End Groups Adjusting the Molecular Nano-Assembly Pattern and Thermal Gelation of Polypeptide Block Copolymer Aqueous Solution

Jin Young Kim,[†] Min Hee Park,[†] Min Kyung Joo,[†] Soo Young Lee,[‡] and Byeongmoon Jeong*[,†]

Department of Chemistry and Nano Science, Department of Bioinspired Science, Ewha Womans University, Daehyun-Dong, Seodaemun-Ku, Seoul, 120−750, Korea, Department of Life Science, Department of Bioinspired Science, Ewha Womans University, Daehyun-Dong, Seodaemun-Ku, Seoul, 120−750, Korea

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ABSTRACT: We are reporting end-capping of the amino groups of a polypeptide block copolymer by hydrophobic alkyl groups including methyl, ethyl, and propyl group. The end-capped polymer aqueous solution undergoes sol-to-gel transition as the temperature increases in a physiologically important temperature range of 10−40 °C, whereas the unmodified polymer does not show the transition. In addition, secondary structure of the polypeptide changed from random coils to β-sheets and the assembled pattern changed from spherical micelles into cylindrical nanofibrous structures in water by the hydrophobic end-capping of the polymer. The enhanced hydrophobic interactions by the alkyl end groups recruit the hydrophobic polypeptides in a more organized way to drive such a conformational change of polypeptides.

Introduction

End-capping of a polymer significantly affects the physicochemical properties of the polymer, thus it has been extensively applied for specialty plastic production.[1−5] For example, poly(oxymethylene) end-capped by ethylene oxide units results in the stabilization of the polymer by blocking the unzipping reaction of the polymer.[3] Recently, the end-capping method has also been applied for the preparation of biomedical polymers. The end-capping of poly(lactic acid) by acetic anhydrides slow down the degradation rate of the poly(lactic acid).[6] The lower critical solution temperature of poly(methyl vinyl ether) decreases from 40 to 15 °C in water by capping the hydroxy end groups with hydrophobic bromoalkyl groups.[7] Compared with polycaprolactone, phosphatidyl choline end-capped polycaprolactone shows an improved antiadsorption of the platelet and prolonged thromoboplastin time.[8] Sol−gel transition temperatures of poly(lactic acid-*co*-glycolic acid)-poly(ethylene glycol)-poly(lactic acid-*co*-glycolic acid) and poly(lactic acid-*co*-caprolactone)-poly(ethylene glycol)-poly(lactic acid-*co*-caprolactone) triblock copolymers were controlled through modifying hydroxyl end groups of the polyester by alkyl group.[9−11] 8-armed star-shape poly(ethylene glycol)-poly(lactic acid) end-capped by cholesterol shows reverse thermal gelation at as low as 3 wt %.[12] They suggested that sol-to-gel transition is facilitated by the association of the hydrophobic alkyl groups.

Here, we are reporting that capping the amino end groups of a polyalanine block copolymer by alkyl groups (1) changes a secondary structure of the polyalanine from random coils to β-sheets in water, (2) changes a self-assembled pattern from spherical micelles to nanofibrous cylinders in water, and (3) induces reverse thermal gelation of the polymer aqueous solutions.

Experimental Section

Synthesis. Poly(alanine)-(propylene glycol)$_{1.75}$-(ethylene glycol)$_{15.5}$-(propylene glycol)$_{1.75}$-poly(alanine) (PA−PLX−PA; 470−900−470: C0) was prepared by ring-opening polymerization of the *N*-carboxy anhydrides of alanine in the presence of α,ω-diamino-(propylene glycol)$_{1.75}$-(ethylene glycol)$_{15.5}$-(propylene glycol)$_{1.75}$ (PLX).[13,14] PLX (6.0 g, 6.7 mmol; MW 900 Da; Aldrich) was dissolved in toluene (100 mL) and the residual water was removed by azeotropic distillation to a final volume of about 10 mL. Anhydrous chloroform/dimethyl formide (60 mL; 2/1 v/v), *N*-carboxy anhydrides of L-alanine (2.0 g, 17.3 mmol), and *N*-carboxy anhydrides of DL-alanine (6.0 g, 52.0 mmol) were added to the reaction mixtures. They were stirred at 40 °C for 24 h. The polymer was purified by repeated dissolution in the chloroform, followed by precipitation into diethyl ether, three times. The yield was 67%. The PA−PLX−PA (470−900−470: C0) (3.0 g; 1.63 mmol) with amino end groups reacted with acetyl chloride (0.45 mL; 6.36 mmol) in chloroform. Triethylamine (0.66 mL; 4.74 mmol) was used as a catalyst. The reaction mixture was precipitated into diethyl ether. Propionyl chloride and butyryl chloride were similarly reacted to prepare C1, C2, and C3 end-capped PA−PLX−PA.

