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Novel Thermoresponsive Polymers Having Biodegradable Phosphoester Backbones

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Introduction. Thermoresponsive polymers are widely studied in both research and technology because of their versatility in many fields. Recent trends in polymer materials are drug delivery,[1] separation of bioactive molecules,[2] and tissue engineering.[3] N-substituted acrylamide polymers have been found to have a phase separation characteristic with changes occurring in their properties upon heating above a certain lower critical solution temperature (LCST).[4−6] In particular *N*-isopropylacrylamide (NIPAAm) is one of the best monomers for accomplishing this and the homopolymer has LCST at 32 °C in aqueous solution.[7] NIPAAm can be applied to polymerization with a wide variety of comonomers and the LCST of the polymers can be controlled around physiological temperatures.[8,9] Furthermore, living radical polymerization has recently been applied with NIPAAm for the preparation of smart polymers with well-defined structures.[10−12] Although NIPAAm is a robust monomer for obtaining thermoresponsive polymer materials such as stimuli-responsive surfaces, particles, and hydrogels, the polymers are not biodegradable.

As well as a stimuli-responsive nature, biodegradability and biocompatibility are important characteristics for polymeric materials used in biomedical fields. While the thermoresponsivity of some biodegradable polymers such as aliphatic polyester block copolymers or polypeptides was recently proposed,[13−16] the molecular design and synthetic process of thermoresponsive biodegradable polymers are still limited. N-substituted acrylamide polymers are thus preferably studied.

Recently, polyphosphoesters have appeared interesting for biological and pharmaceutical applications because of their biocompatibility and structural similarities to naturally occurring nucleic and teichoic acids. Polyphosphoesters have been proposed for use in the field of biomaterials.[17−20] A variety of synthetic routes for polyphosphoesters has been proposed including ring-opening polymerization,[21,22] polycondensation,[23] transesterfication,[24,25] and enzymatic polymerization.[26] There has been a great deal of interest in polyphosphoesters, which are biodegradable through hydrolysis and possibly through enzymatic digestion of phosphate linkages under physiological conditions.[27] Although polyphosphoesters are very interesting polymers, there is no report of any thermoresponsive properties. In current research, thermoresponsive polyphosphoesters are being newly synthesized with simple copolymerization of cyclic phosphoester compounds and their properties are being investigated.

Results and Discussion. 2-Ethoxy-2-oxo-1,3,2-dioxaphospholane (EP) and 2-isopropoxy-2-oxo-1,3,2-dioxaphospholane (IPP) were synthesized by the previously described method.[28] Poly(IPP-*co*-EP) (PI_xE_yP; x = IPP (mol %); y = EP (mol %)) was synthesized by ring-opening polymerization using triisobutyl aluminum (iBu$_3$Al) as an initiator (Scheme 1). The polymerization was homogeneously performed by a solvent-free reaction. The polymers were dissolved in ethanol and purified by reprecipitation into diethyl ether. The range of weight-averaged molecular weights was 1.2×10^4 to 1.5×10^4 g/mol by gel-permeation chromatography through a Polymer Laboratories MIXED-C column using a calibration curve based on linear polystyrene standards and their molecular weight distribution was lower than 1.3. Chloroform was the GPC solvent. The molar fraction of IPP and EP in the copolymer was calculated from a ^{1}H NMR spectrum. ^{1}H NMR (270 MHz, $CDCl_3$): δ = PI_xE_yP: 1.21−1.47 (m; $-CH_3$, 6H in IPP and 3H in EP), 3.95−4.20 (m; $-CH_2-$, 2H in EP and -OCH_2CH_2O-, 4H in backbone), 4.58 (m; $-CH(CH_3)_2$ in IPP, 1H).

The polymerization ratio (r_1/r_2) of IPP and EP was 0.48/2.23 as determined by the Fineman-Ross method. The reactivity of EP was much higher than that of IPP. Chen and co-workers compared the polymerization ability of IPP and EP using stannous octoate as an initiator.[29] In their data, the higher reactivity of EP was also observed.

Figure 1 is a typical photograph of aqueous solutions of $PI_{24}E_{76}P$, which has 24-mol % IPP and 76-mol % EP. The solution was transparent at 20 °C, but it was turbid at 40 °C. The LCST of $PI_{24}E_{76}P$ was 31 °C, as determined from the middle point of the transition state of transmittance using JASCO software.

Figure 2 shows the effect of the composition of the monomer unit on the LCST of the copolymers. The LCST of poly(EP) (PEP) was 38 °C and it linearly decreased with an increase in the composition of IPP. IPP is relatively hydrophobic, the homopolymer of IPP is not soluble in water above 5 °C. Dehydration of the polymer then preferably occurred with the addition of the hydrophobic IPP unit. It is reported that the LCST of thermoresponsive polymers can be controlled by compositions of hydrophobic and hydrophilic units.[8,15] Thermoresponsivity under physiological conditions is effective for drug delivery or tissue engineering applications.[30,31] The thermoresponsivity of polyphosphoesters can also be observed under physiological temperatures. Thus, the polymers are applicable in biomedical field.

