

Dual Thermal- and pH-responsive Polypeptide-based Hydrogels*

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Abstract Smart hydrogels have received increasing attention for their great potential for the applications in many fields. Herein, we report a facile approach to prepare a class of dual-responsive hydrogels assembled from synthetic statistical/block thermal-responsive copoly(L-glutamate)s copolymerized with poly(ethylene glycol), which were prepared by ring-opening polymerization (ROP) and post-modification strategy. The incorporation of oligo(ethylene glycol) (OEG) and glutamic acid residues offers the gels with thermal- and pH-responsive properties simultaneously. We have systematically studied the influence of both temperature and pH on the gelation behaviors of these copolymers. It is found that the increase of glutamic acid content and solution pH values can significantly suppress the gelation ability of the samples. Circular dichroism (CD) results show that the α -helix conformation appears to be the dominant secondary conformation. More interestingly, the gelation property of the block copolymer with statistical thermal-responsive copoly(L-glutamate)s shows greater dependence on pH as compared to that with block segments due to the distinct morphology of the self-assemblies. The obtained hydrogels exhibit pH-dependent and thermal-responsive gelation behaviors, which enable them as an ideal smart hydrogel system for biomedical applications.

Keywords pH-responsive; Thermal-responsive; Hydrogel; Polypeptide

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INTRODUCTION

Hydrogels are rigid and crosslinked networks^[1, 2]. Their high water content and controllable porous nanostructure offer many advantages in biomedical applications, such as tissue engineering, surgical dressings and drug delivery^[3–5]. Both natural polymers and synthetic materials have been used to prepare hydrogels for various applications^[3, 6, 7]. In particular, synthetic polypeptides that combine the advantages of synthetic polymers and biological performances of peptides have received a great deal of interest^[8, 9]. The secondary conformation of polypeptides provides the hydrogels with complementary structural stability^[10, 11]. More importantly, a diversity of amino acid residues derived from natural proteins and functional moieties by post-modification strategy make polypeptides good candidates for stimuli-responsive hydrogels^[12–14].

Stimuli-responsive hydrogels (also referred as smart hydrogels) can rapidly respond in a controlled manner to the external stimuli, including temperature, pH, ionic strength, enzymes, *etc.* Considerable efforts have been contributed to the thermal-responsive hydrogels with sol-to-gel phase transition upon heating to the lower critical

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gelation temperatures (CGTs). The thermally induced gelation allows for incorporating drugs and cells at low temperature before gel formation at elevated temperature, which makes the thermal-responsive hydrogels as promising candidates in the applications of drug delivery and 3D scaffolds. In comparison to the most extensively studied thermal-responsive poly(*N*-isopropyl acrylamide) (PNIPAM) hydrogel, the thermal-responsive hydrogels based on polypeptide provide additional biocompatibility, biodegradability and structural tunability for biomedical applications^[15–19]. For example, Chen and co-workers reported the thermal-responsive hydrogels based on poly(L-glutamate)s bearing hydrophobic alkyl side chains^[20]. Jeong *et al.* synthesized a series of amphiphilic diblock copolypeptides and found that their aqueous solutions underwent sol-to-gel transitions with increasing temperature^[21]. We previously reported the oligo(ethylene glycol) (OEG) functionalized poly(L-glutamate) that showed highly tunable thermal-responsive properties^[22]. A series of thermal-responsive polypeptides and related hydrogels were thereafter prepared by our group^[23, 24]. In addition to thermal-responsive polypeptide hydrogels, pH-responsive polypeptide hydrogels have been widely investigated by incorporating ionic amino acid residues, such as poly(L-glutamate acid) and poly(L-lysine), in the structure of gelators^[25–27]. However, the polypeptide-based hydrogels simultaneously combining temperature- and pH-responsive properties have rarely been documented^[28].

In this work, we report a class of anionic thermal-responsive hydrogels from assembling of synthetic statistical/block copolymer by using poly(ethylene glycol) (PEG) as the macroinitiator. Our design strategy is to incorporate pH- and thermal-responsive polypeptide residues in the structure of polymeric gels. The influences of temperature and pH on the gelation behaviors of different copolymers are systematically studied. It is found that the critical gelation concentration (CGC) increased and the gel strength decreased with increasing the content of glutamic acid and pH of the system. We further demonstrate that the monomer distribution can also influence the gelation behavior of the copolymers, which is possibly due to the distinct morphology of the self-assemblies. In the case of diblock copolymer containing statistical thermal-responsive blocks, long fibril structure was formed to construct the 3D network hydrogel. The secondary conformation and mechanical property of hydrogels were studied by circular dichroism (CD) spectroscopy and rheological measurements, respectively.