^{1}H and ^{13}C NMR Spectra. ^{1}H NMR spectra in CF_3COOD (500 MHz NMR spectrometer; Varian) was used to determine the composition of the polymer. ^{13}C NMR spectral changes of the R−PA−PLX−PA−R (R = C2; 6.0 wt % in D_2O) were investigated as a function of temperature in a range of 10−50 °C with an increment of 10 °C each step. The solution temperature was equilibrated for 20 min at each temperature.

Gel Permeation Chromatography. The gel permeation chromatography system (Waters 515) with a refractive index detector (Waters 410) was used to obtain the molecular weights and molecular weight distributions of the polymers. *N*,*N*-Dimethylformamide was used as an eluting solvent. The poly(ethylene glycol)s in a molecular weight range of 400−20 000 Da were used as the molecular weight standards. An OHPAK SB-803QH column (Shodex) was used.

Circular Dichroism Spectroscopy. Ellipticity of the R−PA−PLX−PA−R (R = C0, C1, C2, or C3) aqueous solution (0.05 wt %) was obtained by a circular dichroism instrument (J-810; JASCO). As for the C2 end-capped polymer, ellipticity of the polymer aqueous solution (0.05 wt %) was investigated as a function of temperature in a range of 10 °C ∼ 50 °C with an increment of 10 °C each step. The aqueous solution was equilibrated for 20 min at each temperature.

FTIR Spectra. IR spectra (FTIR spectrophotometer FTS-800; Varian) of the R−PA−PLX−PA−R (R = C0, C1, C2 or C3)

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* To whom correspondence should be addressed. Fax: 82232772384. Telephone: 82232773411. E-mail: bjeong@ewha.ac.kr.

[†] Department of Chemistry and Nano Science, Department of Bioinspired Science, Ewha Womans University.

[‡] Department of Life Science, Department of Bioinspired Science, Ewha Womans University.

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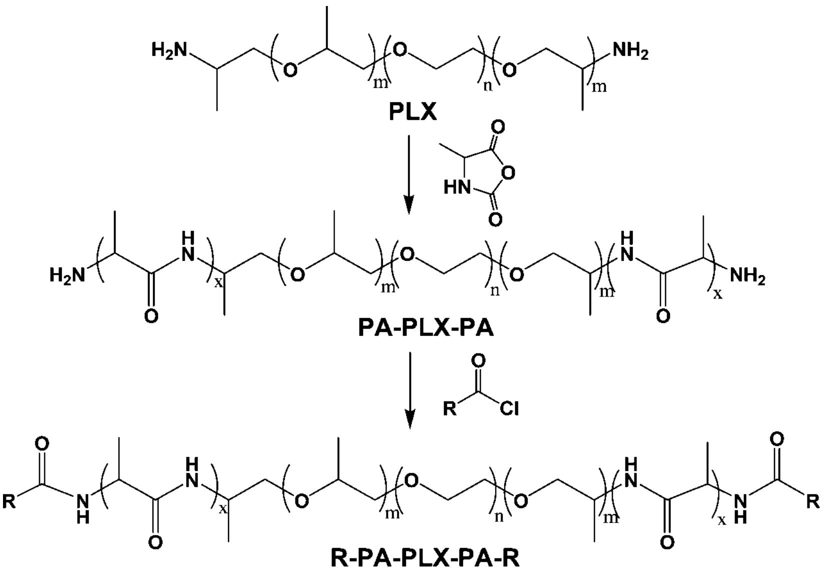
Scheme 1. Synthesis of R−PA−PLX−PA−R[a]



[a] Key: $m = 1.75$. $n = 15.5$, and $x = 6.2$. R is the methyl (C1), ethyl (C2), or propyl (C3) groups.

aqueous solution (6.0 wt % in D_2O) were investigated. As for the C2 end-capped polymer, spectral changes of the polymer aqueous solution (6.0 wt % in D_2O) was investigated as a function of temperature in a range of 10−50 °C with an increment of 5 °C each step. The aqueous solution was equilibrated for 20 min at each temperature.