Figure 3 shows the repeated temperature dependence of the transmittance of light through a $PI_{24}E_{76}P$ aqueous solution. While the hysteresis of change in transmittance between the variations in temperature was observed, the curve coincided well with the variation regardless of the number of repetitions. The polymer associate then completely disintegrated at the low temperature. The hydrodynamic radii (R_h) of $PI_{24}E_{76}P$ in an

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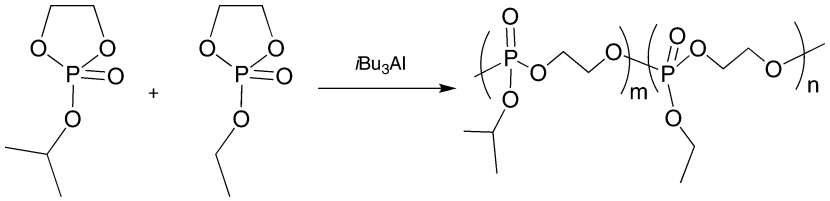
Scheme 1. Synthetic Route of PI_xE_yP



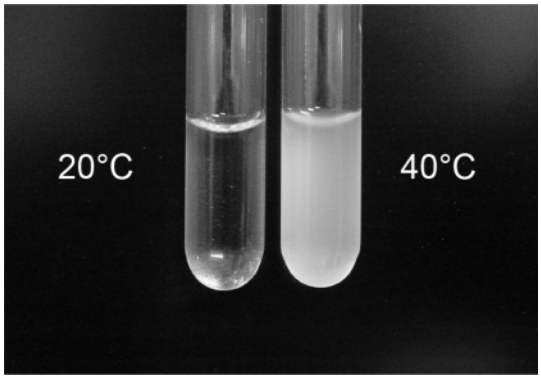


Figure 1. Photograph of $PI_{24}E_{76}P$ aqueous solution at 20 and 40 °C.

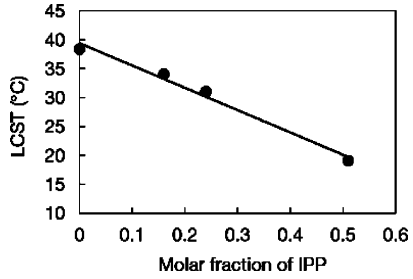


Figure 2. Effect of molar fraction of IPP on LCST of PI_xE_yP.

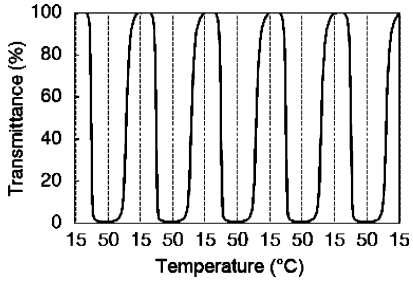


Figure 3. Change in the transmittance of polymer solution by repeated thermal cycling.

aqueous solution were also investigated using the Malvern dynamic light scattering technique. When the R_h of $PI_{24}E_{76}P$ at below 20 °C was 6.7 ± 0.1 nm, the polymer associate with more than 6 μm R_h was formed at 50 °C. The phase separation behavior of the polymer was easily reproducible.

Hydrogels[30,31] and nanoparticles[32,33] have been prepared using polyphosphoesters as macrocross-linkers and macroinitiators, respectively. In the former literatures, the enzymatic digestion and nonenzymatic degradation of polyphosphoesters were investigated. Typically, the rate of degradation of a hydrogel cross-linked with polyphosphoesters was increased with an increase in the concentration of alkaline phosphatase in the soaking medium. Furthermore, the cytotoxicity of the polyphosphoesters and the degradation products were not observed. These polyphosphoesters have been recently studied as artificial cellular matrices and nanoparticles for drug delivery systems.

The possibility of varied molecular designs and changeable solubility of polyphosphoesters can be looked upon as an advantage of the polymers in comparison with conventional biodegradable polymers such as aliphatic polyesters.

In conclusion, thermoresponsive polyphosphoesters were newly obtained from the copolymerization of two cyclic phosphoester monomer components. The LCST of the copolymers could be easily controlled with the composition and the thermoresponsivity of the polymers was ably reproducible. Thermoresponsive polyphosphoesters have great potential as novel smart biomaterials.

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Supporting Information Available: Text giving experimental procedures and synthetic results of polymers, a table of synthetic results, and a figure showing the effect of temperature on light transmittance. This material is available free of charge via the Internet at http://pubs.acs.org.

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