Stereocomplex Formation between Enantiomeric PLA–PEG–PLA Triblock Copolymers: Characterization and Use as Protein-Delivery Microparticulate Carriers

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ABSTRACT: Two enantiomeric triblock ABA copolymers composed of poly(L-lactide)–poly(ethylene glycol)–poly(L-lactide) (PLLA–PEG–PLLA) and poly(D-lactide)–poly(ethylene glycol)–poly(D-lactide) (PDLA–PEG–PDLA) were synthesized with two different middle-block PEG chain lengths by ring-opening polymerization of L-lactide and D-lactide in the presence of PEG, respectively. A pair of enantiomeric triblock copolymers were combined to form a stereocomplex by a solvent-casting method. The triblock copolymers and their stereocomplexes were characterized by ^{1}H- and ^{13}C-NMR spectroscopy and gel permeation chromatography. Their crystalline structures and crystalline melting behaviors were analyzed by the wide-angle X-ray diffraction method and differential scanning calorimetry. The stereocomplex formed between a pair of enantiomeric triblock copolymers exhibited a higher crystalline melting temperature with a distinctive 3/1 helical crystalline structure. PLLA–PEG–PLLA and its stereocomplex with PDLA–PEG–PDLA were used to fabricate a series of microspheres encapsulating a model protein drug, bovine serum albumin (BSA). They were prepared by a double-emulsion solvent-evaporation method. The morphological aspects of the microspheres were characterized and BSA release profiles from them were investigated. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 75: 1615–1623, 2000

Key words: polylactide; poly(ethylene glycol) (PEG); biodegradable; stereocomplex; controlled release; microspheres

INTRODUCTION

The formation of a stereocomplex by blending enantiomeric poly(L-lactide) (PLLA) and poly(D-lactide) (PDLA) was extensively studied during the past decade. A stereocomplex racemic crystallite having a 3/1 helical structure is different from a homopolymer crystallite with a 10/3 helical structure found in individual PLLA or PDLA.[1–4] More compact side-by-side crystallization between the two enantiomeric polymers results in a much higher melting point and lower critical gelation concentration than those of optically pure polymers.[5–9] A new class of biodegradable polymers having higher mechanical strength, improved thermal stability, and a more hydrolysis-resistant property could be obtained by simply blending PLLA and PDLA.[10,11]

Di- or tri-block copolymers composed of PLLA and poly(ethylene glycol) (PEG) have been synthesized to attain versatile biodegradable polymers having more water-absorbing capacity.[12–14] Combination of the hydrophilic PEG segment with the PLLA chain generates biocompatible hydrogel-like biodegradable polymers which have been utilized as drug-delivery carriers and medical devices.[15–17] Additionally, A–B-type amphiphilic diblock copolymers of PLLA–PEG have

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been used for the preparation of polymeric micelles or surface-coating materials for biodegradable nanoparticles.[18] It was also recently reported that the stereocomplex between PLLA–PEG–PLLA and PDLA–PEG–PDLA was readily formed by a solvent-precipitation or casting method. A series of ABA-type triblock copolymers having a single PEG chain length (MW 6000) with different polylactide chain lengths was prepared to study thermal behaviors of their resultant stereocomplexes.[19]

In this article, two enantiomeric triblock copolymers of PLLA–PEG–PLLA and PDLA–PEG–PDLA were synthesized by ring-opening polymerization in the presence of varying PEG chain lengths using stannous octoate as a catalyst. Two relatively short PEG chain lengths (PEG 1000 and 3400) were incorporated as a middle block while keeping molecular weights of the triblock copolymers in the range of 30,000–40,000 in order to determine the PEG chain-length effect on the stereocomplex crystallization behaviors. Triblock copolymers were characterized by ^{1}H- and ^{13}C-NMR and gel permeation chromatography. Thermal properties and crystalline structures of various triblock copolymers as well as their stereocomplexes were determined by differential scanning calorimetry and wide-angle X-ray diffractometry. Triblock copolymers and their stereocomplexes were used to prepare microspheres for delivering a model protein, bovine serum albumin (BSA), in a sustained-release manner. Morphologies of the microspheres were also analyzed.

