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# A Subtle End-Group Effect on Macroscopic Physical Gelation of Triblock Copolymer Aqueous Solutions\*\*

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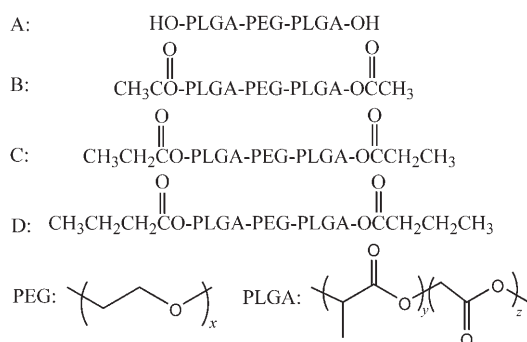
During the last few decades, many researchers have investigated the self-assembly behavior of amphiphilic block copolymers. Most of these studies concerned nanostructures that resulted from self-assembly. In contrast, we have studied the end-group effect on the macroscopic self-assembly, which leads to formation of a biocompatible hydrogel. Although fruitful phenomena related to the end-group effect on microscopic molecular conformation and nanoscale aggregates have been observed in various organic molecules in selective solvents,<sup>[1–4]</sup> reports of the end-group effect on the condensed-state behavior of self-assembled soft matter are rather limited.

As a biomimetic wet material, polymeric hydrogel is a very important type of soft matter. Hydrogels capable of responding to external influences, such as temperature,<sup>[5–8]</sup> pH,<sup>[9]</sup> light,<sup>[10,11]</sup> counterion changes,<sup>[12]</sup> biological molecules,<sup>[13]</sup> and multiple factors,<sup>[14,15]</sup> have been extensively investigated, as they have great potential value in drug-delivery systems and tissue engineering,<sup>[16–19]</sup> sensors and valves,<sup>[20,21]</sup> surface patterning,<sup>[22,23]</sup> and tunable optics.<sup>[24]</sup> Some biodegradable/bioabsorbable hydrogels can form a gel in situ, and therefore are especially attractive as injectable biomaterials.<sup>[16,25–27]</sup> Amorphous and biodegradable polymers with therminduced shape memory are also promising for use in minimally invasive surgery.<sup>[28–30]</sup> Most hydrogels are formed by irreversible chemical cross-linking, but reversible physical gelation is another interesting topic in both fundamental research and potential applications. Recently, a few stimuli-responsive biodegradable physical gels have been reported in some multicomponent polymers, for instance, poly(ethylene glycol)–poly( $\alpha$ -hydroxy acid),<sup>[16,31]</sup> poly(ethylene glycol)–polycaprolactone,<sup>[6,7]</sup> chitosan–glycerol phosphate,<sup>[32]</sup> and copolypeptides.<sup>[33,34]</sup>

Herein, we report the discovery that an alkyl end group can induce a thermoreversible physical gelation in an otherwise sol-like suspension. It is well-known that an end group with an opposite lyophilicity can lead to the formation of nanoparticles through self-assembly.<sup>[35]</sup> Nevertheless, the alkyl end-group effect leading to macroscopic gelation reported herein is rather unique. Such a subtle effect for biodegradable synthetic molecules in water is very interesting and meaningful for biomaterials and other applications.

A series of barbell-like ABA-type triblock copolymers of poly(ethylene glycol) (PEG) and poly(lactic acid-co-glycolic acid) (PLGA), that is, PLGA-PEG-PLGA with different end caps, were synthesized and characterized. PEG is hydrophilic, whereas PLGA is hydrophobic and biodegradable by the hydrolysis of ester bonds.<sup>[19,30,36,37]</sup> Both PEG and PLGA are biocompatible and have been clinically used as implant biomaterials. The block copolymer composed of PLGA and PEG with the composition described is, by itself, not transformed into gels by changing the temperature, but we found that some of its derivatives exhibited a reversible sol–gel transition as a function of temperature. A very subtle end-group effect on the macroscopic self-assembly of soft matter has thus been revealed. The mechanism of the underlying sol–gel transition is also discussed.

The symmetric PLGA-PEG-PLGA copolymer with two hydroxy end groups (sample A) was synthesized by the ring-



opening polymerization of DL-lactide and glycolide in the presence of PEG. Its derivatives with other end-capping groups (samples B–D) were prepared through the alcoholysis of acyl chloride (see Supporting Information). Formation of the PLGA-PEG-PLGA triblock copolymer and its derivatives was confirmed by <sup>1</sup>H NMR spectroscopy in CDCl<sub>3</sub>. The extent of esterification was higher than 90% for all derivatives.

