

EFFECTS OF MOLECULAR WEIGHT ON THERMAL RESPONSIVE PROPERTY OF PEGYLATED POLY-L-GLUTAMATES*

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Abstract We investigated the ring opening polymerization (ROP) of di- and tri-ethylene glycol monomethyl ether functionalized L-glutamate *N*-carboxyanhydrides (NCAs) using hexamethyldisilazane (HMDS) as primary initiator and 1,5,7-triazabicyclo-[4.4.0]dec-5-ene (TBD) as co-initiator. The binary initiator system afforded a living ROP for these pegylated NCAs, and a series of homopolypeptides with controlled molecular weight (MW) and low polydispersity were obtained. We then systematically studied the helical content and clouding point (CP) dependence on polypeptide MW using circular dichroism (CD) spectroscopy and turbidity measurements, respectively. We found that the helical content of both homopolypeptides increased with MW, but the triethylene glycol functionalized poly-L-glutamate (poly-L-EG₃Glu) intended to form more stable α -helical structure than diethylene glycol functionalized counterpart (poly-L-EG₂Glu) at similar MW. Accordingly, the CP of poly-L-EG₂Glu with known end group has strong dependence on its helical content, which is essentially determined by MW. Our results suggested that the thermal responsive properties of these unique pegylated poly-L-glutamates not only rely on their chemical structure but also on their secondary structures, which is different from conventional thermal responsive polymers.

Keywords: *N*-carboxyanhydride (NCA); Ring opening polymerization; Polypeptide; Helicity; Thermal responsive.

Electronic Supplementary Material Supplementary material is available in the online version of this article at <http://dx.doi.org/10.1007/s10118-013-1218-7>.

INTRODUCTION

Stimuli-responsive polymers have attracted great research interest in nano- and biotechnology because of their promising applications^[1–3]. In contrast to conventional polymers, biodegradable stimuli-responsive polymers are desirable for biomedical applications^[4]. Recently, functional polypeptides received extensive research interests for their advantages in biocompatibility and biodegradability^[5–7]. Compared with biodegradable polyesters, the side chains of polypeptides can be chemically modified to introduce specific functional groups for particular applications.

A great deal of efforts has been contributed to synthesize new functional polypeptide materials based on lysine and glutamate^[8–18]. For example, Deming's group made nonionic and water soluble polypeptides derived from oligo(ethylene glycol) functionalized lysine, serine and cysteine amino acids^[18, 19]. Recently, they reported the preparation of glycopolypeptides^[17, 20]. Cheng and coworkers developed a new chemistry to prepare cationic helical polypeptides^[13, 21, 22] and also studied their application in gene delivery. On the other hand, Chen *et al.* used click chemistry to construct sugar and thermal responsive poly-L-glutamate^[14, 15]. Hammond and coworkers used click chemistry to make several different polypeptides with unique properties^[23, 24].

* The work was financially supported by the National Natural Science Foundation of China (Nos. 20974112 and 51225306).

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Received September 14, 2012; Revised September 29, 2012; Accepted October 8, 2012

doi: 10.1007/s10118-013-1218-7

We recently demonstrated that poly-L-EG_mGlu ($m = 2-3$) can display reversible low critical solution temperature (LCST) behaviors in water, and the LCST can be tuned *via* copolymerization of different amino acid monomers^[25]. We initially prepared a series of well-defined poly-L-EG_mGlu using nickel (0) complex as the initiator. One concern is that the residual metal initiator needs to be removed *via* exhaustive dialysis. Considering the promising potential applications of these novel thermal responsive polypeptides, we are determined to optimize the polymerization using organic initiator system. Here, we use a combination of HMDS and TBD to realize controlled ROP of γ -(2-methoxyethoxy)esteryl-L-glutamate *N*-carboxyanhydride (L-EG₂Glu-NCA) and γ -(2-(methoxyethoxy)ethoxy)esteryl-L-glutamate *N*-carboxyanhydride (L-EG₃Glu-NCA). Then, we study the correlation between cloud point (CP) of poly-L-EG_mGlu and molecular weight and try to understand the thermal responsive properties of poly(L-EG_mGlu) on the corresponding helical content.

EXPERIMENTAL

Materials

Hexane (HEX), tetrahydrofuran (THF), dichloromethane (DCM) and *N,N*-dimethylformamide (DMF) were purified first by purging with dry N₂, followed by passing through columns of activated alumina. Hexamethyldisilazane (HMDS) and 1,5,7-triazabicyclo-[4.4.0]dec-5-ene (TBD) were purchased from Sigma-Aldrich and used as received. Deionized water was obtained from a Millipore Milli-Q purification unit. L-glutamic acid was purchased from GL Biochem (Shanghai) Ltd. Di- and tri-ethylene glycol monomethyl ethers were purchased from Aladdin reagent. All commercially obtained reagents were used as received without further purification unless otherwise stated.

