that of PEG-*b*-P(AzoPyl). Other DLS data are summarized in Table S2.[7]

Using DSC, we analyzed the thermal properties of PEG-*b*-P(AzoPyl/C_9COOH) (5-11) (Figures 3a and 3c). DSC curves of bulk PEG-*b*-P(AzoPyl) showed only endothermic and exothermic peaks associated with the melting and crystallization of the PEG unit, respectively (Figures 3b and 3d). In contrast, small exothermic peaks from 55 to 42 °C were observed in a cooling process that the PEG-*b*-P(AzoPyl/C_9COOH) complex underwent (Figure 3, inset). All DSC curves on the cooling process showed the same results in three-repeating measurements. It was reported that the AzoPyl homopolymer, complexed with C_9COOH, had its isotropic–smectic phase transition around 53 °C in cooling.[3] These results indicate that the AzoPyl/C_9COOH complex system exhibited the LC phase-transition behavior even in the PEG-*b*-P(AzoPyl) amphiphilic block copolymer. On the other hand, we observed no endothermic peak caused by the LC phase transition in heating. Probably, the endothermic peak of the LC phase transition overlapped the large peak of the PEG.

Observation of phase-transition behavior in a micellar interior is very attractive for stimuli-responsive polymeric micelles. Both the heating process and the cooling process of a PEG-*b*-P(AzoPyl/C_9COOH) micellar solution exhibited a small endothermic peak at 54 °C and a small exothermic peak at 43 °C (Figure S3[7]). In micellar solutions, the PEG chains are completely hydrated and unable to be crystallized; thus, the outer shell does not exhibit the phase transition. From these facts, we consider that the endothermic peak observed at 54 °C indicates phase transition in the micellar interior of PEG-*b*-P(AzoPyl/C_9COOH). On the other hand, the micelle's exothermic peak at 43 °C corresponded to the bulk sample's peaks ranging from 55 to 42 °C, indicating that the exothermic peak of the micelle might have derived from the LC phase transition of AzoPyl/C_9COOH. These DSC results of the micellar solution samples imply that the PEG-*b*-P(AzoPyl/C_9COOH) micelle solution exhibited the phase-transition behavior in the micellar inner cores.

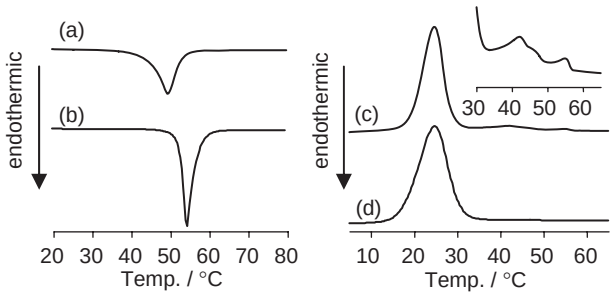

Figure 3. DSC curves (second scan) of bulk PEG-*b*-P(AzoPyl/C_9COOH) (5-11) ((a), (c)) and PEG-*b*-P(AzoPyl) (5-11) ((b), (d)). Here, (a) and (b) were in a heating process whereas (c) and (d) were in a cooling process. Inset shows exothermic peaks of PEG-*b*-P(AzoPyl/C_9COOH) from 30 to 65 °C in an expanded scale.

This polymeric micelle with the LC inner core may be very useful as a component of drug carriers. Furthermore, the nanosized LC inner core of the micelle is very interesting in regard to the physicochemical sciences. Regarding PEG-*b*-P(AzoPyl/carboxylic acid) complex micelles, future studies should investigate (1) control of drug incorporation, (2) drug release by temperature, and (3) possible evaluations of the LC phase transition in micellar inner cores.

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7 Supporting Information is also available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

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