Gel-permeation chromatography (GPC) was used to determine the molecular weight of the polymers and its distribution. All of the GPC traces of the triblock copolymer and its derivatives were unimodal with a polydispersity of less than 1.2. Data for the four samples are listed in Table 1.

The original PLGA-PEG-PLGA block copolymer is quite hydrophilic, and thus sample A was soluble in water (Figure 1a). However, a physical gel was observed at room temperature when the hydroxy end groups were replaced with acetate groups (Figure 1b). The sample with propionate end groups also formed a gel (Figure 1c). The end-group effect is

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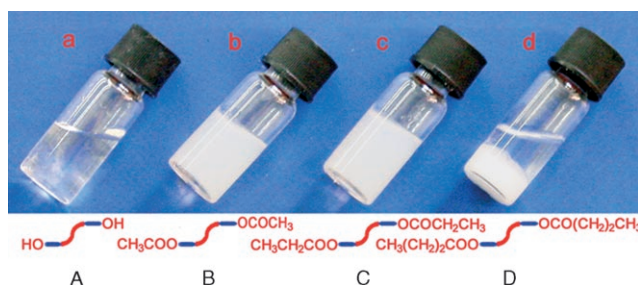
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**Table 1:** Characteristics of the four samples A–D.

Sample	$M_n^{[a]}$	LA/GA [mol/mol] <sup>[b]</sup>	$M_n^{[c]}$	$(M_w/M_n)^{[c]}$	CMC [wt%] <sup>[d]</sup>	CGC [wt%] <sup>[e]</sup>
A	900-1000-900	1.6	3172	1.14	$0.047 \pm 0.005$	–
B	943-1000-943	1.6	3240	1.13	$0.034 \pm 0.003$	16
C	957-1000-957	1.6	3389	1.15	$0.027 \pm 0.002$	14
D	971-1000-971	1.6	3484	1.11	–	–

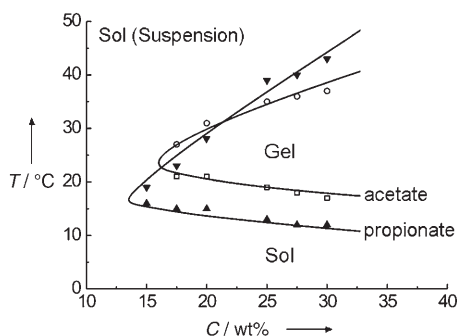
[a] The number-average molecular weight ( $M_n$ ) of the central block PEG was provided by Aldrich. [b] The molar ratio of lactide/glycolide (LA/GA) and thus the  $M_n$  of each PLGA block was calculated from  $^1\text{H}$  NMR experiments. [c] The  $M_n$  of the copolymers and their polydispersity indexes ( $M_w/M_n$ ) were measured by GPC. [d] The critical micelle concentration (CMC) was measured by the method of hydrophobic probe solubilization at 25 °C. [e] The critical gel concentration (CGC) was obtained from the phase diagram in Figure 2.



**Figure 1.** a–d) Images of the triblock copolymer/water mixtures showing the sol, gel, gel, and precipitation states for samples A–D, respectively. Concentration: 25 wt%; temperature: 25 °C.

so subtle that the butyrate end groups led to precipitation in the whole temperature region (from 0 to 50 °C, Figure 1d).

The condensed states of samples B and C were highly dependent on temperature. The phase diagrams of the PLGA-PEG-PLGA derivatives with acetate and propionate end groups are shown in Figure 2. The sol–gel transition was determined by the test-tube inversion method.<sup>[31]</sup> The sample was regarded as a gel if no flow was observed 30 s after the vial was inverted. Both the upper critical solution temperature (UCST) and lower critical solution temperature (LCST) were established. The polymer–water mixtures had three



**Figure 2.** Phase diagrams of PLGA-PEG-PLGA block copolymers B and C in water at various temperatures  $T$  and concentrations  $C$ . The symbols  $\blacktriangle$  and  $\blacktriangledown$  indicate the boundaries of sol–gel and gel–sol (suspension) for sample B, while  $\square$  and  $\circ$  indicate these boundaries for sample C.