EXPERIMENTAL

Materials

L-Lactide and D-lactide were purchased from Purac Biochem (Gorinchem, Netherlands) and recrystallized from ethyl acetate before use. PEG with molecular weight (MW) 1000 and 3400, and PLLA acid (MW 50,000) supplied from Polysciences (Warrington, PA) were used by drying under reduced pressure at 70°C without further purification. Stannous octoate [$Sn(Oct)_2$: stannous 2-ethylhexanoate] was obtained from Sigma (St. Louis, MO) and toluene was dehydrated with 3 Å molecular sieves (Aldrich, Milwaukee, WI) without distillation. The polystyrene molecular weight standards (MWs 3700, 13,700, 18,700, 29,300, 44,000, and 114,200) were obtained from Aldrich. Poly(vinyl alcohol) (PVA: MW 31,000–50,000) and BSA were from Sigma.

Synthesis of Triblock Copolymers

A total of 30 g of L-lactide or D-lactide plus PEG was used for the polymerization. The amounts of the two different PEG, 1000 and 3400, were adjusted at 3 and 10% (w/w) concentration to a total feed amount, respectively. The mixture was heated to 120°C under nitrogen purging for complete melting. Stannous octoate was added at a 0.05% (w/w) level of the total feed amount. After degassing for 1 h, the mixture was sealed under vacuum and slowly heated to 180°C for 4 h. The synthesized polymer was dissolved in chloroform, precipitated in cold diethyl ether, and reprecipitated in cold methanol and dried under vacuum.

Film Preparation

PLLA–PEG–PLLA or PDLA–PEG–PDLA dissolved in chloroform [10% (w/v)] was cast onto presilanized Petri dishes at 4°C for slow solvent evaporation. Films composed of individual triblock copolymers were not formed because of the brittleness of the resulting films. A stereocomplex between the enantimeric PLA–PEG–PLA triblock copolymers, however, was successfully made in a film type by mixing equal amounts of PLLA–PEG–PLLA and PDLA–PEG–PDLA.

Characterization

NMR spectra were taken with a Brucker DRX 300 operating at 300.13-MHz ^{1}H-NMR and 75.48-MHz ^{13}C-NMR. Chemical shifts were measured in ppm using tetramethylsilane (TMS) as an internal reference. Gel permeation chromatography (GPC) was carried out with a Waters 510 HPLC pump system connected with 410 differential refractometer using a Shodex GPC K-803 column. Chloroform was used as a mobile phase at a flow rate 1.0 mL/min. Specific optical rotation values, $[\alpha]^{25}$, of various PLA–PEG–PLA copolymers were determined in chloroform at the concentration of 1 g/dL at 25°C using a polarimeter (Rudolph Autopol 111, USA) at a center wavelength of 589 nm (sodium D line). $[\alpha]^{25}$ values of PDLA and PLLA were approximately +150° and −150°, respectively. X-ray diffraction analysis was performed on a wide-angle goniometer (D/Max-3C, Rikagu, Japan) with a CuKα (λ = 0.154 nm) source. Thermograms were obtained by using a differential scanning calorimeter (DuPont 2000, USA). Heat-

Table I Molecular Characteristics of PLA_x–PEG_y–PLA_x Triblock Copolymers Prepared by Melt Polymerization

Polymer	DP_{PEG}[a]	DP_{PLA}[a]	M_n[a]	M_n[b]	M_w[b]	M_w/M_n[b]	$[\alpha]_D^{25}$ (deg)[c]
$PLLA_{200}$–PEG_{77}–$PLLA_{200}$	77	200	32,200	37,300	38,000	1.02	−147
$PDLA_{208}$–PEG_{77}–$PDLA_{208}$	77	208	33,300	33,900	37,500	1.11	+151
$PLLA_{250}$–PEG_{23}–$PLLA_{250}$	23	250	37,100	37,700	40,700	1.08	−149
$PDLA_{262}$–PEG_{23}–$PDLA_{262}$	23	262	38,800	34,400	35,500	1.03	+152