EXPERIMENTAL

Materials and Methods

γ -Benzyl-L-glutamate *N*-carboxyanhydride (BLG-NCA) and γ -propargyl-L-glutamate *N*-carboxyanhydride (PLG-NCA) were synthesized according to the method described in the literature^[29]. Hexane and tetrahydrofuran (THF) were purified by passing through activated alumina columns prior to use. *N,N*-dimethylformamide (DMF) was stored over calcium hydride (CaH₂) and purified by vacuum distillation with CaH₂. Triethylene glycol monomethyl ether and benzoin dimethyl ether (DMPA) were purchased from Aladdin Ltd. α -Methoxy- ω -amino poly(ethylene glycol) (PEG-NH₂, $M_n = 2000 \text{ g}\cdot\text{mol}^{-1}$) was purchased from JenKem Technology Co, Ltd. Benzyl-L-glutamic acid and L-glutamic acid were purchased from GL Biochem (Shanghai) Ltd. All other chemicals were purchased from commercial suppliers and used without further purification unless otherwise stated. ¹H-NMR spectra were recorded on Bruker AV500 FT-NMR spectrometer. Tandem gel permeation chromatography (GPC) was performed at 50 °C using an SSI pump connected to Wyatt Optilab DSP with 0.02 mol·L⁻¹ LiBr in DMF as an eluent at flow rate of 1.0 mL·min⁻¹. All GPC samples were prepared at the concentration of about 5 mg·mL⁻¹. Circular dichroism spectra were recorded on an Applied Photophysics Chirascan CD Spectrometer. The solution was placed into a quartz cell with a pathlength of 0.1 cm. For the temperature-dependent experiments, the temperature of sample chamber, where the quartz cell was placed, was controlled by a water bath (from 25 °C to 65 °C). The samples were equilibrated at each temperature for at least 10 min before the CD measurements. Ellipticity ($[\theta]$ in deg·cm²·dmol⁻¹) = (milli degrees × mean residue weight)/(pathlength in millimeters × concentration of polypeptide in mg·mL⁻¹). The α -helix contents of the polypeptides were calculated by the following equation: α -helix content (%) = $(-[\theta_{222}] + 3000)/39000$.

Synthesis of Diblock/Triblock Copolymers

Diblock copolymer poly(ethylene glycol)-b-poly(γ propargyl-L-glutamate-co- γ benzyl-L-glutamate) (PEG-b-Poly(PLG-co-BLG))

mPEG-NH₂ ($M_n = 2000 \text{ g}\cdot\text{mol}^{-1}$) was heated at 50 °C and dried under high vacuum for 12 h, and then dissolved in anhydrous DMF in a Schlenk flask. A mixed solution of PLG-NCA (100 mg·mL⁻¹ in DMF) and BLG-NCA (100 mg·mL⁻¹ in DMF) with given ratio were added to the reaction flask. The polymerization was performed under a N₂ atmosphere for 72 h at room temperature, and then the solution was precipitated in an excess amount of diethyl ether. The product was washed twice with diethyl ether and dried for two days under vacuum, yield: 69%.

Triblock copolymer poly(ethylene glycol)-b-poly(γ propargyl-L-glutamate)-b-poly(γ benzyl-L-glutamate) (PEG-b-PPLG-b-PBLG)

mPEG-NH₂ was first heated at 50 °C and dried under high vacuum for 12 h, and then dissolved in anhydrous DMF in a Schlenk flask. The solution of PLG-NCA (100 mg·mL⁻¹ in DMF) was added to the reaction flask. The polymerization was performed under a N₂ atmosphere and monitored by observing the disappearance of characteristic peaks from NCA (1790 and 1850 cm⁻¹) using an FTIR spectrometer. After the peaks disappeared, additional BLG-NCA (100 mg·mL⁻¹ in DMF) was added. After stirring for 72 h at room temperature, the product was precipitated in an excess amount of diethyl ether, washed twice with diethyl ether and dried for two days under vacuum, yield: 67%.

Deprotection of block copolypeptides

The benzyl protective groups on PEG-b-Poly(PLG-co-BLG) were removed by reacting 4 equiv. of HBr (in HAc, $c = 33 \text{ wt}\%$) with BLG repeat units in CF₃COOH (40 mg·mL⁻¹) at room temperature for 2.5 h. The product of poly(ethylene glycol)-b-poly[(γ propargyl-L-glutamate)-co-(L-glutamic acid)] (PEG-b-Poly(PLG-co-GA)) was precipitated with an excess of diethyl ether and dried overnight under vacuum at 40 °C. The deprotection of block copolymers was carried out using the same protocol to give poly(ethylene glycol)-b-poly(γ propargyl-L-glutamate)-b-poly(L-glutamic acid) (PEG-b-PPLG-b-PGA).

General procedure for modification with OEG-SH

PEG-b-Poly(PLG-co-PGA) and DMPA ([DMPA]₀/[C $\equiv$ C]₀ = 0.05, molar ratio) were dissolved in DMF before the addition of OEG-SH ([SH]₀/[C $\equiv$ C]₀ = 10, molar ratio). The reaction mixture was carefully degassed through three cycles of freeze/thaw/pump and then irradiated by UV light for 3 h. The mixture was dialyzed against water for 3 days and freeze-dried to yield PEG-b-Poly[(PLG-g-EG)-co-PGA] as a white solid.

Hydrogel Preparation

Each sample with a given concentration was dissolved into the solvents with desired pH values, which was adjusted by HCl or NaOH solutions. The exact pH values of solutions were measured using a calibrated pH-electrode. The sol-to-gel transition temperature of the copolymers in water was determined by inverted tube method with a temperature increment of 1 °C per step. It was recorded if no flow was observed within 30 s.

Rheological Measurements

Rheological measurements were performed on a TA DHR-1 rheometer. A flate plate with a diameter of 40 mm was employed. In each measurement, ~150 μ L of a polymer solution was loaded onto the plate by a dropper. A strain amplitude of $\gamma = 0.2\%$ was used in temperature sweeps to ensure that the deformation was within the linear viscoelastic regime. Strain sweeps were measured at an oscillation frequency of 6 Hz.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra were recorded from 2000 cm⁻¹ to 1400 cm⁻¹ on a HORIBA-JOBIN YVON FluoroMax-4 spectrofluorometer. The solution samples at different pHs were cast on KBr plates before measurement and the sample at high temperature was prepared in an oven.