Characterization

¹H-NMR spectra were recorded on a Bruker AV400 FT-NMR spectrometer (400 MHz). Tandem gel permeation chromatography/light scattering (GPC/LLS) was performed at 50°C using an SSI pump connected to Wyatt Optilab DSP and Wyatt DAWN EOS light scattering detectors with DMF containing 0.02 mol/L LiBr salt as eluent at a flow rate of 1.0 mL/min, and separations were achieved using 10⁵, 10⁴, and 10³ Å Phenomenex Phenogel 5 µm columns. All GPC/LLS samples were prepared at concentrations of about 5 mg/mL. The refractive index increment (dn/dc) of poly-L-EG_mGlu homopolypeptide was measured using a Wyatt's rEX DRI detector and an Astra software dn/dc template. The polypeptide solutions with different concentrations were sequentially injected into the DRI injector. The measured refractive index values were plotted versus concentration and the dn/dc values were obtained from the slope of linear fit. The dn/dc values of poly-L-EG₂Glu and poly-L-EG₃Glu in DMF containing 0.02 mol/L LiBr salt at wavelength of 658 nm are 0.0582 mL g⁻¹ and 0.0613 mL g⁻¹, respectively. UV-Vis spectra were measured by monitoring the transmittance of a 500 nm light beam through a quartz sample cell at concentration of 2 mg/mL on a Shimadzu UV-2550 spectrophotometer equipped with a temperature controller S-1700. The solution was heated at a rate of 0.5 K/min between 20°C and 80°C. The cloud point (CP) was defined as the temperature corresponding to 50% transmittance of aqueous solution during the heating process. Circular dichroism (CD) 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. Ellipticity ($[\theta]$ in deg·cm² dmol⁻¹) = (millidegrees × mean residue weight)/(pathlength in millimeters × concentration of polypeptide in mg mL⁻¹). The α -helical contents of the polypeptides were estimated using the equation: α -helix% = $(-[\theta_{222}] + 3000)/39000$ ^[26]. FTIR measurements were performed using a Nicolet Avatar 330 FT-IR spectrometer operated under OMNIC software. Liquid samples were analyzed by direct injection of the reaction medium into a 0.1 mm liquid cell with KBr windows. The transmittance intensity of C=O stretching band (≈ 1850 cm⁻¹), which has linear dependence on NCA concentration within studied range, was used to monitor the relative change of NCA concentration versus conversion.

Synthesis of L-EG_mGlu-NCAs

The detailed preparation of L-EG₂Glu-NCA and L-EG₃Glu-NCA were reported elsewhere^[25]. Results of ¹H-NMR and FTIR characterization were in agreement with expected values.

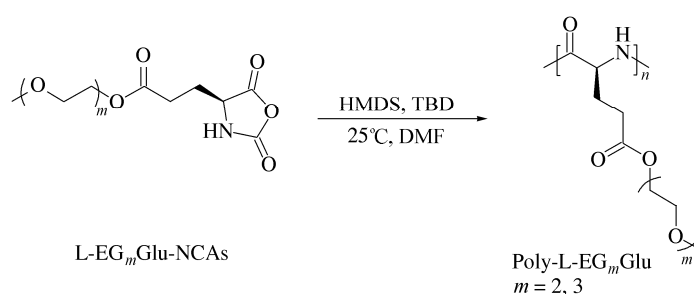
Polymerization of L-EG_mGlu-NCAs

All polymerizations were performed in a glove box under high purity N₂ atmosphere. Typically, a solution of HMDS (0.1 mol/L in DMF) was added to a solution of L-EG_mGlu-NCA in DMF (50 mg mL⁻¹) using micropipette. After mixing, a prescribed TBD solution (0.1 mol/L in DMF) was added into the mixture under stirring. The reaction was then stirred at room temperature, *ca.* 25°C. At regular intervals, aliquots of sample solution were sampled to determine NCA concentration using FTIR. The conversion of NCA was estimated by comparing the transmittance of carbonyl stretch at 1850 cm⁻¹ normalized to pristine solution prior to polymerization. Another aliquot of reaction solution was then diluted to about 10 mg mL⁻¹ using DMF for GPC/LLS characterization. The remaining samples were mixed with tetrabutylammonium fluoride solution (1.0 mol/L, 100 μL), diisopropylethylamine (60 μL) and benzyl chloroformate (200 μL) to end-cap the polymer chain^[21]. The mixture was stirred at room temperature for about 3 h before being precipitated into ethyl ether. Crude polymers were collected by centrifugation. The crude polypeptides were redissolved in CH₂Cl₂ and precipitated into ethyl ether followed by centrifuge. The products were dried in vacuum oven to yield white sticky solid (80% yield). Poly-L-EG₂Glu: ¹H-NMR (400 MHz, CF₃COOD, δ), 7.30 (br, 0.13H, Ar H), 4.72 (br, 1H; CH), 4.40 (br, 2H; CH₂), 3.93–3.84 (br, 6H; CH₂–O–CH₂–CH₂), 3.54 (br, 3H; CH₃), 2.64 (br, 2H; CH₂), 2.27–2.11 (br, 2H; CH₂); Poly-L-EG₃Glu: ¹H-NMR (400 MHz, CF₃COOD, δ), 7.23 (br, 0.17H, Ar H), 4.64 (br, 1H; CH), 4.32–4.28 (br, 2H; CH₂), 3.85–3.75 (br, 10H; CH₂–O–CH₂–CH₂–O–CH₂–CH₂), 3.46 (br, 3H; CH₃), 2.55 (br, 2H; CH₂), 2.17–2.02 (br, 2H; CH₂).

