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Synthesis and characterization of di- and triblock copolymers of poly(ethylene oxide) and poly(DL-valine-*co*-DL-leucine)

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Abstract

Di- and triblock copolymers of poly(ethylene oxide) (PEO) and poly(DL-valine-*co*-DL-leucine) were synthesized by ring-opening polymerization of *N*-carboxyanhydride (NCA)s of DL-valine and DL-leucine initiated by amine-terminated PEOs. The resulting block copolymers were characterized by ^{1}H NMR, FT-IR, DSC, and wide-angle X-ray diffraction (WAXD). Differential scanning calorimeter revealed that the melting temperature of triblock copolymer was lower than that of diblock copolymer and the study of WAXD patterns of triblock copolymer indicated that PEO segments are stretched during the copolymerization as shown in the formation of complex. The self-assembly behaviors of these di- and triblock copolymers in the aqueous solution showed various forms.

Keywords: Leucine and valine *N*-carboxyanhydride; Block copolymer; Self-assembly

1. Introduction

Various polymer systems based on poly(ethylene oxide) (PEO) have been the subject of many studies particularly in the area of biomedical applications such as drug delivery systems, protein modification etc. [1–4] Because PEO is well known to be a non-toxic and non-immunogenic water soluble polymer. Recently, the amphiphilic block copolymers consisted of polypeptide and PEO were synthesized and interesting morphological modifications (vesicles, nanoparticles etc.) were observed with these polymers [5–8]. However, the structural variations have been rather limited and it would be interesting to synthesize different kind of peptide-containing block copolymers and observe further the morphological behaviors of those resulting polymers.

In the present work, we have prepared di- and triblock copolymers by polymerization of DL-valine and DL-leucine NCAs by primary amine terminated PEO. DL-valine and DL-leucine were used as hydrophobic block because they contain isopropyl and isobutyl side chains to increase the hydrophobicity and the polypeptide synthesized by DL-amino acid would not form crystalline polymer so that it would help to increase the solubility. Therefore, we studied the self-assembly behavior of these resulting polymers in the aqueous solution. The result of the first phase of the work is described in the present presentation.

2. Experimental part

2.1. Materials

DL-valine *N*-carboxylanhydride (V-NCA, **2a**) and DL-leucine *N*-carboxylanhydride (L-NCA, **2b**) were purchased from Korea Fine Chemical Co., Korea. α-Methyl-ω-amino-poly(ethylene oxide) (mPEOA, **1a**,$M_n = 5,000$) and α,ω-diamino-poly(ethylene oxide) (DAPEO **1b**,$M_n = 3,400$) were purchased from the Shearwater Corp., USA. All of the above materials were used as received. Dialysis tube was purchased from Spectrum Corp., USA. Reagent-grade *N*,*N*-dimethylformamide (DMF) was dried with $MgSO_4$ and then distilled under reduced pressure over P_2O_5.

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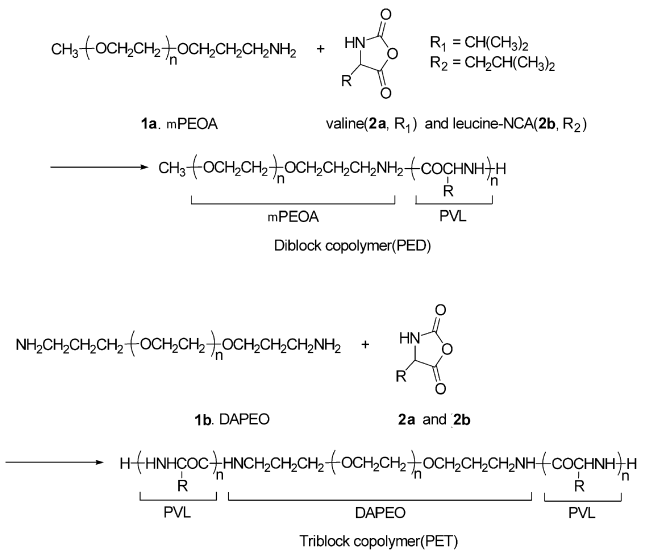

Scheme 1. Synthetic routes of di- and triblcok copolymers.