Transmission Electron Microscopy (TEM)

TEM images of the samples were recorded on FEI TECNAI 20. Hydrogels were diluted to 0.1 wt% using deionized water, and then negatively stained by 2% uranyl acetate. The sample at high temperature was prepared in an oven.

RESULTS AND DISCUSSION

mPEG-NH₂ ($M_n = 2000 \text{ g}\cdot\text{mol}^{-1}$) was used as a macroinitiator to obtain the diblock copolymer by ROP of a mixture of γ propargyl-L-glutamate NCA and γ benzyl-L-glutamate NCA. The polypeptide block is a random mixture of two monomers^[29, 30]. The ¹H-NMR spectra show that all of the peaks of block copolymers are well assigned, confirming the corresponding chemical structures (Fig. 1A). The poly(ethylene glycol)-*b*-poly(γ propargyl-L-glutamate)-*b*-(γ benzyl-L-glutamate) (PEG-*b*-PPLG-*b*-PBLG) triblock copolymer was synthesized by the sequential ROP of γ propargyl-L-glutamate NCA and γ benzyl-L-glutamate NCA, respectively, with

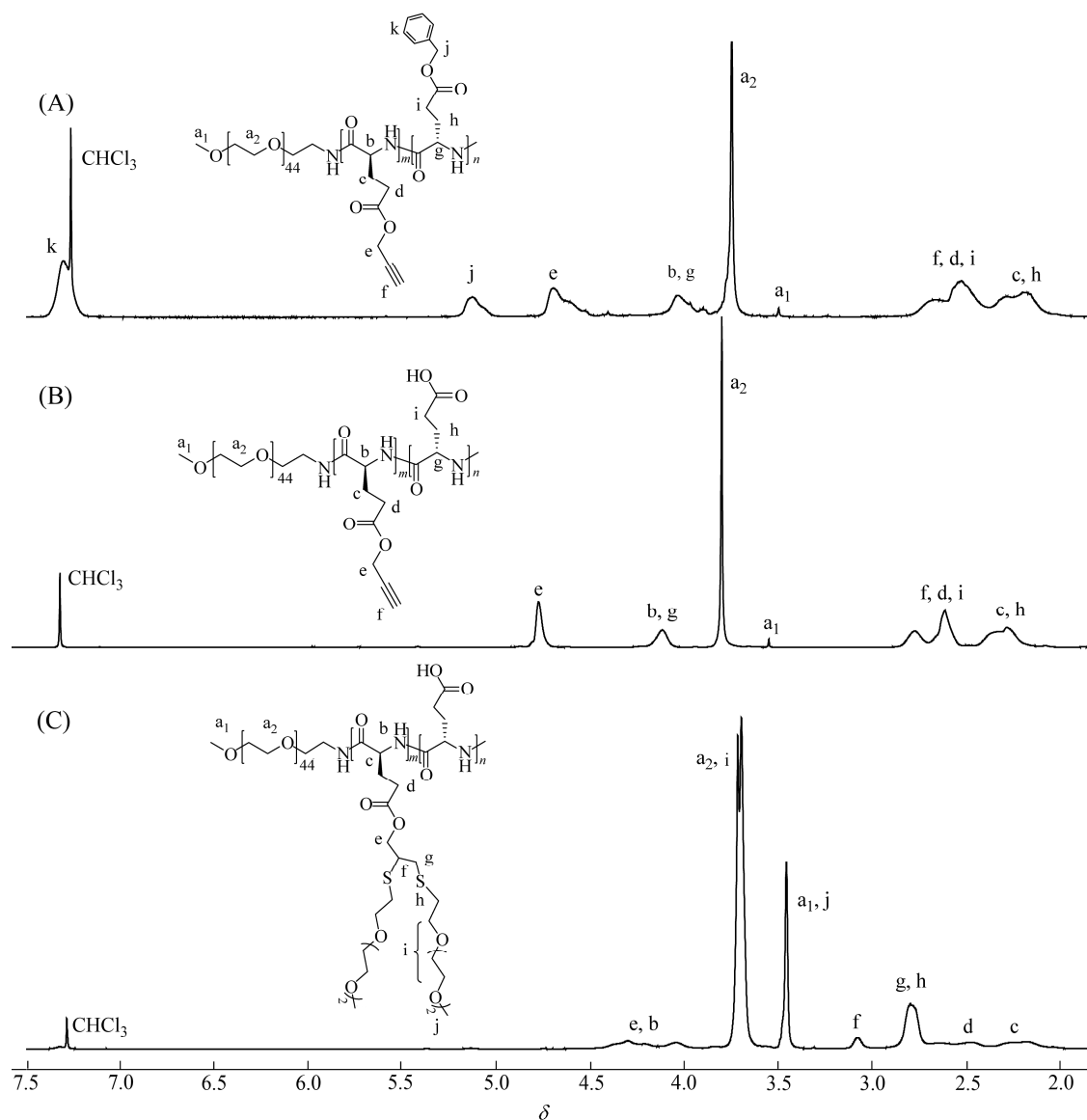


Fig. 1 ¹H-NMR spectra of (A) PEG-*b*-Poly(PLG_m-co-BLG_n), (B) PEG-*b*-Poly(PLG_m-co-GA_n) and (C) PEG-*b*-(PPLG-*g*-EG)-*b*-PGA in CDCl₃/CF₃CO₂D (*V*:*V* = 10:1)