Transmission Electron Microscopy. The R−PA−PLX−PA−R (C0, C1, C2, and C3) aqueous solution (2.0 wt %; 20 μL) at 15 °C was placed on the carbon grid and the excess solution was blotted with filter paper. The grids were dried at 15 °C for 24 h. The microscopic image was obtained by JEM-2100F (JEOL) with an accelerating voltage of 200 kV.

Dynamic Mechanical Analysis. Changes in modulus of the polymer aqueous solutions were investigated by dynamic rheometry (Rheometer RS 1; Thermo Haake). The aqueous polymer solution was placed between parallel plates of 25 mm diameter and a gap of 0.5 mm. During the dynamic mechanical analysis, the samples were located inside of a chamber with water-soaked cotton to minimize the water evaporation. The data were collected under a controlled stress (4.0 dyn/cm^2) and a frequency of 1.0 rad/s. The heating rate was 0.5 °C/min.

Results and Discussion

Synthesis of the end-capped PA−PLX−PA (R−PA−PLX−PA−R) is described in scheme 1. PA−PLX−PA (M_n = 1840 Da by ^{1}H NMR, M_w/M_n = 1.3 by GPC) was synthesized by ring-opening polymerization of *N*-carboxy anhydrides of L-alanine/DL-alanine (25/75 by mole) in the presence of α,ω-diamino-(propylene glycol)$_{1.75}$-(ethylene glycol)$_{15.5}$-(propylene glycol)$_{1.75}$.[13,14] End-capping of the PA−PLX−PA (C0) was carried out by reacting the polymer with acetyl chloride (C1), propionyl chloride (C2), or butyryl chloride (C3) in chloroform. Triethyl amine was used as a catalyst. The polymers end-capped with alky group larger than pentyryl chloride was partially soluble in water. Therefore, the end-capped polymers of C0 (unmodified), C1, C2, and C3 that were completely soluble in water were compared in this study. ^{1}H NMR and gel permeation chromatogram of the end-capped polymer (R−PA−PLX−PA−R) confirmed the polymer structure (Supporting Information: Figure S1a). The methylene group next to the carbonyl appears at 2.3−2.6 ppm. The terminal methyl end group appeared at 1.1−1.7 ppm. The gel permeation chromatogram of R−PA−PLX−PA−R appeared at a slightly earlier retention time than the unmodified polymer (C0), suggesting better solvation of the end-capped polymer in dimethyl formamide than the unmodified polymer (Supporting Information: Figure S1b).

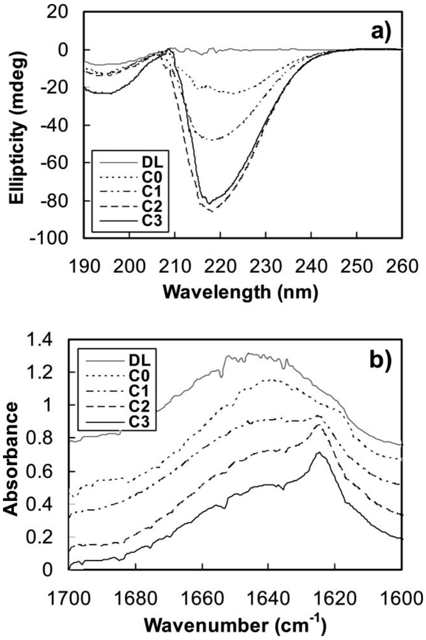


Figure 1. (a) Circular dichroism spectra of R−PA−PLX−PA−R aqueous solutions (0.05 wt %) at 20 °C. (b) FTIR spectra of R−PA−PLX−PA−R aqueous solutions (6.0 wt % in D_2O) at 20 °C. C0, C1, C2, and C3 indicate $-NH_2$ (unmodified PA−PLX−PA), $-NHCOCH_3$, $-NHCOCH_2CH_3$, and $-NHCOCH_2CH_2CH_3$ end-capped polymers, respectively. DL indicates a reference polymer of DL-poly(alanine)-PLX-DL-poly(alanine).