2.2. Polymerization and characterization

2.2.1. Synthesis of diblock copolymers of PEO and poly(leucine and valine) (PED)

The preparative procedure for PED-1 diblock copolymers is shown in Scheme 1. DL-leucine *N*-carboxyanhydride (26 mg, 0.2 mmol) and DL-valine *N*-carboxyanhydride (59 mg, 0.5 mmol) were first dissolved in 0.7 ml of *N*,*N*-dimethylformamide (DMF), followed by addition of 2 ml of distilled chloroform. MPEOA (0.5 g, 0.1 mmol) was then dissolved in 2 ml chloroform and added to the above NCA solution. The reaction mixture was stirred at 20 °C under the stream of dry nitrogen until NCA reacted completely as ascertained by tracing the characteristic carbonyl 1850 and 1790 cm^{-1} in IR spectrum. The reactions were complete in 8 h. When complete the reaction mixture was poured into a 10-fold volume of diethyl ether and the precipitate formed was collected by filtration and washed with diethyl ether and with water to remove the unreacted PEO, followed by drying in vacuum. The conversion was virtually quantitative.

2.2.2. Synthesis of triblock copolymers (PET)

The preparation of PET triblock copolymers is outlined in Scheme 1. The synthesis of PET triblock copolymers were carried out according to the same procedure to that employed for the syntheses of PED-1 diblock copolymers.

2.2.3. Characterization

Gel permeation chromatography (GPC) analysis was performed with PL-200 equipped with 10 μm MIXED-B X2.500A column at a flow rate 1 ml min^{-1} using DMF with 0.05 M LiBr as eluent. NMR spectra were recorded on Bruker AM-300 (300 MHz for ^{1}H and 75.1 MHz for ^{13}C) instrument with $CDCl_3$ solvent. Both ^{1}H and ^{13}C NMR measurements were carried out at room temperature with tetramethylsilane as an internal standard. FT-IR spectra of the compounds were obtained with a Bruker EQUINOX-55 spectrometer using KBr pellet. Differential scanning calorimeter (DSC) analysis was performed on a TA 2200 thermal analyzer system and measurements were made using a closed cell at heating rate of 10 °C/min in N_2 atmosphere. Wide-angle X-ray diffraction (WAXD) data were collected with a PC-MRD software that has a user interface based on a reciprocal space model. Samples of PET and PEO standard were irradiated with Cu Kα X-rays (wavelength $\lambda = 1.5406$ Å) at a scan rate of 2 °C/min. Data were taken over a diffraction angle range 10–50° (2θ). The hydrodynamic radii, RH, of the PED and PET nanoparticles (0.1 wt% concentration) prepared by dialysis method were measured using Dynamic Light Scattering (DLS, Brookharven Instruments) with an argon laser ($\lambda_o = 514.5$ nm). Transmission electron microscopy (TEM) images were obtained from an EM 912 Omega operating at 120 kV.

2.3. Preparation of PED and PET nanoparticles

Preparation of PED and PET nanopaticles was carried out by the dialysis method. Five mg of PED-1 were dissolved in 5 ml of DMF. The solution was introduced into the dialysis tube (molecular cutoff 3,400 and 10,000 g/mol) and dialyzed 3 times against 1.0 l of distilled water for the time period of 3 h. Thus organic solvent was removed completely.

Table 1
Characterization of di- and triblock copolymers

Sample	{[valine]/[leucine]}/[PEO][a]		$(\eta)^b$ (dl/g)	$M_n{}^a$	$M_n{}^c$	PDI
	Reactant	Product[a]				
PED-1	91.9/8.1	89.9/10.1	0.19	6077	6070	1.04
PED-2	95.8/4.2	96.2/3.8	0.17	5382	5400	1.06
PET-1	84.6/13.4	90.3/9.7	0.15	5133	5140	1.03
PET-2	88.5/11.5	93.0/7.0	0.14	4136	4180	1.04

[a] Composition and molecular weight were obtained by ^{1}H NMR(M_n = molecular weight of valine NCA × 0.69 × mole ratio of valine + molecular weight of leucine NCA × 0.72 × mole ratio of leucine + molecular weight of PEO).
[b] Intrinsic viscosity was measured in dichloroacetic acid at 20 °C using a Cannon–Freske viscometer.
[c] Determined by GPC using DMF with 0.05% LiBr as eluent at 35 °C and polystyrene standard.

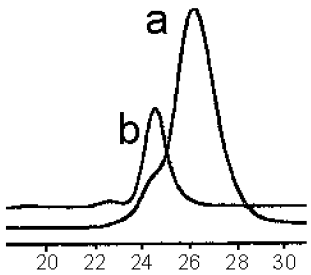

Fig. 1. GPC chromatographs of DAPEO (a) and PET-1 (b).