mPEG-NH₂ as a macroinitiator. FTIR was used to monitor the conversion of NCA monomers to polypeptides. The chemical structure and degree of polymerization (DP) were confirmed by ¹H-NMR measurements (Fig. S1 in supporting information, SI). A series of triblock copolymer were synthesized by changing the ratio of NCAs to mPEG-NH₂ macroinitiator. The average DP of PPLG was 55 for most of the samples, and the average DP of PBLG was ranging from 12 to 52. The molecular characteristics of the copolymers along with their abbreviations are shown in Table 1. The diblock and triblock copolymers are denoted as PEG-*b*-Poly(PLG_{*m*}-*co*-BLG_{*n*}) and PEG-*b*-PPLG_{*m*}-*b*-PBLG_{*n*}, where the subscript *m* and *n* represent the average DP of PBLG and PPLG segments, respectively. The GPC traces indicated the narrow molecular weight distribution with dispersity (*D*) ≤ 1.26 for all of the block copolymers (Fig. S2 in SI).

Table 1 Molecular parameters of block copolypeptides

Samples	Feed ratio ^a	<i>m/n</i> ^b	<i>M_n</i> ^b (kDa)	<i>M_n</i> ^c (kDa)	Dispersity (<i>D</i>) ^c
PEG- <i>b</i> -Poly(PLG ₅₃ - <i>co</i> -BLG ₅₂)	60/60	53/52	22.9	23.1	1.26
PEG- <i>b</i> -Poly(PLG ₅₀ - <i>co</i> -BLG ₂₅)	60/30	50/25	15.8	14.9	1.26
PEG- <i>b</i> -Poly(PLG ₅₅ - <i>co</i> -BLG ₁₈)	60/20	55/18	14.9	17.4	1.16
PEG- <i>b</i> -Poly(PLG ₅₅ - <i>co</i> -BLG ₁₂)	65/15	55/12	13.7	16.7	1.16
PEG- <i>b</i> -PPLG ₅₄ - <i>b</i> -PBLG ₂₅	50/25	54/25	17.7	18.5	1.22

^a Feed molar ratio of PLG-NCA/BLG-NCA; ^b Calculated from ¹H-NMR spectra; ^c Determined from GPC

The protecting group of PBLG was removed by acidolysis to yield PEG-*b*-Poly(PLG-*co*-GA) with protonated PGA^[31], which was then modified with thiol-terminated triethylene glycol monomethyl ether (OEG-SH) to yield PEG-*b*-Poly[(PLG-*g*-EG)-*co*-GA]^[29]. The chemical structure was confirmed by ¹H-NMR. Figure 1(b) shows a typical ¹H-NMR spectrum of PEG-*b*-Poly(PLG-*co*-GA) diblock copolymer. The disappearance of proton signals at $\delta = 5.21$ and 7.01 suggests the complete deprotection of benzyl groups (Fig. 1B). Furthermore, the same integrals of other peaks indicate the negligible degradation of ester groups of PLG residues during the deprotecting process. The protons of OEG moiety at $\delta = 2.69$ –3.03, 3.45, and 3.70 are observed, and the protons of propargyl group at $\delta = 4.72$ and 2.71 in PEG-*b*-Poly[(PLG-*g*-EG)-*co*-GA] copolymer completely disappear, indicating the quantitative conversion of alkynyl groups. The chemical structure of modified triblock copolymer PEG-*b*-(PPLG-*g*-EG)-*b*-PGA is also confirmed by ¹H-NMR spectrum (Fig. 1C).

All of the PEG-*b*-Poly[(PLG-*g*-EG)-*co*-GA] copolymer samples can readily dissolve in neutral and alkaline solutions at the concentration up to 10 wt%. At low pH, the samples spontaneously form self-supported hydrogels with a concentration of 2.5 wt% or higher upon heating. The gelation is reversible by cooling. The inverted tube method was used to determine the critical gelation concentration^[32]. It is expected that the PPLG-*g*-EG and PGA residues can provide thermal- and pH-responsive properties^[29, 33], as Poly[(PLG-*g*-EG)-*co*-GA] copolymers show a pH-responsive LCST behavior in aqueous solution.

The CD spectroscopy was used to study the influence of secondary structure on the gelation behaviors of the samples. The CD spectra of PEG-*b*-Poly[(PLG₅₅-*g*-EG)-*co*-GA₁₈] at different temperatures (pH 2.8) are shown in Fig. 2(a), which exhibits two negative peaks at $\lambda \sim 208$ and 222 nm and one positive peak at $\lambda \sim 195$ nm, indicating a α -helical conformation^[34]. The CD signal of the sample is significantly reduced upon heating to 65 °C, possibly due to the phase transition induced sample aggregation. Note that no precipitates were observed in solution during heating. Further, the conformation can be fully recovered upon cooling^[29]. As pH increases to 4.4, the CD signal of α -helix conformation barely varies during the heating process (Fig. 2b), indicative of negligible aggregation. The α -helix content was further calculated and the relationship between α -helicity and temperature is given in Fig. 2(c) and Fig. S3 (in SI). In the acidic solution, the α -helicity of the samples decreases largely as the temperature increases. In contrast, the α -helicity at high pH shows a modest decrease with temperature change, suggesting less dependence of the secondary conformation on the temperature. The sample PEG-*b*-Poly[(PLG₅₀-*g*-EG)-*co*-GA₂₅] shows similar behavior (Fig. S4 in SI). In the case of PEG-*b*-(PPLG₅₄-*g*-EG)-*b*-PGA₂₅ triblock copolymer with a thermal-responsive PPLG₅₄-*g*-EG block, the

