Thermoresponsive Polyphosphoesters Bearing Enzyme-cleavable Side Chains

Yasuhiko Iwasaki,*[1] Takashi Kawakita,[1] and Shin-ichi Yusa[2]
[1]*Faculty of Chemistry, Materials and Bioengineering, Kansai University, 3-3-35 Yamate-cho, Suita 564-8680*
[2]*Graduate School of Engineering, University of Hyogo, 2167 Shosha, Himeji 671-2280*

(Received July 29, 2009; CL-090708; E-mail: yasu.bmt@ipcku.kansai-u.ac.jp)

Thermoresponsive polyphosphoesters bearing acetoxymethyl (AM) groups were newly synthesized. The polymers had the LCST-type cloud point in aqueous media, and the thermoresponsive phenomena was influenced by the condition of the medium. About 40% of the AM groups in the polymers were cleaved in contact with esterase for 24 h in phosphate buffer solution. This was significant compared with the spontaneous cleavage in the buffer solution. After the cleavage of the AM groups, the thermoresponsivity of the polyphosphoesters was diminished.

Thermoresponsive polymers are being widely studied in both research and technology because of their versatility in many fields. We have recently discovered that aqueous solutions containing polyphosphoesters bearing simple alkyl chains demonstrated LCST (lower critical solution temperature)-type phase separation behavior.[1] Polyphosphoesters have high importance in bio-related fields because of their biocompatibility and structural similarities to naturally occurring nucleic and teichoic acids.[2,3] The phosphoester backbone is degradable through spontaneous hydrolysis; the degradation is accelerated by enzymatic treatment.[4] In addition, a variety of polyphosphoesters can be designed in comparison with conventional biodegradable polymers because cyclic phosphates are obtained as monomers from the condensation of alcohol and chloro-cyclic phosphate.[5] Through the use of some alcohol compounds, biodegradable macrocrosslinkers[4] and macroinitiators for atom-transfer radical polymerization[6] have already been prepared. These polyphosphoesters can serve as building blocks for constructing novel biomaterials.

Very recently, Wang and co-workers have synthesized well-defined block copolymers of poly(ethylene glycol) and polyphosphoester.[7] The block copolymers can form core–shell polymeric micelles in an aqueous medium with the incrementation of temperature due to self-association of the polyphosphoester block. Although it is clear that polyphosphoester is a new candidate thermoresponsive polymer,[1,7] the solution properties partially evaluated.

In bioscientific applications, thermoresponsive polymers have great potential.[8] In particular, the selective delivery of drugs to target sites through hyperthermia could be performed.[9] However, heat treatment might induce adverse effects on normal tissue, and limitations remain in terms of selectivity. We then synthesized a new polymer whose thermoresponsivity can be regulated by enzymatic treatment.

Figure 1 shows the chemical structure of a polyphosphoester bearing acetoxymethyl ester groups (PEHA).[10] The acetoxymethyl bromide partially reacts with the phosphate units generated by deprotection of the benzyl groups in the side chains. The final molar fraction of the monomer units of PEHA was 0.92/0.02/0.06 (EP/–OH/AM). The range of M_n was 6.0×10^3–7.0×10^3 g mol^{-1} and no significant change in molecular weight was observed during immobilization.

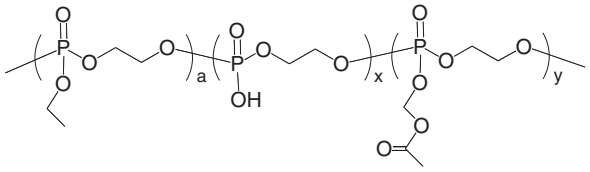

Figure 1. Chemical structure of PEHA.

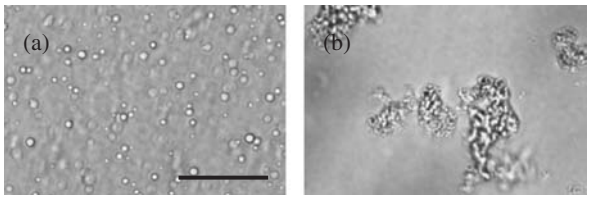

Figure 2. Phase contrast micrographs of PEHA in (a) 150 mM NaCl aqueous solution and (b) PBS at above LCST (bar = 50 μm).