RESULTS AND DISCUSSION

Synthesis of Polypeptides

The synthesis of L-EG_m-Glu amino acid monomers was reported elsewhere^[25, 27]. After purification, they were converted into corresponding NCAs, which were purified by flash column chromatograph prior to subsequent ROP^[28]. Recently, Cheng and coworkers demonstrated that addition of small amount of strong nucleophiles such as TBD can effectively accelerate the polymerization rate of polar NCA monomers initiated by HMDS^[21]. We thus decided to extend such strategy to L-EG_mGlu-NCAs by using HMDS as primary initiator and TBD as co-initiator (Scheme 1). We kept the ratio of HMDS/TBD being 100, and only varied the reaction time to determine the monomer conversion versus time. To more accurately measure MW, we determined the dn/dc values of poly-L-EG₂Glu and poly-L-EG₃Glu in DMF containing 0.02 mol/L LiBr. The dn/dc values of poly-L-EG₂Glu and poly-L-EG₃Glu are 0.0582 mL g⁻¹ and 0.0613 mL g⁻¹, respectively.



Scheme 1 HMDS/TBD mediated ROP of L-EG_mGlu-NCAs

At pre-determined time intervals, we quenched a small portion of reaction solution to obtain a series of homopolypeptides. Their MWs and molecular weight distributions were characterized using GPC/LLS with known dn/dc values. These data are summarized in Table 1. Figure 1(a) shows correlation between conversions and reaction time. For both NCA monomers, the conversions increase almost linearly for first 12 h. The corresponding conversion is about 63% and 52% for L-EG₂Glu-NCA and L-EG₃Glu-NCA, respectively. Further extension of reaction time causes leveling off from linear dependence, indicating that polymerization rate slows down after 12 h. The reason we assume is due to the conformation change with elongation of polypeptide

chains^[29, 30]. Figures 1(b) and 1(c) show the corresponding number averaged molecular weight (M_n) versus monomer conversion. Apparently, the M_n of poly-L-EG₂Glu increases linearly with conversion of L-EG₂Glu-NCA, which indicates a controlled ROP for L-EG₂Glu-NCA (Fig. 1b). Also, the polydispersity index (PDI) of MW is relatively low (PDI < 1.2). For poly-L-EG₃Glu (Fig. 1c), the M_n increases monotonically with NCA conversion except that its values are slightly lower than the theoretical ones under the same reaction conditions. The experimental MW lower than the theoretical value means that there are probably more initiation reactions than expected based on the prescribed monomer-to-initiator ratio. It was well known that NCA monomer can be initiated by both nucleophiles and strong base *via* normal-amine (NA) and activated monomer (AM)^[31, 32], respectively. A possible reason is that TBD might be involved in the initiation of NCA *via* AM mechanism because TBD is a strong base. However, the trend of MW versus conversion is similar to poly-L-EG₂Glu, and the PDI of obtained polypeptides is still low with PDI < 1.2 (Table 1). These results suggested a controlled ROP for L-EG₃Glu-NCA. It is worth noting that the participation of TBD initiation can be suppressed as demonstrated in the case of L-EG₂Glu-NCA and others reported in literature^[12, 21]. In brief, we can obtain good control over MW and PDI for both poly-L-EG₂Glu and poly-L-EG₃Glu by using HMDS and TBD co-initiator system. The chemical structure of obtained products are further confirmed using ¹H-NMR (Figs. S1 and S2). Note that the resonances of benzyl groups are clearly observed in the corresponding ¹H-NMR spectra, which suggests the successful end-capping of polypeptides using benzyl chloroformate.