[a] Determined by measuring the integration ratio of resonances due to PEG blocks at 3.64 ppm (—O—CH_2CH_2) and to PLA blocks at 1.58 ppm (—CH_3—) in the 300.13-MHz ^{1}H-NMR spectra. (DP_{PEG}: 3400/44 = 77, 1000/44 = 23).
[b] Measured by GPC using a SHODEX GPC K-803 column (exclusion limit: 70,000).
[c] Performed by a polarimeter using a center wavelength 589 nm (sodium) at a concentration 1 g/dL in chloroform at 25°C.

ing was performed under a flow of nitrogen gas at a rate of 10°C/min. Samples were first heated up to 250°C, followed by a rapid quenching using liquid nitrogen, and then a second heating was recorded for detecting any thermal change.

Microsphere Preparation

Microspheres encapsulated with BSA were prepared by using enantiomeric PLLA–PEG–PLLA containing two different middle-block PEG chains (PEG 1000 and 3400), their stereocomplexes, and PLLA (MW 50,000). BSA microspheres were obtained by a double-emulsion solvent-evaporation method as previously described.[20,21] BSA (112.5 mg) dissolved in 0.75 mL of 33 m*M* phosphate buffered saline (PBS, pH 7.2) was emulsified in 6 mL of a methylene chloride solution containing the polymer (0.9 g). The primary *W*/*O* emulsion was made by using a high-speed homogenizer (Tekmar Co. Model SDT 1810, USA) for 1 min, and the stabilized primary emulsion was poured into 450 mL of 0.3% (w/v) aqueous PVA solution and further stirred at 700 rpm for 3 h. Hardened microspheres were collected by centrifugation at 10,000 rpm for 30 min, washed with deionized water three times, freeze-dried, and stored at −20°C until use. The surface and cross-sectional morphology and average size distribution of the microspheres were observed using scanning electron microscopy (SEM, Philips 535M).

Determination of BSA Loading Within Microspheres

Ten milligrams of BSA encapsulated microspheres was suspended in 3 mL of an aqueous alkali–surfactant solution containing 1*N* NaOH and 0.5% sodium dodecyl sulfate at 37°C until the solution became transparent due to complete degradation and dissolution of the polymer. The BSA amount in the solution was then determined spectrophotometrically at 280 nm in duplicate. The theoretical loading in the microspheres was 11.1% (w/w).

In Vitro BSA Release of Microspheres

One hundred milligrams of freeze-dried microspheres suspended in 4 mL of 33 m*M* phosphate buffer saline (pH 7.2) containing 0.05% (w/v) sodium azide was incubated by shaking at 200 rpm, 37°C. At preset time intervals, after a brief centrifugation at 3000 rpm for 15 min, 2 mL of the incubation medium was collected and then the same volume of fresh buffer medium was replenished. The amount of released BSA in the collected medium was measured spectrophotometrically at 280 nm using a Beckman DU 650 spectrophotometer.

RESULTS AND DISCUSSION

Two sets of enantiomeric triblock copolymers of PLLA–PEG–PLLA and PDLA–PEG–PDLA were synthesized by varying the PEG chain length in the middle block. The amount of the two different PEGs, 1000 and 3400, was adjusted to 3 and 10% (w/w), respectively, to a total feed amount in order to narrowly control the molecular weights of the triblock copolymers. Table I shows the molecular weights of the triblock copolymers determined by ^{1}H-NMR and GPC. The molecular weights of the synthesized triblock copolymers were within a confined range between 32,000 and 38,000 with a narrow M_w/M_n distribution. Typical ^{1}H- and ^{13}C-NMR spectra of PLLA–PEG–PLLA are shown in Figure 1, which are in good agreement with the