Circular dichroism shows the increases in the negative Cotton band at 218 nm as the end group varies from C0, C1, C2, and C3. C2 and C3 showed a similar ellipticity (Figure 1a). The absorption peak at 1625 cm^{-1} of the FTIR spectra increases as the end group vary from C0, C1, C2 and C3 (Figure 1b). C2 and C3 were similar in absorbance. The negative Cotton band at 218 nm (circular dichroism) and absorption peak at 1620−1630 cm^{-1} (FTIR) are characteristics of a β-sheet structure of polypeptides.[15−17] DL in Figure 1 is the reference unmodified

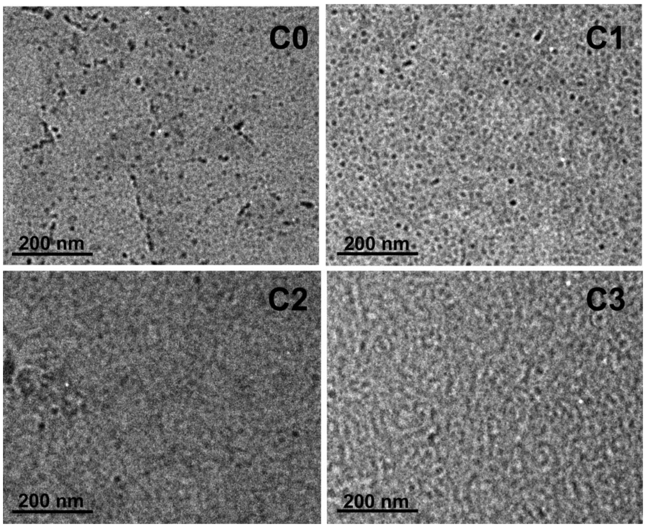


Figure 2. Transmission electron microscopic image of the R−PA−PLX−PA−R. Polymer aqueous solutions (2.0 wt %) were air-dried at 15 °C. The scale bar is 200 nm. C0, C1, C2, and C3 indicate $-NH_2$ (unmodified), $-NHCOCH_3$, $-NHCOCH_2CH_3$, and $-NHCOCH_2CH_2CH_3$ end-capped polymers respectively.

PA−PLX−PA where the PA consists solely of DL-alanine. The circular dichroism (small negative Cotton band at 195 nm) and FTIR spectra (broad amide I band centered at 1640 cm^{-1}) of the polymer show a characteristic random coil conformation of the DL-polyalanine. Therefore, both circular dichroism and FTIR spectra suggest the development of β-sheet structure by end-capping of the PA−PLX−PA. Development of the β-sheet conformation from human proteins with random coil or α-helix structure are reported for the major mechanism of mad cow disease and Alzheimer's disease.[18,19] Current study might suggest that the alkylation of a protein by toxic metabolites or compounds might trigger a conformational change of protein to direct a pathological protein development.[20,21]

The transmission electron microscopic images of the assembled polymer were developed from the polymer aqueous solution. The images show dominantly spherical micellar structures of the C0 (unmodified) and C1, whereas the ethyl (C2) and propyl (C3) modified polymers show coexistence of spherical micellar structures and fibrous cylindrical structures (Figure 2). This observation suggests that β-sheeted polypeptides tend to form a fibrous nanostructure.[22,23]

^{13}C NMR spectra (in D_2O) of end-capped PA−PLX−PA show that the PLX peak is broadened as the temperature increases (Figure 3), suggesting that molecular motion of PLX decreases and dehydration of PLX is involved in the sol-to-gel transition, similar to other reverse thermogelling polymers.[14,24,25] However, a negative Cotton band at 218 nm and FTIR peak at 1625 did not significantly change in a temperature range of 0 ∼ 50 °C (Supporting Information: Figure S2a and Figure S2b).

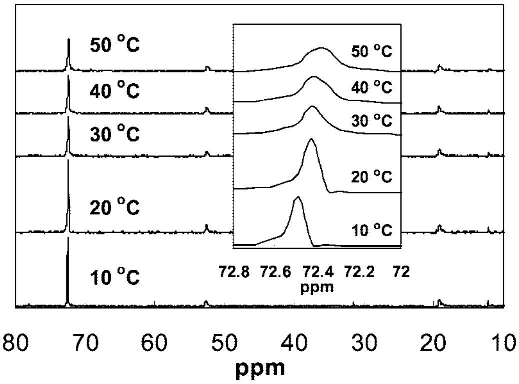


Figure 3. ^{13}C NMR spectra of $CH_3CH_2CONH-PA-PLX-PA-NHCOCH_2CH_3$ (C2) aqueous solutions (6.0 wt % in D_2O) as a function of temperature.