α -helicity has a slight decrease as the temperature increases (Fig. S5 in SI), indicating less aggregation in the system with increasing temperature. However, it was difficult to characterize the secondary structure dependence on temperature due to the reduced signals. We thus performed FTIR measurement to characterize these samples. The FTIR spectra of PEG-*b*-Poly[(PLG₅₅-*g*-EG)-*co*-GA₁₂] diblock copolymers are shown in Fig. 3. It can be seen that the amide I band of the samples at different pHs locates at 1653 and 1550 cm⁻¹ (20 °C), indicating the dominant α -helix conformation. As temperature increases to 50 °C, the sample shows similar adsorption peaks at 1653 and 1550 cm⁻¹ but with a broader shoulder at lower wavenumber. This may be attributed to the presence of non-hydrogen bonded carbonyls^[35], indicative of dehydration of OEG groups. Note that the conformation variation upon heating is pH independent. All the other samples show the similar behaviors (Fig. S6 in SI).

We further studied the influence of temperature and pH on the gelation of PEG-*b*-Poly[(PLG-*g*-EG)-*co*-GA] diblock polymers. A typical photo of a hydrogel formed from PEG-*b*-Poly[(PLG₅₅-*g*-EG)-*co*-GA₁₈] at a concentration of 6 wt% is shown in Fig. S7 (in SI). The sample remains sol at 25 °C over the entire experimental pH window. As the temperature increases to 50 °C, a hydrogel forms at pH 2.7. Further increasing temperature to 65 °C, the hydrogels form at pH 3.2 and 3.8. However, the sample remains a sol state at pH 4.3. It is known that the PGA block, with a pK_a of ~4.3, usually adopts negative charged random coil at high pH and uncharged

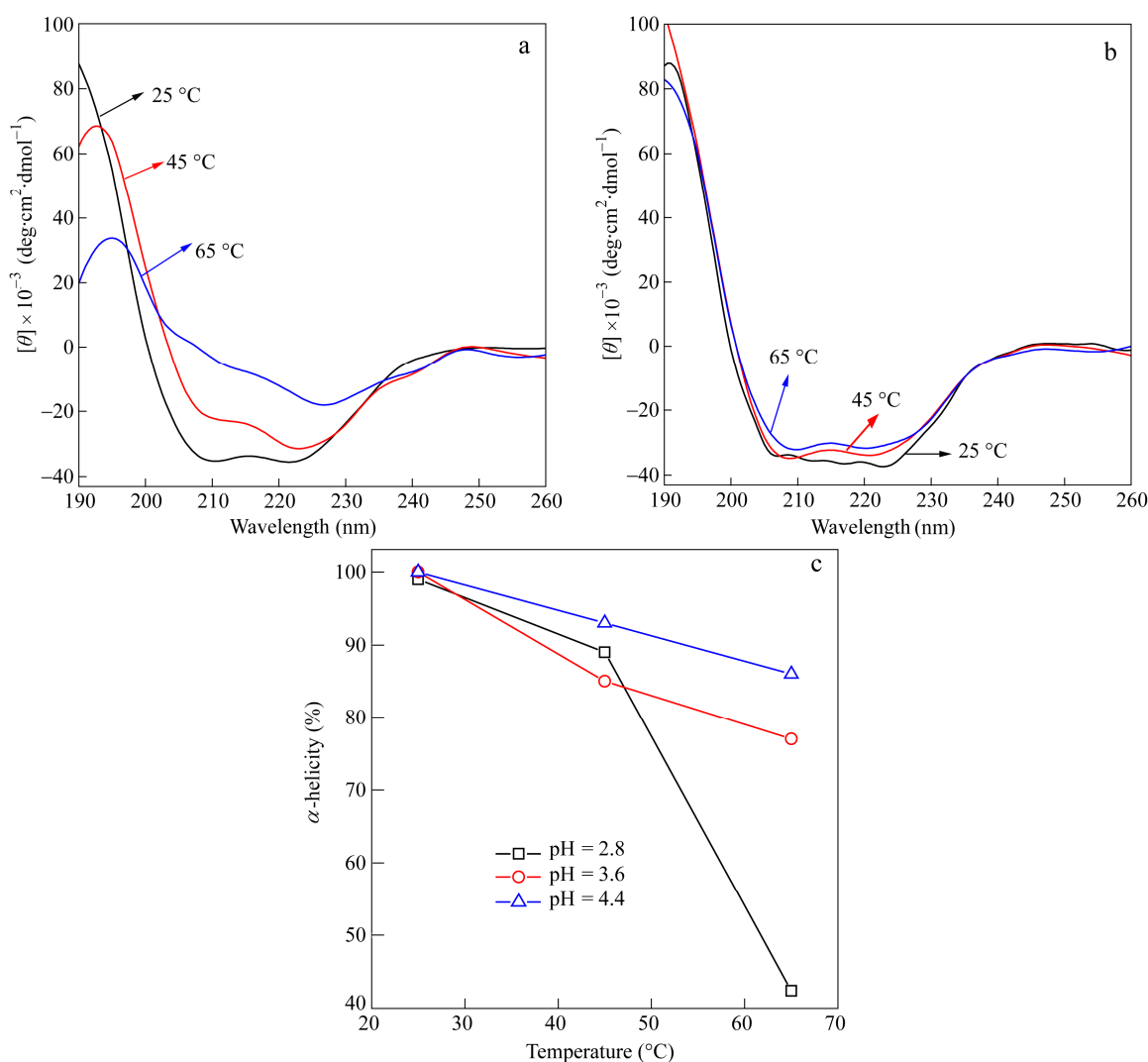


Fig. 2 The dependence of secondary structure of PEG-*b*-Poly[(PLG₅₅-*g*-EG)-*co*-GA₁₈] copolymer on temperature at a concentration of 0.2 mg·mL⁻¹: (a) pH 2.8, (b) pH 4.4 and (c) α -helicity dependence on temperature at different pHs