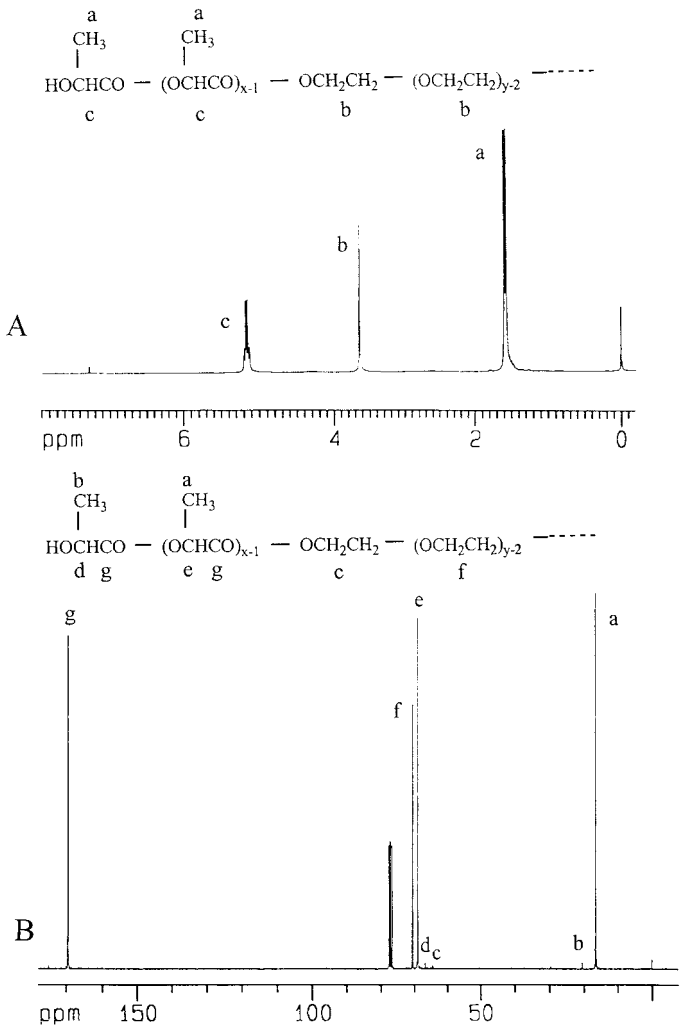

Figure 1 (A) 300.13-MHz ^{1}H-NMR spectra and (B) 75.48-MHz ^{13}C-NMR spectra of $PLLA_{200}$–PEG_{77}–$PLLA_{200}$ in $CDCl_3$.

spectra previously reported.[12] GPC profiles demonstrated a single polymer peak, suggesting that triblock copolymers were successfully synthesized without the presence of the homopolymer of PL(D)LA and PEG.

Figure 2 shows wide-angle X-ray diffraction patterns of enantiomeric triblock copolymers and their stereocomplexes with varying the two PEG chain lengths. Diffraction 2θ peaks of the triblock copolymers appear at 15°, 16.8°, 19.1°, and 22.5°, which agree well with the reported values for PLLA homopolymers.[1] On the other hand, those of the stereocomplexes exhibit 2θ values at 11.9°, 20.7°, and 23.9°, indicating that the preferential formation of racemic crystallites of the 3/1 helical structure between the two enantiomeric triblock copolymers occurred rather than the homopolymer crystallization having a 10/3 helical structure.[3,4] The X-ray diffraction patterns suggest that the crystallization behavior of the stereocomplex formed between the triblock copolymers of PL(D)LA–PEG–PL(D)LA does not significantly differ from that formed between the homopolymers of PLLA and PDLA even in the presence of PEG in the block copolymer structure. In addition, there is no evidence of the crystallized PEG middle-block segment in the samples of the triblock copolymers and their stereocomplexes.