3. Results and discussion

3.1. Synthesis of the PED and PET di- and triblock copolymers

PED and PET copolymers were obtained by ring-opening polymerization of NCAs of amino-acid initiated by the amine end-groups of PEOs. The mechanism of amine-initiated polymerization of NCAs is well known [9,10]. The compositions and molecular weights of the di- and triblock copolymers were summarized in Table 1. The composition of a copolymer was determined from the ratio of the integrated peak areas of the six methyl protons of leucine and valine and four methylene protons of PEO assuming that the relativities of leucine and valine NCAs are equal. As shown in Table 1, the conversions were virtually quantitative. The smaller feed ratio NCA/PEO gave the lower molecular weight polymers as anticipated. Molecular weights of copolymers were calculated by the composition of copolymers based on the PEO used, assuming that all the polymers have the expected structure. Fig. 1 represents the results of GPC analysis. The polydispersity of PEOs and block copolymers were low values in the GPC measurements. This fact indicated that unreacted PEOs were completely removed and the resulting block copolymers exhibited very narrow molecular weight distribution. The resulting polymers were soluble in DMF, and DMAc.

3.2. Characterization of the di- and triblock copolymers

The chemical structures of copolymers were confirmed by IR, ^{1}H NMR spectra. The IR spectra of di- and triblock copolymers exhibited absorption bands at 1655 and 1543 cm^{-1} attributable to amide bond. IR absorptions of 1860 and 1785 cm^{-1}, which are characteristic of NCAs of DL-leucine- and DL-valine-NCA, were clearly disappeared. However, it was expected that since DL-mixture of NCAs was used, the formation of a α-helical structure by the polypeptide chain would not be observed. The ^{1}H NMR spectrum of di- and triblock copolymers showed two methyl group protons (0.8–0.9 ppm), methyne in leucine and valine (1.23 ppm), methylene in leucine (1.6–1.8 ppm), and methylene in poly(ethylene glycol) (3.6 ppm).

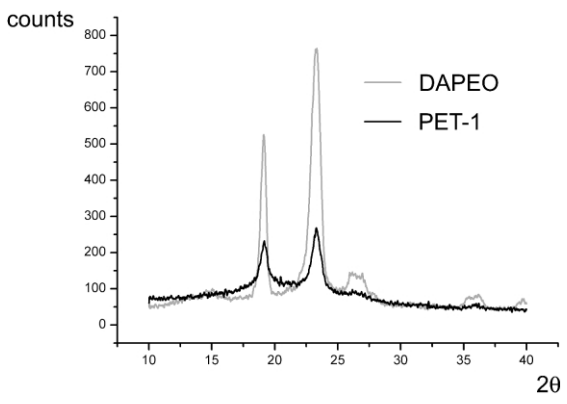

Fig. 2. WAXD curves of PED-1 (bottom) and DAPEO (top).

Table 2
Relative intensities and *d* spacing of the WAXD peaks of PED-1 and DAPEO

PED-1		PED-2		DAPEO	
d Spacing (Å)	Rel. int. (%)	*d* Spacing (Å)	Rel. int. (%)	*d* Spacing (Å)	Rel. int. (%)
4.64	80.8	4.63	86.9	4.65	68.1
3.83	100	3.81	100	3.81	100
3.33	30	3.31	34.1	3.31	19.1
				2.48	11.0

A comparison of the WAXD of PET-1 and DAPEO showed (Fig. 2 and Table 2) the following differences: (1) slight reduction in the intensity of the peak at 3.81 Å; (2) large increase in the density of the peak at 4.65 Å; (3) decrease in *d* spacing. Pure DAPEO has two prominent peaks at 4.65 and 3.81 Å with relative intensities 68.1 and 100%, respectively. Reduced intensity of the peak at 3.81 Å and increased in the intensity of peak at 4.65 Å observed for PET-1 block copolymers may be attributed to a change in packing distance between the PEO chains on $x-y$ plane after block polymerizations and structural rearrangements similarly occur as shown in the case of the formation of PEO/sodium poly(glutamate) (PGNA) complex [11].