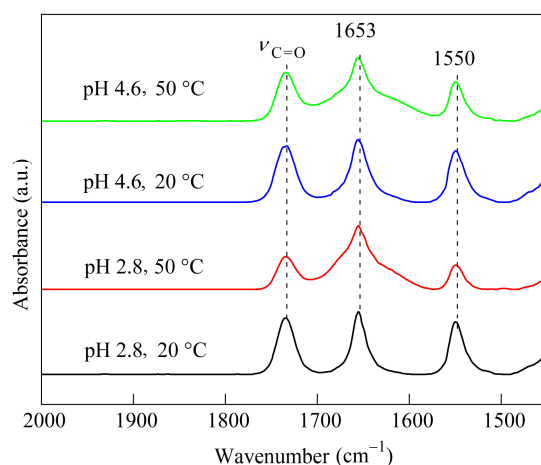


Fig. 3 FTIR spectra of PEG-*b*-Poly[(PLG₅₅-*g*-EG)-*co*-GA₁₂] at different conditions

α -helix at low pH^[36]. As pH increasing, the α -helicity of the sample decreases, which facilitates the dissolving of copolymers and results in a sol state.

Phase diagrams of all the block copolymers at different concentrations are shown in Fig. 4 and Fig. S8 (in SI). Table S1 summarizes the sol-to-gel transition temperatures of all samples at different pHs. In all cases, the sol-to-gel transition temperature decreases with the decrease of pH values. This is mainly due to the increase of α -helicity content of PGA component, which results in the decreases of the sample's solubility at low pH^[31]. FTIR spectra show that the dehydration is present at both low and high pHs. We therefore demonstrate that the hydrophilic-hydrophobic balance of samples dominates the sol-to-gel transition temperature. It is noted that the sol-to-gel transition temperature levels off as the pH value further decreases, possibly due to the less solubility of the samples in water. The sol-to-gel transition temperature decreases with increasing concentration of the polymers in all cases, consistent with the previous results^[28]. Interestingly, it is observed that the sol-to-gel transition temperature of PEG-*b*-(PPLG₅₄-*g*-EG)-*b*-PGA₂₅ triblock copolymer with thermal-responsive PPLG₅₄-*g*-EG block shows greater dependence on pH as compared to that of PEG-*b*-Poly[(PLG₅₅-*g*-EG)-*co*-GA₂₅] diblock polymer with statistical polypeptide block. At pH = 3.8, the PEG-*b*-Poly[(PLG₅₅-*g*-EG)-*co*-GA₂₅] diblock copolymer solution remains a gel state at a concentration ≥ 5 wt%; however, the PEG-*b*-(PPLG₅₄-*g*-EG)-*b*-PGA₂₅ triblock copolymer turns to sol state as the concentration increased up to 10 wt%, suggesting its weak gelation ability. These results are not consistent with the LCST behaviors of both polymers without attaching PEG segments. In comparison to the Poly[(PLG-*g*-EG)-*co*-GA] random copolymer, the LCST of (PPLG-*g*-EG)-*b*-PGA block copolymer shows less pH dependence. We attribute this to the different aggregation of two systems, which is consistent with the CD results. The relationship between the sol-to-gel transition temperature of PEG-*b*-Poly[(PLG-*g*-EG)-*co*-GA] (concentration of 6 wt%) versus average DP of PGA units is shown in Fig. 5(a). In all cases, the sol-to-gel transition temperature increases with average DP of PGA units increasing, particularly in the high pH range. This is mainly due to the increase of charged PGA units that can increase the solubility of copolymers. At high pH, only the polymer with the lowest PGA content, *i.e.* PEG-*b*-Poly[(PLG₅₅-*g*-EG)-*co*-GA₁₂], shows the sol-to-gel transition. All the other samples remain the sol state over the entire experimental windows. The correlation of lowest critical gelation concentration (CGC) and average DP of PGA units of all samples at pH 2.7 is shown in Fig. 5(b). It is observed that the CGCs of the samples generally increase with increasing PGA units, indicating that the incorporation of PGA residues suppresses the gelation ability of the samples^[28].

The rheology measurements were performed to investigate the mechanical properties of the hydrogels. We showed a dynamic strain sweep for the hydrogel from PEG-*b*-Poly[(PLG₅₅-*g*-EG)-*co*-GA₁₈] diblock polymer in the strain range of 0.01%–100% with $\omega = 6 \text{ rad}\cdot\text{s}^{-1}$ at 45 °C (Fig. 6a). The G' is $\sim 1196 \text{ Pa}$, which is approximately one order of magnitude larger than the G'' , indicative of the solid gel state. The gel network can