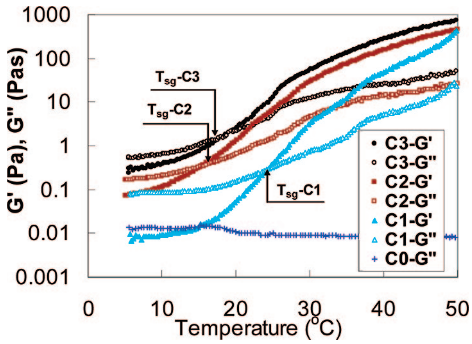


Figure 4. Increases in storage modulus (G') and loss modulus (G'') of the R−PA−PLX−PA−R aqueous solutions (6.0 wt %) during the sol-to-gel transition. G' of C0 is negligibly small (<0.001 Pa s) and does not cross G' in a temperature range of 0−50 °C and is not shown in the graph.

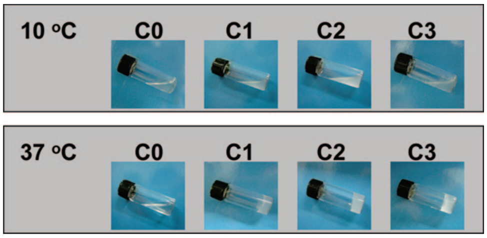


Figure 5. Photos of the R−PA−PLX−PA−R aqueous solutions (6.0 wt %) at 10 °C and at 37 °C. C0, C1, C2, and C3 indicate $-NH_2$ (unmodified PA−PLX−PA), $-NHCOCH_3$, $-NHCOCH_2CH_3$, and $-NHCOCH_2CH_2CH_3$ end-capped polymers, respectively.

An elastic component (G') crosses over the viscous component (G'') of the complex modulus of the polymer aqueous solutions (6.0 wt %) as the temperature increases, suggesting that sol-to-gel transition occurs for end-capped polymers of C1, C2, and C3 (Figure 4).[26,27] However, the unmodified PA−PLX−PA (C0) did not undergo sol-to-gel transition in the same temperature range of 0−50 °C. The elastic component of the unmodified PA−PLX−PA aqueous solution (6.0 wt %) was less than 0.001 Pas and did not cross the viscous component (data not shown). The sol-to-gel transition temperature defined by the crossover point of G' and G'' of C1 (24 °C) is higher than C2 (16 °C) and C3 (17 °C). Considering an increase in hydrophobicity and partial development of β-sheet secondary structure of polypeptide from the random coil structure, a drastic change in aqueous polymer solution property by end-capping the amino end group (C0) with acetyl (C1) or propionyl (C2) group is apparent. C2 and C3 end-capped polymer showed a similar sol-to-gel transition temperature. The gel modulus at 37 °C increased in an order of C1 < C2 < C3 end-capping, suggesting that both hydrophobic interactions and secondary structure are important in gel packing density or gel modulus. When the poly(ethylene glycol)-poly(propylene glycol)-poly(ethylene glycol) was end-capped by short polycaprolactone (PCL_4) or poly(lactic acid) (PLA_6), the polymer decreased in gel modulus and increased in sol−gel transition temperature.[28] Ding group end-capped block copolymers of PLGA−PEG−PLGA by methyl, ethyl, and propyl groups.[9,10] They found that an otherwise sol system could, in water, exhibit the sol−gel transition upon increase of temperature in the cases of methyl and ethyl capped samples, and the propyl-ended block copolymer precipitated in water. The present work introduced similar end groups to a different block copolymer, and we confirmed the change from a sol to a