Digestion of the AM groups of the PEHA was determined by treatment of porcine liver esterase (Sigma-Aldrich, St. Louis, MO, USA). PEHA (0.5 g) was dissolved in PBS (9 mL) and then 1 mL of esterase (400 U mL^{-1}) was added. The samples for structural analysis were corrected after purification with ultrafiltration (MWCO: 10000) at 4 °C and freeze-dried.

The cloud points of PEHA and esterase-treated PEHA in PBS were determined by transmittance measurements using a JASCO V-650 UV–vis spectrophotometer (Tokyo, Japan). PEHA was dissolved in an aqueous medium, and a transmittance curve through the solution at a wavelength of 500 nm was recorded as the temperature was varied (0.2 °C min^{-1} increments).

Figure 2 shows phase-contrast micrographs of the aqueous PEHA solution. In 1 wt % PEHA polymer solution containing 150 mM NaCl, liquid–liquid phase separation occurred above the LCST. On the other hand, precipitation of PEHA occurred in phosphate buffer saline (PBS; [NaCl] = 136.9, [KCl] = 2.7, [KH_2PO_4] = 1.5, and [Na_2HPO_4] = 8.1 mM). Two major phenomena occur in thermoresponsive polymers. One is coil–globule phase transition, which is observed in poly[*N*-isopropylacrylamide (NIPAAm)]. The other is the exhibition of coacervation accompanying the liquid–liquid phase separation. The polyphosphoesters bearing alkoxy groups such as ethoxy and isopropoxy groups formed coacervation in PBS and did not cause precipitation above the LCST. In contrast, aggregation involving coil–globule transition was observed in PBS containing PEHA, as shown in Figure 2. The small amount of phosphates in PBS might affect the thermoresponsive phenomena of PEHA because no aggregation of PEHA was observed in 150 mM aqueous NaCl

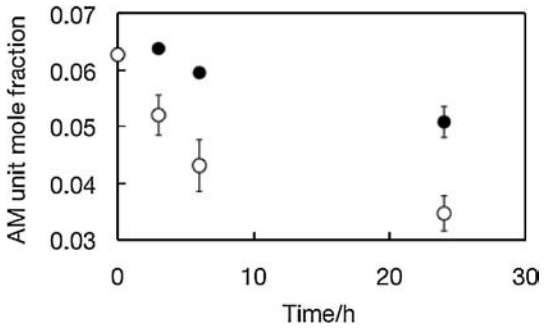

Figure 3. Change in unit mole fraction of acetoxymethyl ester group of PEHA in contact with porcine liver esterase (●) in PBS, (○) in esterase solution, [Esterase] = 40 U mL^{-1}.

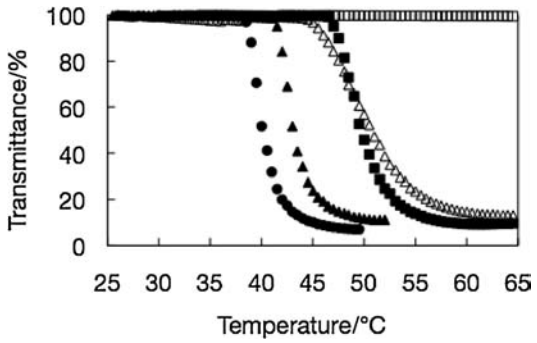

Figure 4. Thermoresponsivity of PEHA in PBS before and after incubation with porcine liver esterase for 6 and 24 h. (●) 0, (▲) 6, and (■) 24 h in PBS; (△) 6 and (□) 24 h in esterase solution.

solution. The phase separation temperature of polymer solutions was commonly decreased with an increase in the ion strength and polymer concentration. A more detailed study of the mechanism of thermoresponsivity will be reported later.