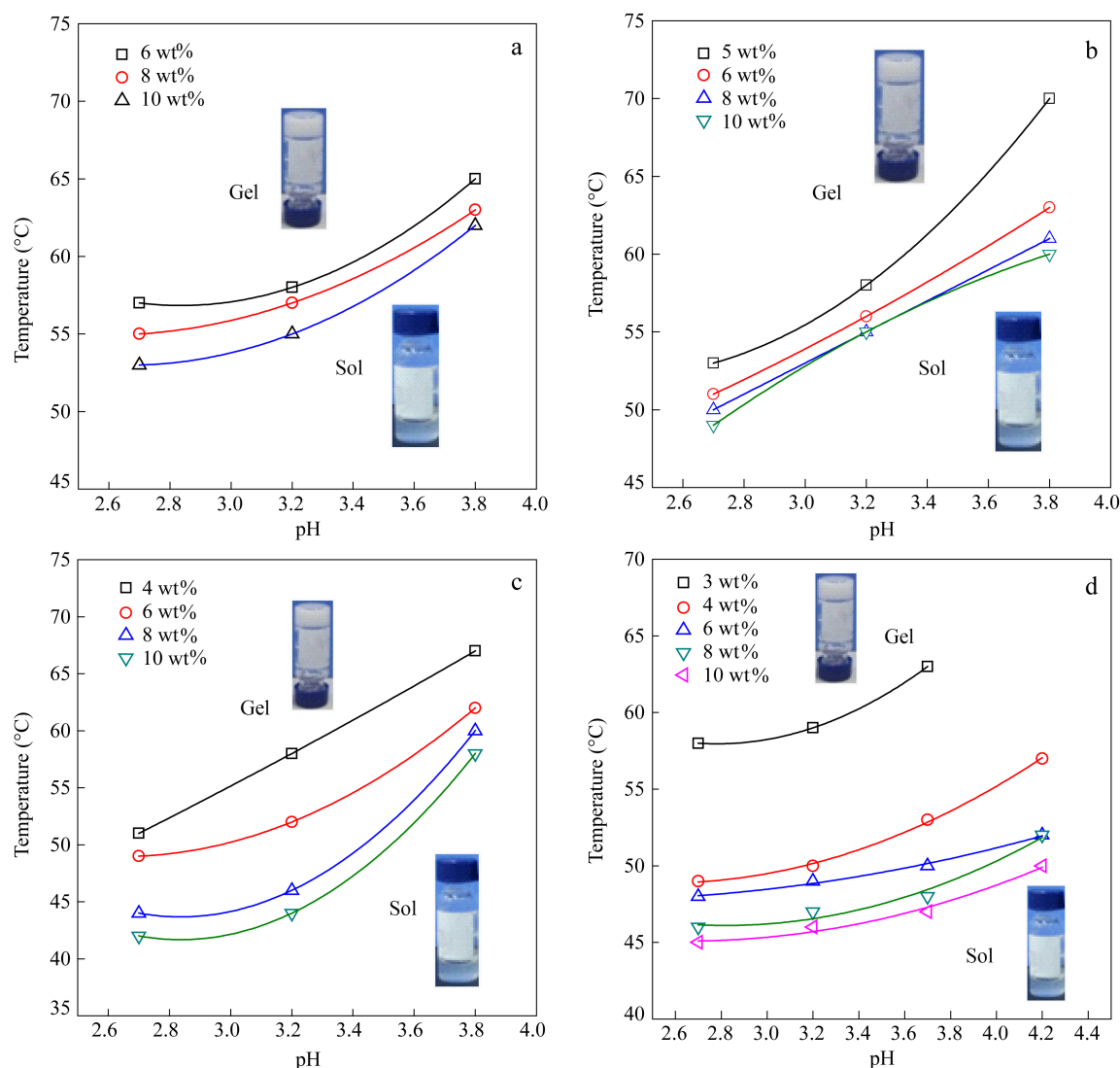


Fig. 4 Sol-to-gel phase diagrams of copolypeptides at different pHs: (a) PEG-*b*-Poly[(PLG₅₃-g-EG)-*co*-GA₅₂], (b) PEG-*b*-Poly[(PLG₅₀-g-EG)-*co*-GA₂₅], (c) PEG-*b*-Poly[(PLG₅₅-g-EG)-*co*-GA₁₈] and (d) PEG-*b*-Poly[(PLG₅₅-g-EG)-*co*-GA₁₂] (The online version is colorful.)

be easily disrupted under large strain, suggesting the relatively weak hydrogel. Temperature-induced increase of the gel strength is given in Fig. 6(b). Both G' and G'' of the PEG-*b*-Poly[(PLG₅₅-g-EG)-*co*-GA₁₈] hydrogel increase substantially from 10^2 Pa to 2×10^3 Pa, and 50 Pa to 370 Pa, respectively at ~ 40 °C. This is consistent with the result obtained from the inverted tube method, further confirming the sol-to-gel transition.

To directly observe the nanostructure of the hydrogels, TEM was used to examine the morphology of samples prepared from dilute copolymer solutions at 50 °C which is above the cloud point of thermal-responsive segment^[32]. It is observed that the diblock copolymer PEG-*b*-Poly[(PLG₅₅-g-EG)-*co*-GA₁₂] forms long fibril structures with a diameter of ~ 4 nm at pH 3.0 (Fig. 7). We therefore demonstrate that the formation of 3D network is based on the self-assembly of 1D fibril nanostructures, possibly driven by both of the inter/intra hydrogen bonding and collapse of PPLG residues upon heating. In comparison, the PEG-*b*-(PPLG₅₄-g-EG)-*b*-PGA₂₅ triblock copolymer with the thermal-responsive segment mainly forms spherical morphology in acidic solution at elevated temperature (Fig. S9 in SI), which explains the remained sol state in most cases.