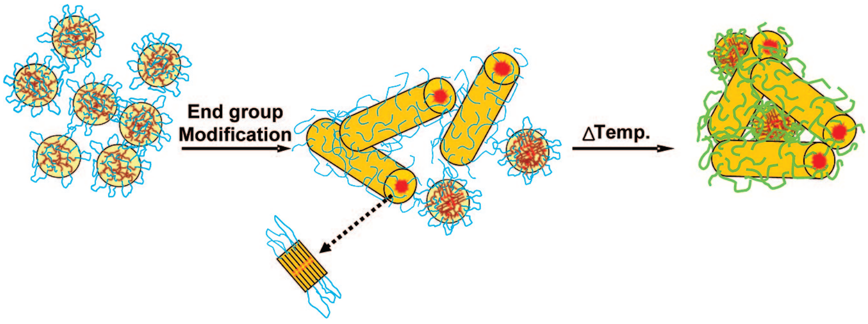


Figure 6. Schematic presentation of end group effects of R−PA−PLX−PA−R on sol−gel transition. Blue lines (hydrophilic PLX), brown lines (hydrophobic PA), and red circles (end-capping alkyl group) are shown. Yellow spheres and cylinders indicate the hydrophobic domains consisting of polyalanines. End-capping by alkyl groups increases β-sheet conformation of polypeptides and cylindrical nanofibrous assembly of the polymers. The assembled polymers undergoes sol-to-gel transition, which involves dehydration of hydrophilic blocks.

sol−gel transition from the virgin polymers to the methyl, ethyl or propyl end-capped polymers. The gel-to-sol transition of methyl and ethyl capped PLGA−PEG−PLGA upon decreasing the temperature was reported, suggesting that the physical gelation of the alkyl-group-capped PLGA−PEG−PLGA is reversible.[9,10] Whereas the present block copolymers R−PA−PLX−PA−R take on thermogelling, the gel−sol transition is reversible but, however, requires a long time. The present research extends the end-capping induced sol−gel transition to polypeptide-based block copolymers, and our polymer is unique in that end-capping (1) increased the gel modulus, (2) decreased the sol−gel transition temperature, and (3) triggered the formation of β-sheets and possibly even nanofibrils involved in the physical hydrogel.

Figure 5 shows the photos of the polymer aqueous solution (6.0 wt %) at 10 and 37 °C. Unmodified polymer (C0) is in a sol state both at 10 and 37 °C, whereas C1, C2, and C3 modified polymer showed a sol state at 10 °C, and a gel state at 37 °C. A polymer aqueous solution with such a phase behavior is suggested to be promising as a minimally invasive injectable system.[12,25,29,30]

On the basis of the circular dichroism spectra, FTIR spectra, transmission eletron mocroscopic images, ^{13}C NMR spectra, and dynamic mechanical analysis, the effect of end-capping by alkyl group of the PA−PLX−PA is schematically presented (Figure 6). As the polymer amino end groups are capped by hydrophobic alkyl groups, the increased hydrophobic intertractions changes the molecular assembly of the polymers from random coils to β-sheets. As the β-sheet conformation of the polypeptide increases, the self-assembly pattern of the polymer changes from spherical micelles to nanofibrous structures. The nanofibrous structure facilitates the reverse thermal gelation of the polymer, and thus the sol-to-gel transition of the end-capped polymer occurs in a physiologically important temperature range of 10−40 °C, even though the unmodified polymer does not show sol-to-gel transition in this temperature range.

Conclusions

To conclude, the amino end group of a polyalanine block copolymer were capped by hydrophobic alkyl groups. As the alky group length increased from C0 (unmodified) to C1, C2, and C3, the polypeptide increased the β-sheet conformation in water and the self-assembled morphology of the polymer changed from spherical micelles to nanofibrous structure. Such changes drives the end-capped polymer aqueous solutions to undergo a sol-to-gel transition as the temperature increases in a physiologically important range of 10−40 °C. This paper suggests that the simple hydrophobic end-capping not only changes the secondary structure and the self-assembly pattern of polypeptides but also induces thermosensitivity of the polymer aqueous solution. Aside from the characteristics as a biomaterial, current study might suggest that the hydrophobic alkylation of a protein may trigger a conformational change of the protein to direct a pathological protein development.