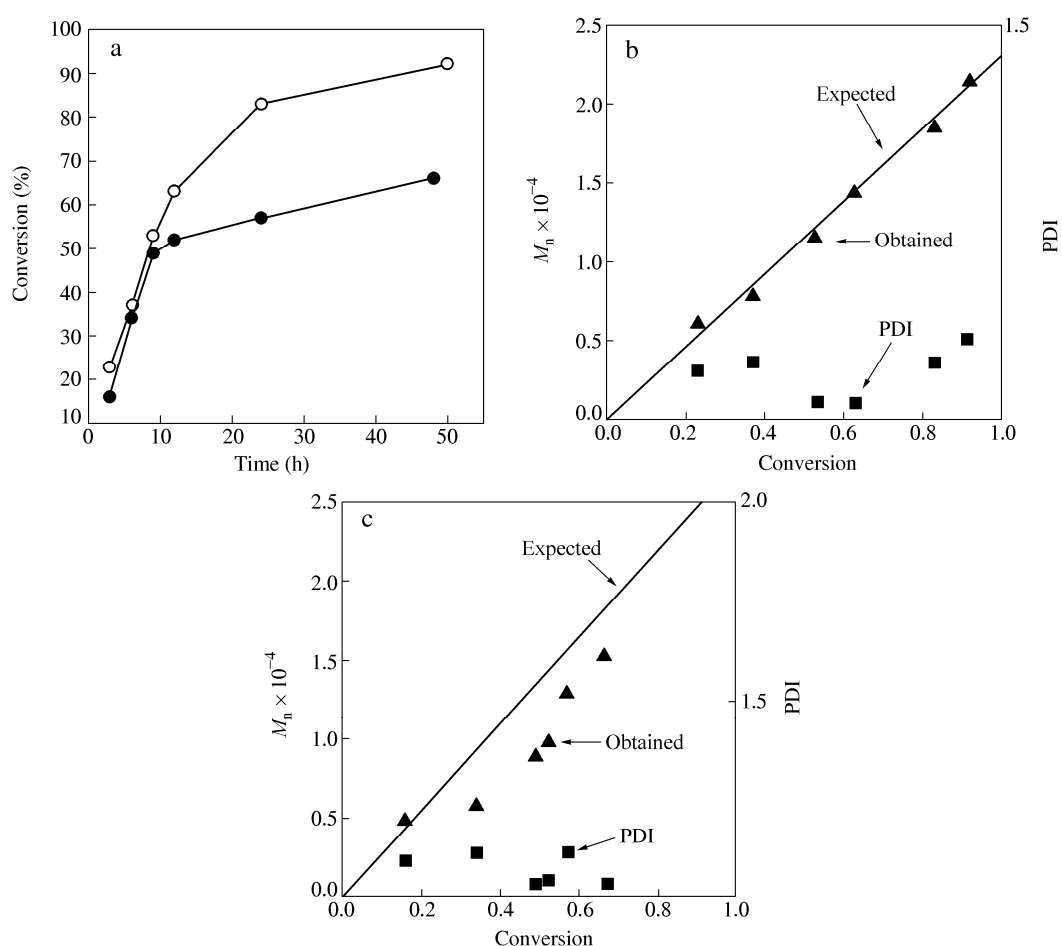


Fig. 1 (a) The monomer conversion dependence on reaction time in the HMDS/TBD mediated ROP of L-EG_mGlu NCA: (○) $m = 2$, (●) $m = 3$; Plots of M_n and PDI versus conversion in the HMDS/TBD mediated ROP of L-EG_mGlu-NCA: (b) $m = 2$, (c) $m = 3$

Table 1. Characterization results of ROP of L-EG_mGlu-NCAs and corresponding homopolypeptides ^a

Sample	Time (h)	Conversion ^b (%)	$M_n \times 10^{-3}$	DP ^c	PDI	CP ^d (°C)
Poly-L-EG ₂ Glu	3	23	5.98	26	1.07	–
	6	37	7.79	34	1.08	54
	9	53	11.5	50	1.03	48
	12	63	14.4	62	1.03	41
	24	83	18.5	80	1.08	38
	50	92	21.4	93	1.05	36
	3	16	4.32	17	1.10	–
Poly-L-EG ₃ Glu	6	34	5.08	21	1.12	–
	9	49	7.94	32	1.04	–
	12	52	8.71	35	1.05	–
	24	57	11.5	46	1.12	–
	48	66	13.6	55	1.04	72
	144	90	22.5	86	1.09	70

^a All reactions were conducted at 25°C in DMF with $[M]_0/[I]_0 = 100$ and $[M]_0 = 0.2$ mol/L. The samples were obtained by quenching stock reaction mixture into benzyl chloroformate solution at different time intervals; ^b Conversion was calculated by normalizing NCA concentration to initial NCA concentration prior to ROP from the FTIR spectrum; ^c DP was calculated from GPC/LLS with known dn/dc values; ^d CPs were defined as the temperature corresponding to 50% transmittance of aqueous solution during the heating process.

Secondary Structure Dependence on MW

It was known that poly(γ -benzyl L-glutamate) (PBLG) adopts α -helical conformation in most organic solvents. For its helical structure, it has a pitch of about 3.6 residuals^[33, 34]. The helical stability of PBLG not only depends on solvent, but also relies on polypeptide chain length^[35]. After obtaining a series of narrow dispersed poly-L-EG₂Glu and poly-L-EG₃Glu homopolypeptides, we then investigate the secondary structure dependence on MW using CD spectroscopy.