Analysis of the thermal transition behavior of PED, PET, and PEO was carried out by DSC measurements. Thermograms of PED, PET, and PEO are shown in Fig. 3. The

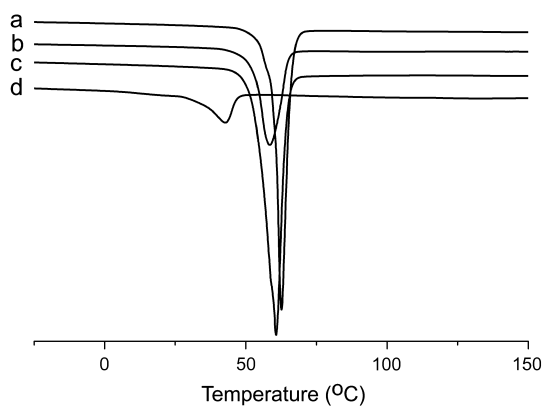

Fig. 3. DSC thermograms of (a) mPEOA, (b) PED-1, (c) DAPEO, and (d) PET-1.

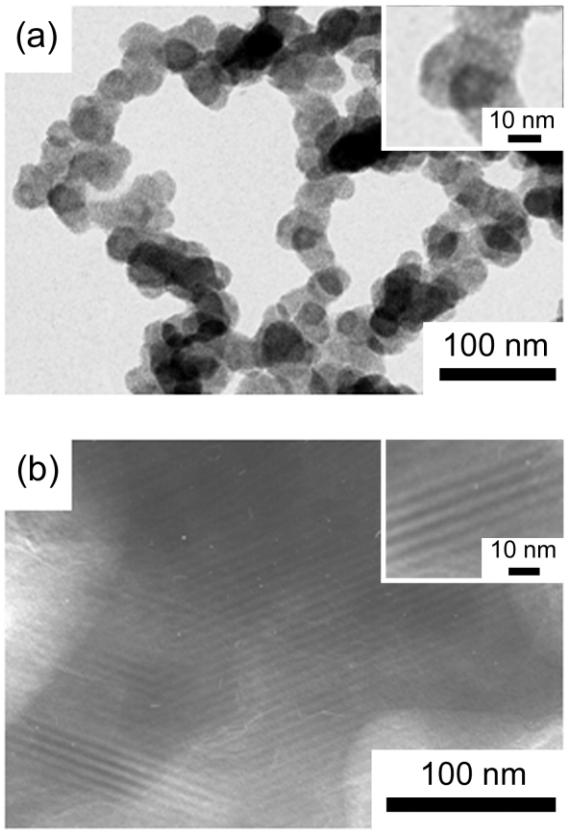

Fig. 4. Transmission electron micrographs of (a) PED-1 and (b) PET-1.

melting points of the PED and PET were somewhat lower than the melting point of pure PEO, confirming that the PEO was attached to the PED and PET and rearranged in the crystalline segment after block copolymerization. Similar results obtained with triblock random copolymers prepared by glycolide, carprolacton and poly(ethylene oxide) [12,13].

Narrow polydispersity is helpful for the formation of self-assembled structure [14]. Fig. 4 showed relatively regular structure of micelle and lamellar. DLS measurements of PED-1 diblock copolymers showed that the effective diameter of nanoparticles was about 50 nm. This value is a little larger than that obtained from TEM images which are shown in Fig. 4. This discrepancy may be attributed to the volume expansion by hydration of particles. Fig. 4(b) shows striations with a line width of ca. 6.4 nm. These observations may be taken as that while diblock copolymers form micellar structures as shown in Fig. 5(a), the triblock copolymers form lamellar structures of PEO and PVL blocks as shown in Fig. 5(b).

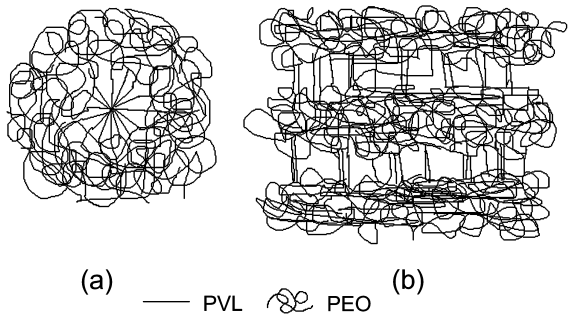

Fig. 5. Schematic illustration of PEO and polypeptide blocks (a) di- and (b) triblock copolymers.

4. Conclusion

Di-and triblock copolymers of PEO and poly(DL-valine-*co*-DL-leucine) were prepared by polymerization of NCA mixtures of DL-valine and DL-leucine with amine-terminated PEOs. While the amphiphilic diblock copolymers formed micellar nanoparticles, the triblock copolymers formed multi-lamellar structures. Further investigations in the present peptide/PEO block copolymer systems are in progress and the results will be published elsewhere.

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