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Supporting Information Available: ^{1}H NMR spectra (CF_3COOD) and GPC chromatogram (DMF), FTIR, and circular dichroism spectra of C2−PA−PLX−PA−C2 aqueous solutions as a function of temperature. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Hilf, S.; Grubbs, R. H.; Kilbinger, A. F. M. *J. Am. Chem. Soc.* **2008**, *130*, 11040–11048.
(2) Brzezinska, K. R.; Curtin, S. A.; Deming, T. J. *Macromolecules* **2002**, *35*, 2970–2976.
(3) Masamoto, J.; Matsuzaki, K.; Iwaisako, T.; Yoshida, K.; Kagawa, K.; Nagahara, H. *J. Appl. Polym. Sci.* **2003**, *50*, 1317–1329.
(4) Okamato, M. *Polymer* **2001**, *42*, 8355–8359.
(5) Fleischer, C. A.; Morales, A. R.; Koberstein, J. T. *Macromolecules* **1994**, *27*, 379–385.
(6) Bigg, D. M. *Adv. Polym. Technol.* **2005**, *24*, 69–82.
(7) Durme, K. V.; Mele, B. V.; Bernaerts, K. V.; Verdonck, B.; Du Prez, F. E. *J. Polym. Sci. Part B. Polym. Phys.* **2005**, *44*, 461–469.
(8) Meng, S.; Zhong, W.; Chou, L. L.; Wang, Q.; Liu, Z.; Du, Q. *J. Appl. Polym. Sci.* **2007**, *103*, 989–997.
(9) Yu, L.; Zhang, H.; Ding, J. *Angew. Chem., Int. Ed.* **2006**, *45*, 2232–2235.
(10) Yu, L.; Chang, G.; Zhang, H.; Ding, J. *J. Polym. Sci. Polym. Chem.* **2007**, *45*, 1122–1133.
(11) Jo, S.; Kim, J.; Kim, S. W. *Macrmol. Biosci.* **2006**, *6*, 923–928.
(12) Nagahama, K.; Ouchi, T.; Ohya, Y. *Adv. Funct. Mater.* **2008**, *18*, 1220–1231.
(13) Oh, H. J.; Joo, M. K.; Sohn, Y. S.; Jeong, B. *Macromolecules* **2008**, *41*, 8204–8209.
(14) Choi, Y. Y.; Joo, M. K.; Sohn, Y. S.; Jeong, B. *Soft Matter* **2008**, *4*, 2383–2387.
(15) Ozbas, B.; Kretsinger, J.; Rajagopal, K.; Schneider, J. P.; Pochan, D. J. *Macromolecules* **2004**, *37*, 7331–7337.
(16) Yang, H.; Pritzker, M.; Fung, S. Y.; Sheng, Y.; Wang, W.; Chen, P. *Langmuir* **2006**, *22*, 8553–8562.
(17) Hamley, I. W.; Ansari, I. A.; Castelletto, V.; Nuhn, H.; Rosler, A.; Klok, H. A. *Biomacromolecules* **2005**, *6*, 1310–1315.
(18) Burkoth, T. S.; Benzinger, T. L. S.; Jones, D. N. M.; Hallenga, K.; Meredith, S. C.; Lynn, D. G. *J. Am. Chem. Soc.* **1998**, *120*, 7655–7656.
(19) Maya, Y.; Ono, M.; Watanabe, H.; Haratake, M.; Saji, H.; Nakayama, M. *Bioconj. Chem.* **2009**, *20*, 95–101.
(20) Liu, H.; Lightfoot, R.; Stevens, J. L. *J. Biol. Chem.* **1996**, *271*, 4805–4812.
(21) He, K.; He, Y. A.; Szklarz, G. D.; Halpert, J. R.; Correia, M. A. *J. Biol. Chem.* **1996**, *271*, 25864–25872.

(22) Lim, Y. B.; Lee, M. *J. Mater. Chem.* **2008**, *18*, 723–727.
(23) Lim, Y. B.; Moon, K. S.; Lee, M. *Angew. Chem., Int. Ed.* **2009**, *48*, 1601–1605.
(24) Jeong, B.; Wang, L. Q.; Gutowska, A. *Chem. Commun.* **2001**, 1516–1517.
(25) Park, S. H.; Choi, B. G.; Joo, M. K.; Han, D. K.; Sohn, Y. S.; Jeong, B. *Macromolecules* **2008**, *41*, 6486–6492.
(26) Sarvestani, A. S.; He, X.; Jabbari, E. *Biomacromolecules* **2007**, *8*, 406–415.
(27) Li, L.; Liu, E.; Lim, C. H. *J. Phys. Chem. B.* **2007**, *111*, 6410–6416.
(28) Xiong, X. Y.; Tam, K. C.; Gan, L. H. *Polymer* **2005**, *46*, 1841–1850.
(29) Liu, L.; Ding, J. *Chem. Soc Rev.* **2008**, *37*, 1473–1481.
(30) Zhang, H.; Yu, L.; Ding, J. *Macromolecules* **2008**, *41*, 6493–6499.

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