As shown in Figs. 2(a) and 2(b), the CD spectra of poly-L-EG₂Glu and poly-L-EG₃Glu exhibit two negative dichronic bands at 222, 208 nm and one positive band near 190 nm, which are characteristic of α -helical structure^[36]. The corresponding molar ellipticity decreases with increase of chain length. These results suggest that the α -helix content increases with samples' MW for both polypeptides, but poly-L-EG₃Glu has stronger dependence on MW than poly-L-EG₂Glu (Fig. 2c). For poly-L-EG₂Glu, increase of MW only has small contribution to the corresponding helical content. For example, poly-L-EG₂Glu₃₄ with DP = 34 has about 19% helical content, and poly-L-EG₂Glu₅₀ with DP = 50 has about 20% helical content. In contrast, poly-L-EG₂Glu₉₃ with almost double MW has helicity of only about 32%. Further increase of MW does not cause substantial increase of its helicity^[25]. Quantitative analysis using DICHROWEB K2D method^[37] reveals that the secondary structure of poly-L-EG₂Glu is mainly composed of random coil, *ca.* 50%. The corresponding results are summarized in Table S1. Specifically, the random coil component does not change with increase of DP, because the increase of helical content is compensated by the decrease of β -sheet content. It is worth noting that the conformation of poly-L-EG₂Glu can slowly evolve into β -sheet with time or change of external conditions^[25]. A possible reason for this is that the diethylene glycol side chains are not long enough to stabilize the α -helical conformation. Such point was further supported from the physical properties of poly-L-EG₁Glu, which only has one ethylene glycol unit as side chain and forms predominate β -sheet structure after polymerization as revealed by FTIR measurements (Fig. S3).

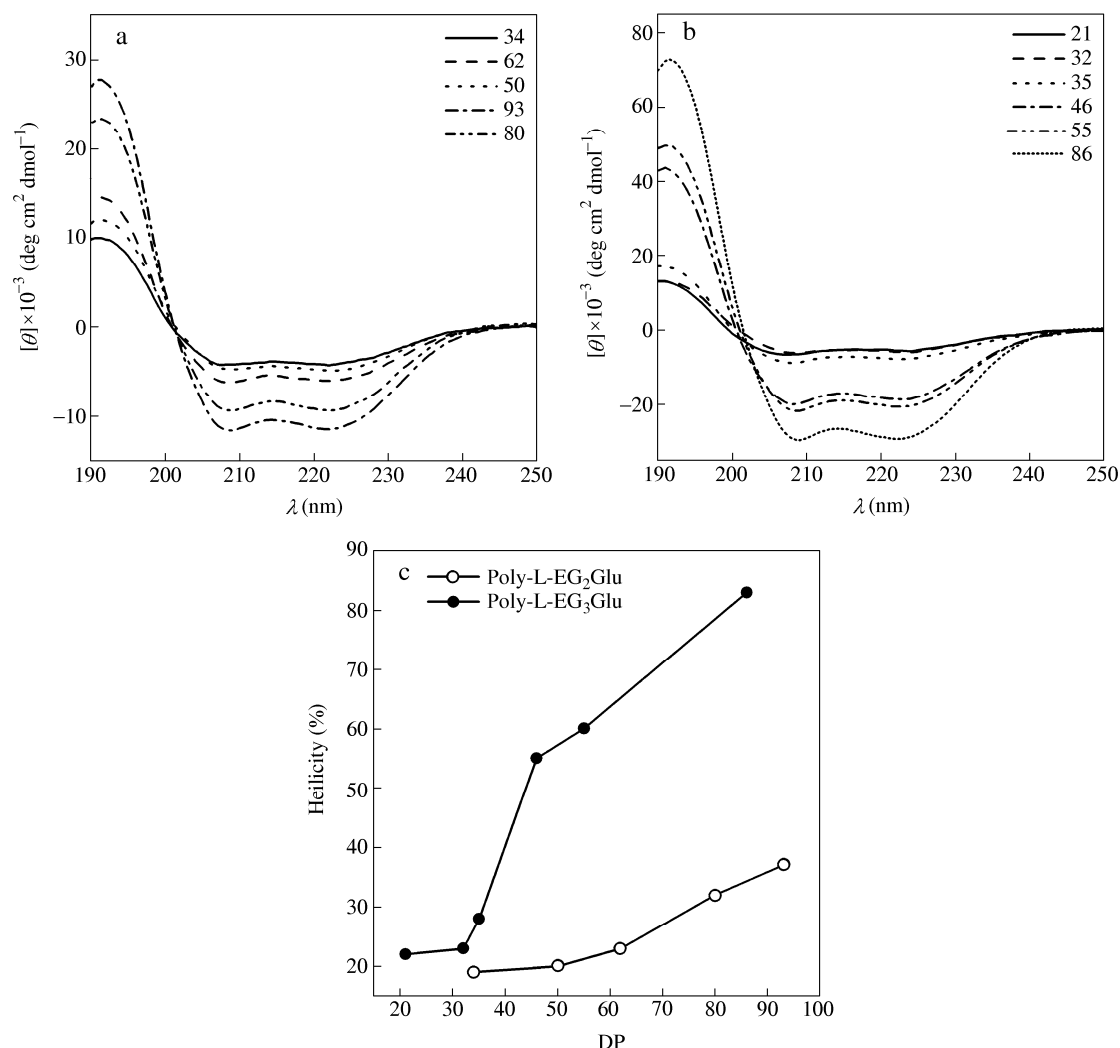
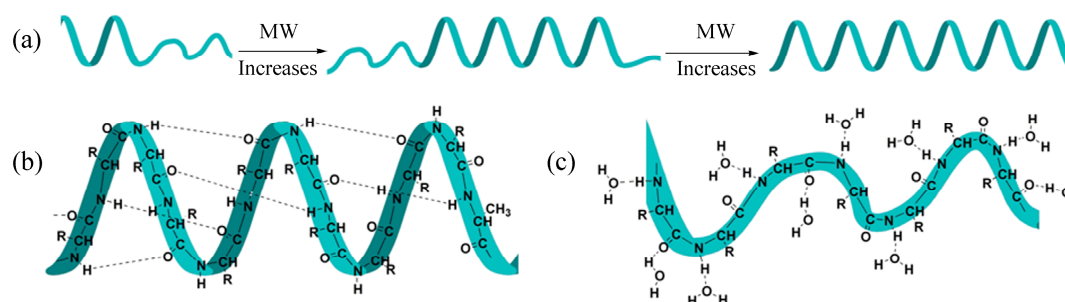