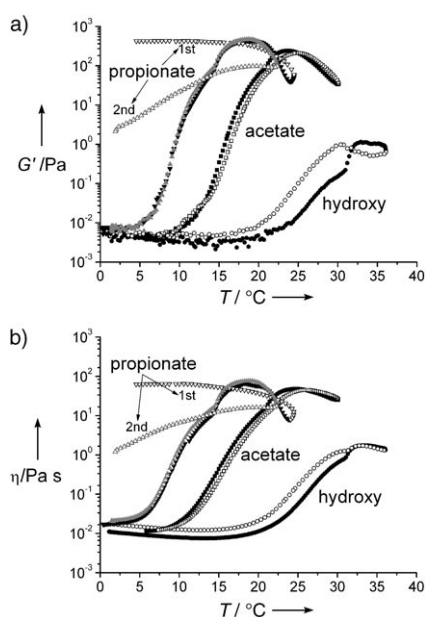
physical states at 0 to 50 °C: solution, gel, and re-solution again (the re-solution was actually a flowable suspension that eventually led to precipitation). The degradation time was on the order of weeks (data not shown), whereas the measurement of a phase diagram lasted for 8–10 h. Material degradation during the measurement should be insignificant, and therefore the approach to determining the phase diagrams is valid even for such a biodegradable material.

Figure 2 also indicates that the critical gel concentration (CGC) drops from 16–14 wt% on changing the end group from the acetate to the propionate group. The increase of hydrophobicity of the end group strengthens the hydrophobic interaction between chains, which is responsible for the decrease of the CGC and also of the sol–gel phase-transition temperature.

Rheological measurement or dynamic mechanical analysis was used to obtain the storage modulus  $G'$  and viscosity  $\eta$  (associated with dissipative modulus  $G''$ ).  $G'$  and  $\eta$  are measures of the load-bearing capacity and dissipated energy, respectively, when a material undergoes a cyclic deformation. An abrupt increase of both  $G'$  and  $\eta$  was found with an increase in temperature. The rheological measurements demonstrated that even the original PLGA-PEG-PLGA block copolymer could exhibit significant thermosensitivity. (The highest viscosity was not large enough to allow the aqueous solution to act like a “gel”, and thus the thermoinduced transition of the virgin polymer was hidden in the test-tube inversion experiment.) The data from different samples were similar at low temperatures, but were very different at high temperatures. The results at low and high temperatures (Figure 3) indicate that the thermosensitivity of the derivative capped with alkyl groups was much more significant than that of the virgin polymer with the hydroxy end groups, as the mechanical quantities spanned more than four orders of magnitude for samples B and C but just two orders of magnitude for sample A. Figure 3 also reveals that the transition temperature was lowered with an increase in hydrophobicity of the end groups, which was consistent with the measurements from the test-tube inversion experiments (Figure 2).

Our rheological measurements confirm that despite hysteresis, the sol–gel transition is basically thermoreversible. Recovery to the sol state on cooling sample C occurred rather slowly because of the more hydrophobic propionate end groups, and thus a very striking hysteresis was observed during the first heating and cooling cycle. Nevertheless, after further cooling for a sufficiently long time, the second heating process resulted in a good consistency with the first heating cycle (Figure 3). Hence, the sol–gel transition in our samples is thermodynamically reversible.

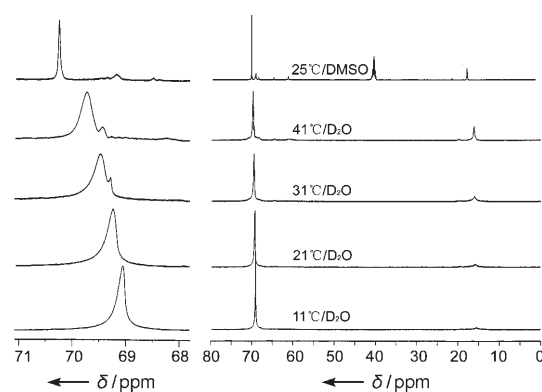
A question now arises: do the amphiphilic molecules form a gel straightforwardly or through a hierarchical mechanism, namely, first forming micelles and then forming a gel by further aggregation of percolated micelles? A comparison of the critical micelle concentration (CMC) and CGC should thus be performed. The CMC was determined by the hydrophobic dye solubilization method with 1,6-diphenyl-1,3,5-hexatriene (DPH) as the probe,<sup>[38]</sup> and the CGC was obtained from the phase diagrams in Figure 2 by the test-tube inversion



**Figure 3.** a) Storage modulus  $G'$  and b) viscosity  $\eta$  of the block copolymers PLGA-PEG-PLGA with hydroxy, acetate, and propionate end groups in aqueous solution (20 wt %) as a function of temperature  $T$  in dynamic rheological measurements. Heating and cooling rates:  $0.5 \text{ K min}^{-1}$ ; oscillatory frequency:  $10 \text{ rad s}^{-1}$ . The filled and empty symbols represent the data resulting from heating and cooling, respectively. After the first heating and cooling cycle, sample C and the coaxial Couette cylinder were placed in a refrigerator at  $4^\circ\text{C}$  for 12 h; then a second heating and cooling cycle was recorded.

method. Table 1 summarizes the data for the PLGA-PEG-PLGA triblock copolymer and its derivatives. According to these results, the CGC is much higher than the CMC for all of the available samples. Therefore, micelles must have been formed before gelation.