The enzymatic digestion of acetoxymethyl esters from PEHA was evaluated in contact with porcine liver esterase for a given time. Figure 3 shows the time dependence of the relative fraction of the acetoxymethyl groups on the EP units. The data are represented as the mean from 4 samples. When PEHA was treated with the enzyme, the decrease in the fraction of AM groups was dramatic compared to that soaked in PBS for 24 h. The fraction then gradually decreased over time. Geurtsen and co-workers reported that the activity of porcine liver esterase decreased during the first 24 h to approximately 40% and then remained constant for up to 6 days.[11] Even in synthetic polymer systems, the effect of esterase has been observed. The AM groups spontaneously degraded in PBS. The rate of degradation at the early stage was much slower than that of the esterase treatment.

The thermoresponsivity of PEHA before and after contact with protease is shown in Figure 4. The PEHA/PBS showed the LCST-type cloud point at 40 °C. In both PBS and that with esterase, the temperature of the cloud point increased with an increase in incubation time. In particular, the PEHA treated with esterase for 24 h did not have a cloud point between 20 and 65 °C. The density of AM groups on the polymer influenced the thermoresponsivity of the polymer; that is, the clouding phenomena could be controlled by acetoxymethylation of the polyphosphoesters. In addition, the polymer before acetoxymethylation (PEH) did not have a LCST (data not shown). While the cloud point of PEHA synthesized in this study was beyond physiological condition (>40 °C), it could be adjusted by introducing more hydrophobic units into the polymer as described in previous literature.[6] Because the block copolymers composed of polyphosphoesters and poly(ethylene glycol) form a micelle structure above the LCST,[7] PEHA will serve as building blocks for making enzyme-responsive micelles.

In summary, we synthesized thermoresponsive polyphosphoesters bearing AM groups as side chains. The thermoresponsivity of the polymers in aqueous solution depended on the concentration of the AM groups. Cleavage of the AM group and degradation of the polymer chain were accelerated with esterase treatment. The AM group is widely used for prodrugs and for fluorescence probes for cell imaging.[12] This group effectively induces cell membrane penetration and is rapidly cleaved intracellularly.[13] The use of polyphosphoesters bearing AM groups as drug carriers will require further molecular design to obtain self-assembly, stealth, and targeting characteristics. However, the newly designed structure is appealing as a basic motif for application.

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References and Notes

1 Y. Iwasaki, C. Wachiralarpphaithoon, K. Akiyoshi, *Macromolecules* **2007**, *40*, 8136.
2 S. Penczek, J. Pretula, K. Kaluzynski, *Biomacromolecules* **2005**, *6*, 547.
3 Z. Zhao, J. Wang, H. Q. Mao, K. W. Leong, *Adv. Drug Delivery Rev.* **2003**, *55*, 483.
4 C. Wachiralarpphaithoon, Y. Iwasaki, K. Akiyoshi, *Biomaterials* **2007**, *28*, 984.
5 R. S. Edmundson, *Chem. Ind. (London)* **1962**, 1828.
6 Y. Iwasaki, K. Akiyoshi, *Macromolecules* **2004**, *37*, 7637.
7 Y.-C. Wang, L.-Y. Tang, Y. Li, J. Wang, *Biomacromolecules* **2009**, *10*, 66.
8 L. Klouda, A. G. Mikos, *Eur. J. Pharm. Biopharm.* **2008**, *68*, 34.
9 A. Chilkoti, M. R. Dreher, D. E. Meyer, D. Raucher, *Adv. Drug Delivery Rev.* **2002**, *54*, 613.
10 Supporting Information is available electrically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.
11 W. Geurtsen, G. Leyhausen, F. Garcia-Godoy, *Dent. Mater.* **1999**, *15*, 196.
12 S. J. Hecker, M. D. Erion, *J. Med. Chem.* **2008**, *51*, 2328.
13 C. Schultz, M. Vajanaphanich, A. T. Harootunian, P. J. Sammak, K. E. Barrett, R. Y. Tsien, *J. Biol. Chem.* **1993**, *268*, 6316.

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