Fig. 2 CD spectra of poly-L-EG_mGlu with various DP in water (0.2 mg/mL at 25°C): (a) $m = 2$, (b) $m = 3$; (c) The helicity of dependence of poly-L-EG_mGlu on DP

In contrast, the side chain of poly-L-EG₃Glu homopolypeptide has one more ethylene glycol unit than that of poly-L-EG₂Glu, and the helicity of poly-L-EG₃Glu increases more obviously with MW (Fig. 2c). When the DP < 35, the helicity increases slowly with DP. When the DP > 35, the helicity increases from 28% for DP = 35 to 55% for DP = 46 and to 83% for DP = 86, respectively. The reason we believe is due to the increase of side chain length, which can stabilize the α -helical conformation and prevent forming intermolecular hydrogen bonding. We also used DICHROWEB K2D method to analyze the secondary composition for poly-L-EG₃Glu (Table S1). For poly-L-EG₃Glu homopolypeptide, both β -sheet and random coil contents decrease with increase of MW when helical content increases accordingly.

For poly-L-EG_mGlu homopolypeptide, the helical content dependence on MW is schematically illustrated in Scheme 2(a). To form α -helical structure, the amide bonds of polypeptide backbone form regular intramolecular hydrogen bonding. In contrast, random coil polypeptides are rich in intermolecular hydrogen bonding between amide bonds and water molecules. For the former, its solubility is determined mainly by the hydrophilicity of side chains. For the latter, the intermolecular hydrogen bonding will enhance its solubility in water. The competition between such intramolecular and intermolecular hydrogen bonding essentially determines their thermal responsive properties for both poly-L-EG₂Glu and poly-L-EG₃Glu as discussed below.



Scheme 2 (a) Schematic illustration of helicity increase with molecular weight, (b) intramolecular hydrogen bonding illustration of helical polypeptide, and (c) intermolecular hydrogen bonding for random coil polypeptide

Cloud Point Dependence on MW

Generally, the characteristic physical properties of thermal responsive polymers are determined by the chemical structure of polymer backbone^[38, 39]. Whereas the nature of polymer end groups such as their size and hydrophobicity has significant effects on the CP of thermal responsive polymers, particularly for low MW samples. Hydrophilic end-groups tend to increase LCST while hydrophobic end-groups tend to decrease polymer LCST^[40–45]. Therefore, contributions from end-groups should be considered when studying the MW dependence of LCST. Following literature protocol, we end-capped the poly-L-EG_mGlu using benzyl chloroformate^[21]. The incorporation of end groups is confirmed from ¹H-NMR analysis (Figs. S1 and S2).