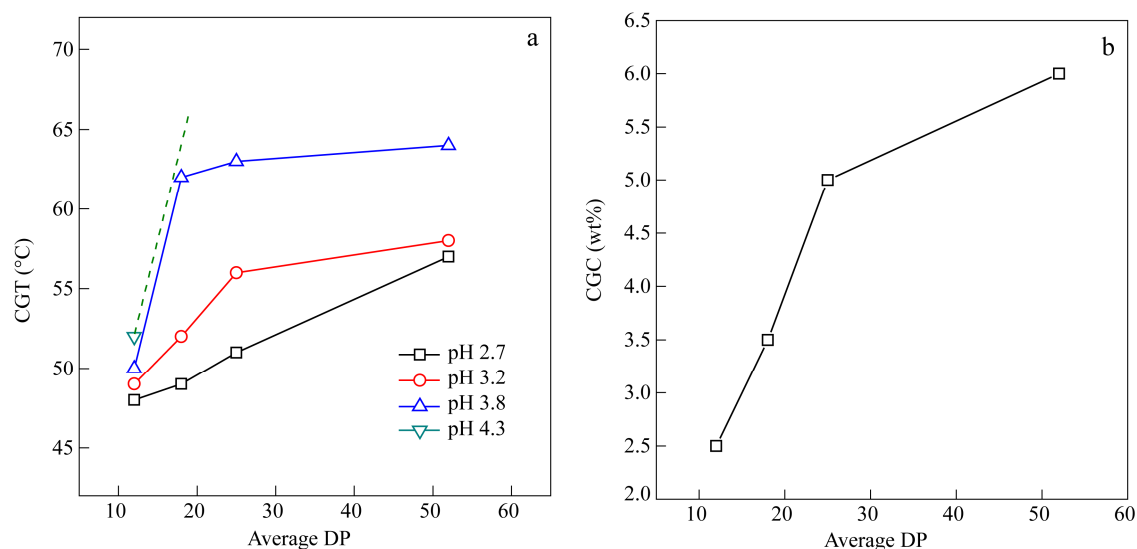


Fig. 5 (a) Plots of CGT of copolypeptides with statistical copoly(L-glutamate)s as a function of average DP of PGA units (6 wt%) at different pHs; (b) A plot of CGC of random copolypeptides as a function of average DP of PGA units at pH 2.7

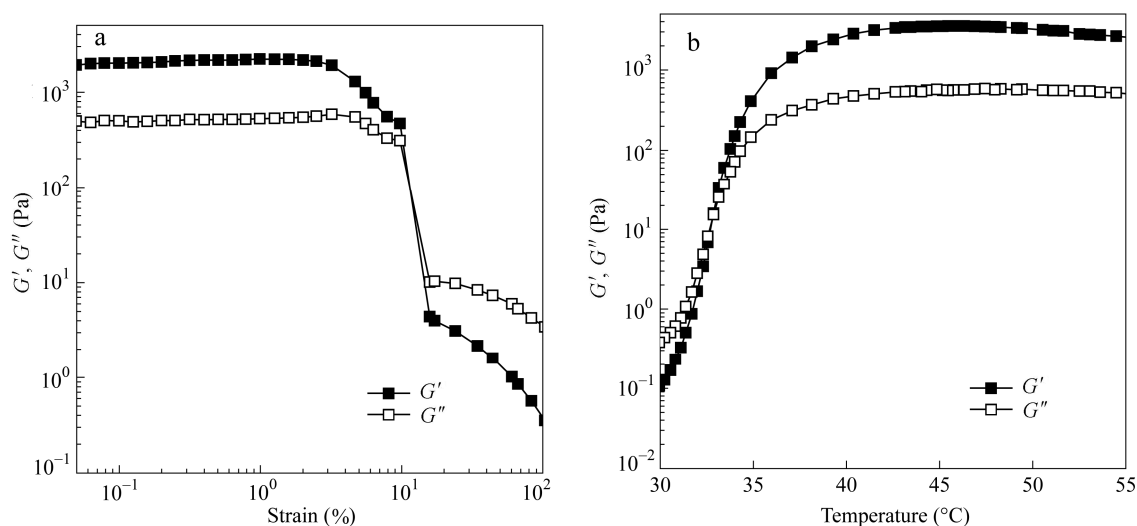


Fig. 6 (a) G' and G'' as a function of strain for the sample of PEG-*b*-Poly[(PLG₅₅-g-EG)-*co*-GA₁₈] (pH 3.2, 10 wt%) in the gel state; (b) G' and G'' as a function of temperature for the sample of PEG-*b*-Poly[(PLG₅₅-g-EG)-*co*-GA₁₈] (10 wt%) at pH 3.2

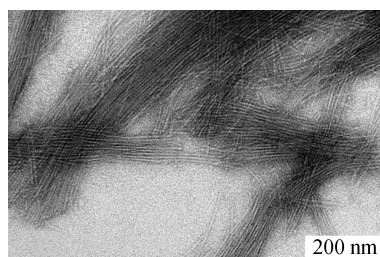


Fig. 7 TEM image of PEG-*b*-Poly[(PLG₅₅-g-EG)-*co*-GA₁₂] hydrogel at a concentration of 0.5 mg·mL⁻¹, pH 3.0 and 50 °C (The sample was stained with 2% uranyl acetate.)

CONCLUSIONS

In conclusion, we prepared a class of dual-responsive hydrogels by ring-opening polymerization (ROP) and subsequent modification. The incorporation of oligo(ethylene glycol) (OEG) and glutamic acid residues endows the gel with thermal- and pH-responsive properties. Increasing the amounts of PGA units and pH results in the polymers with reduced gelation ability. Interestingly, the gelation property is also dependent on the morphology of the self-assemblies. The as-prepared hydrogels from hybrid peptide show dual-responsive gelation behaviors, which may find potential applications for biomedicine.

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