Dynamic light scattering showed that the micelles from samples A–C had average hydrodynamic radii of 11.3, 18.6, and 22.2 nm, respectively, measured at  $14^\circ\text{C}$  at a low concentration (0.5 wt %). Micellar aggregates were also detected with an increase of temperature or concentration. Strong evidence for the integrality of micelles in aggregation came from NMR experiments. To interpret the molecular mechanism of the sol–gel phase transition, the  $^{13}\text{C}$  NMR spectra<sup>[31]</sup> of the polymers were measured in both water and dimethyl sulfoxide (DMSO; Figure 4). DMSO is a good solvent for all of the copolymer blocks, so both the methyl group of PLGA and the ethylene group of PEG are shown as sharp peaks in  $[\text{D}_6]\text{DMSO}$ . Water is a good solvent for PEG but a poor solvent for PLGA. Therefore in water, the methyl group (15–16 ppm) of PLGA appears as a broadened and collapsed peak in the sol state ( $11^\circ\text{C}$ ), whereas the ethylene group (69–70 ppm) of PEG appears as a sharp peak. During the sol–gel transition ( $21^\circ\text{C}$ ), these characteristics in the NMR spectra remained unchanged, thus indicating no considerable change in the core–corona micellar structure during physical gelation. On increasing the temperature to  $31^\circ\text{C}$  (the gel state), the PEG peak split into two separate subpeaks, with a strong subpeak at  $\delta = 69.5 \text{ ppm}$  and a weak one at  $\delta = 69.3 \text{ ppm}$ , and the height of the peak from the methyl group of



**Figure 4.**  $^{13}\text{C}$  NMR spectra of PLGA-PEG-PLGA copolymer with acetate end groups (sample B) in  $\text{D}_2\text{O}$  (25 wt %) at the indicated temperatures. The solution was equilibrated for 20 min at the setting temperature before measurement. The spectrum in  $[\text{D}_6]\text{DMSO}$  is shown as control.

PLGA was slightly enhanced. This behavior might have been caused by an increase in the thermal motion of the hydrophobic chain and the disturbance of the micellar structure. At a higher temperature ( $41^\circ\text{C}$ ) the PEG peak split further, whereas the height of the peak from the methyl group of PLGA increased significantly. This finding suggests that the core–corona structure of the micelles in the aqueous environment was broken and the hydrophobic blocks were exposed to water, which led to a re-sol state at  $41^\circ\text{C}$  as shown in the phase diagram (Figure 2).

Combination of the data in Figures 1 to 4 confirms the hierarchical mechanism for the physical gelation studied herein: the amphiphilic block copolymers are first assembled into micelles; then the micelles are further aggregated to form a macroscopic gel, and an intact micellar structure is kept during the sol–gel transition. Although the quantitative theoretical prediction of the phase-transition point is still open, the LCST behavior implies that both micellization and gelation are driven by an entropy effect that results from the hydrophobic interaction. The alkyl group is rather hydrophobic and the gelation is very sensitive to the end groups. The sol–gel transition cannot happen unless the hydrophobic interaction is strong enough to induce the large-scale self-assembly of micelles. Gelation also cannot occur when the micellar structure is broken at higher temperatures, or when the block copolymer derivative is over-hydrophobic and leads to macroscopic phase separation between polymer and water. Therefore, the end-group effect is especially significant when an amphiphilic block copolymer has a moderate lyophilicity with a subtle balance between hydrophilicity and hydrophobicity.

In summary, PLGA-PEG-PLGA and its derivatives were synthesized, and a surprising end-group effect on the macroscopic self-assembly behavior was found. The aqueous solution of the PLGA-PEG-PLGA triblock copolymer exhibited a sol state in the temperature range examined. In contrast, the polymer derivatives end-capped with acetate or propionate groups underwent a reversible sol–gel transition as a function of temperature, whereas the block copolymer capped with the butyrate group precipitated in water. The

mechanism of the sol–gel transition of the thermogelling derivatives was further discussed. Our study also reveals that a small end group in a moderately lyophilic polymer can adjust not only the microscopic self-assembled nanostructures, but also the macroscopic condensed-state behavior of the underlying soft matter as a result of large-scale self-assembly. Thus, this research gives some new insights into the relationship between physical gelation and chemical composition, and affords a molecule–design approach to preparing novel injectable biomedical materials and intelligent wet devices.

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