After obtaining a series of poly-L-EG₂Glu and poly-L-EG₃Glu homopolypeptides with known end groups, we study the cloud point dependence on MW. Figure 3(a) shows the transmittance dependence on temperature for poly-L-EG₂Glu with different DP. With temperature increasing, all samples display LCST type phase transition albeit having different transition ranges. When DP < 50, the solution transmittance of poly-L-EG₂Glu solution (2 mg/mL) drops from 100% to about 30% without showing macroscopic phase separation during heating from RT to above CP. In contrast, when DP > 60, the transmittance of poly-L-EG₂Glu solution drops from 100% at RT to below 10% above CP. The LCST transition range is about 10 K. If we define the CP as the temperature corresponding to 50% transmittance, the correlation of CP versus DP can be plotted for poly-L-EG₂Glu shown in Fig. 3(c). The CP of dilute poly-L-EG₂Glu solution decreases monotonically with increase of DP. In particular, CP of the poly-L-EG₂Glu₃₄ is 54°C, while CP of the poly-L-EG₂Glu₉₃ has decreased to 36°C. Further increase MW did not induce significant decrease of CP as reported before. The CP of poly-L-EG₂Glu₂₉₉ is about 32°C^[25].

In contrast, poly-L-EG₃Glu needs relative longer chain to show thermal responsive properties (Fig. 3b). When DP < 32, poly-L-EG₃Glu does not display LCST transition below boiling point of water. When DP is larger than 46, poly-L-EG₃Glu starts to show thermal responsive properties however with CP above 80°C, which exceeds the instrument limit. Only when DP > 55, poly-L-EG₃Glu homopolypeptides start to show well-defined LCST transitions. The corresponding CP for poly-L-EG₃Glu₅₅ is about 72°C. Notably, the transition range is much narrower than that of poly-L-EG₂Glu with comparable MW. With increasing the DP to 160, we observed CP close to 56°C^[25].

For both poly-L-EG₂Glu and poly-L-EG₃Glu, such distinct CP dependence on the MW contrasts sharply with the well-studied poly(*N*-isopropylacrylamide) (PNIPAM). Given known end group, the CP of PNIPAM only changed from 39.9°C for DP = 50 to 36.9°C for CP = 100^[46]. A surprising result is that the CP change is about 18 K when the DP of poly-L-EG₂Glu increases from 34 to 93. Such big CP jump indicates there must be additional factors that determine the thermal responsive properties in addition to end groups. Compared to synthetic polymers, enantiomeric pure polypeptides have specific secondary structures such as α -helix or β -sheet, which corresponds to intramolecular and intermolecular hydrogen bonding, respectively. Hence, the above-discussed characteristic thermal properties of poly-L-EG_mGlu might be associated with their secondary structure dependence on MW.

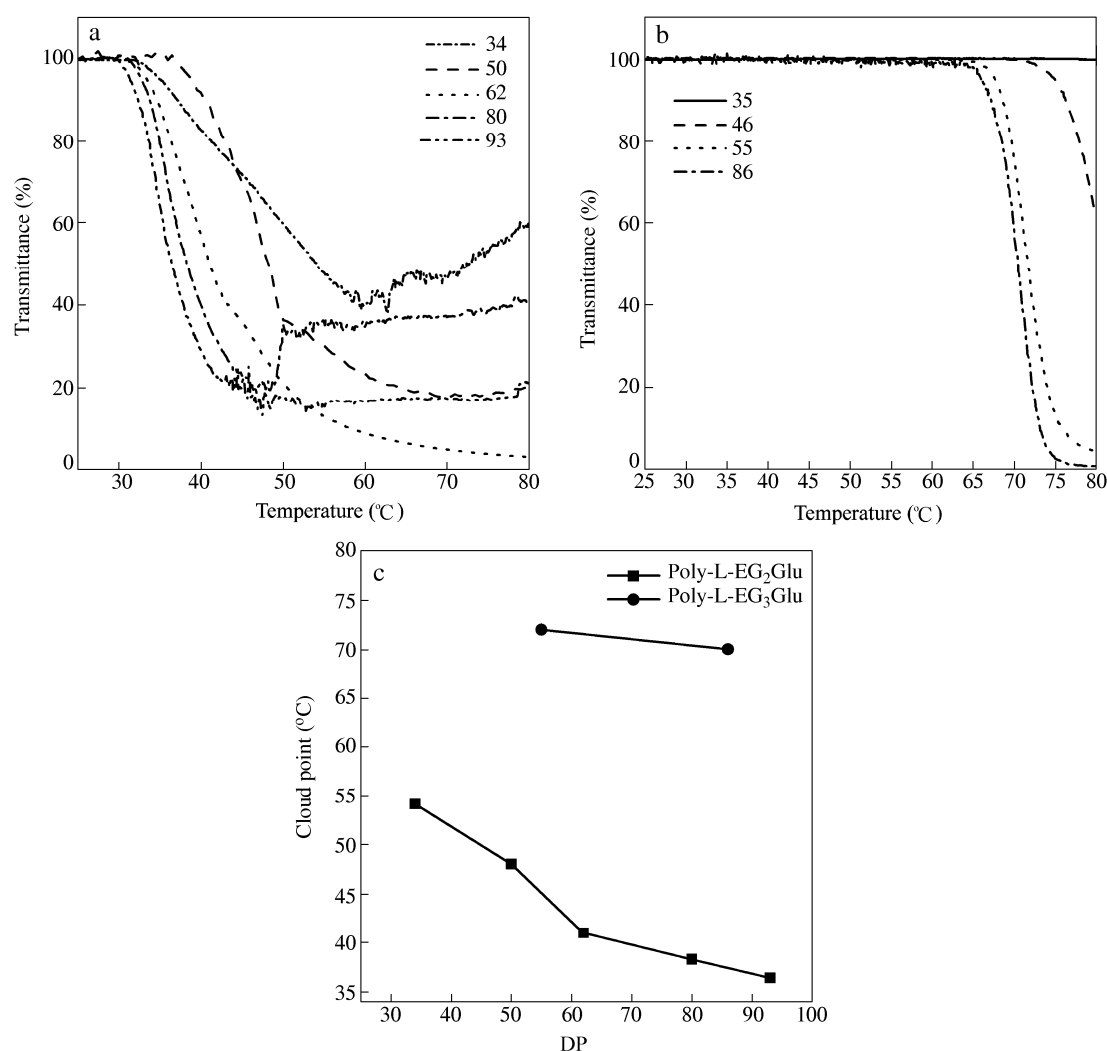


Fig. 3 The light transmittance of aqueous solutions of poly-L-EG_mGlu with various DP versus temperature at the heating rate of 0.5 K·min⁻¹: (a) $m = 2$, (b) $m = 3$; (c) The cloud point dependence on DP for poly-L-EG_mGlu

We have discussed in previous section that the helicity of poly-L-EG_mGlu increases with the corresponding chain length. For poly-L-EG_mGlu homopolypeptides, there are two functional groups that can interact with water to form hydrogen bonding. One is the amide bond within the backbone; the other is the ethylene glycol unit on side chain. If poly-L-EG_mGlu adopts helical conformation, the amide bonds within polypeptide backbone form the intramolecular hydrogen bonding, which practically minimizes their interactions with water (Scheme 2b). In this case, the reversible dehydration/rehydration of di- or triethylene glycol chains is responsible for polymers thermal responsive properties^[25]. As far as the thermal energy does not disrupt the ordered secondary structure, the amide bonds won't interact with water molecules. In contrast, if poly-L-EG_mGlu homopolypeptide adopts a random coil conformation, the amide bond can form strong hydrogen bonding with water in addition to ethylene glycol unit (Scheme 2c). Because of this, their solubility in water is significantly enhanced. Therefore, it is expected that the thermal responsive property of poly-L-EG_mGlu will depend on the regularity of rigid helical conformation of the polypeptide chain. Short poly-L-EG_mGlu polypeptides have more random coil component than helical component, which makes them more hydrophilic. On the other hand, increase of MW induces more and more amides bonds transformed from random coil conformation to ordered helical structure as illustrated in

Scheme 2(a). Thus, the shorter poly-L-EG_mGlu polypeptides are more hydrophilic than the longer poly-L-EG_mGlu polypeptides from the secondary structure point-of-view. So the CP of poly-L-EG_mGlu has stronger dependence on MW as compared to PNIPAM. For poly-L-EG₃Glu, its side chain is longer and more hydrophilic than that of poly-L-EG₂Glu, so it needs even larger MW to display thermal responsive properties.

Following the above discussion, we can expect that disruption of the enantiomeric purity will not only decrease the samples' helicity, but also induce loss of thermal responsive properties because of the transition from intramolecular to intermolecular hydrogen bonding for the backbone amide bonds. If we used equal amount of D-EG_mGlu NCA and L-EG_mGlu NCA to do ROP, we obtained racemic homopolypeptides, which was found to completely lose thermal responsive properties. In contrast, enantiomeric pure poly-D-EG_mGlu and poly-L-EG_mGlu displayed reversible LCST transitions in water^[25].

CONCLUSIONS

We successfully synthesized a series of thermal responsive homopolypeptides *via* ROP of di- and triethylene glycol monomethyl ether L-glutamate NCAs. The combination of HDMS and TBD allowed well controlled ROP for these two NCA monomers. Their molecular weight can be controlled by monomer to initiator ratio, and the obtained samples had narrow molecular weight distribution. We also demonstrated that the helicity of poly-L-EG_mGlu increases with MW, and the helical regularity ultimately determined the thermal responsive properties of the obtained polypeptides. We found that the longer the polypeptide chain, the more the helical component, the less the intermolecular hydrogen bonding, and the lower the cloud point. The primary reason is that the amide bonds in helical polypeptide form intramolecular hydrogen bonding. In contrast, random coil segments in poly-L-EG_mGlu are more hydrophilic because the existence of rich intermolecular hydrogen bonding with water.

ACKNOWLEDGEMENTS Z.B.L thanks Prof. Dongsheng Liu in Tsinghua University for providing CD facility. S.S.Z. thanks Dr. Hua Lu in Scripps Research Institute for discussion about synthesis of polypeptides.

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