Thermal characteristics of the triblock copolymers and their stereocomplexes determined by DSC are shown in Figure 3. All the DSC thermograms were obtained from a second heating procedure. It can be seen that the stereocomplexes containing PEG 3400 and 1000 exhibit typical 3/1

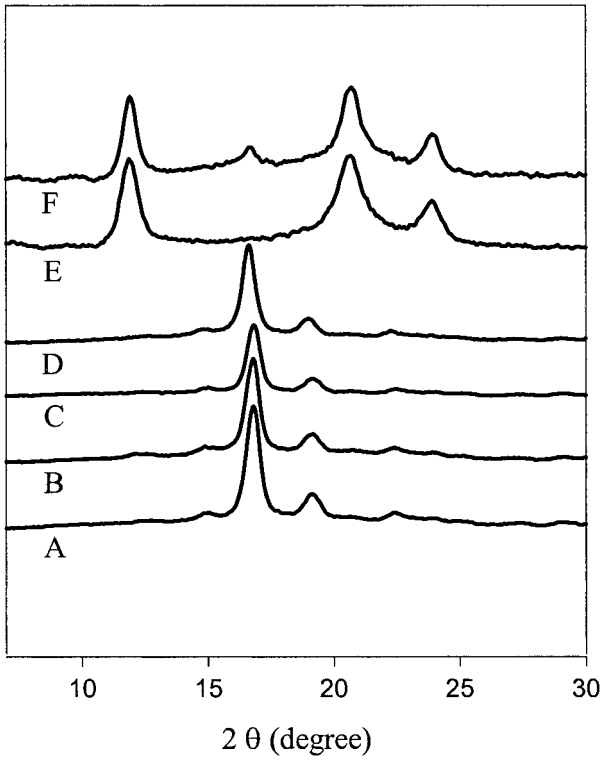

Figure 2 Wide-angle X-ray diffraction patterns of (A) PLLA–PEG (3400)–PLLA, (B) PDLA–PEG (3400)–PDLA, (C) PLLA–PEG (1000)–PLLA, (D) PDLA–PEG (1000)–PDLA, (E) PEG (3400) stereocomplex, and (F) PEG (1000) stereocomplex.

helical racemic crystalline melting temperatures of 214.9 and 223.4°C, respectively, while enantiomeric triblock copolymers show 10/3 helical crystalline melting temperatures between 165 and 168°C. The stereocomplex formed from a pair of enantiomeric triblock copolymers having PEG 1000 also exhibits a minor homopolymer crystallinity in addition to racemic crystallinity. This homopolymer crystallinity can also be seen in the X-ray diffraction pattern in Figure 2(F). It was reported that as the molecular weights of the enantiomeric homopolymers were varied racemic crystallization predominantly takes place relative to the homopolymer crystallization in the lower molecular weight range below about 40,000.[9] Thus, it seems reasonable to say that a pair of PEG 3400 enantiomeric triblock copolymers having respective molecular weights of 32,200 and 33,300 tended to more preferentially form a racemic crystalline structure than did the PEG 1000 triblock copolymers having those of 37,100 and 38,800. This might be one reason for the additional formation of homopolymer crystallization observed in the stereocomplex with PEG 1000. The stereocomplex formed between PEG 1000 triblock copolymers also has a slightly higher melting temperature (223.4°C) for racemic crystallites compared to that (214.9°C) for the stereocomplex sample of PEG 3400. The effect of the middle-block PEG chain length on the melting temperature of the racemic crystalline structure can be explained by the relative molecular weight of the two triblock copolymers. Increase in the melting temperature of the racemic crystallinity for the PEG 1000 triblock copolymers is thus likely due to the higher molecular weights of PL(D)LA in the triblock copolymer structure. Additionally, the longer PEG chain length, being more flexible and mobile, was likely to interfere with the more compact crystallization of the stereocomplex. On the other hand, the incorporation of PEG between two PL(D)LA chains does not change the crystalline melting temperature of the triblock copolymers, but affects the glass transition temperature to a great extent. The triblock copolymers containing PEG 3400 have a significantly lower glass transition temperature (34.5–36.3°C) than that of those containing PEG 1000

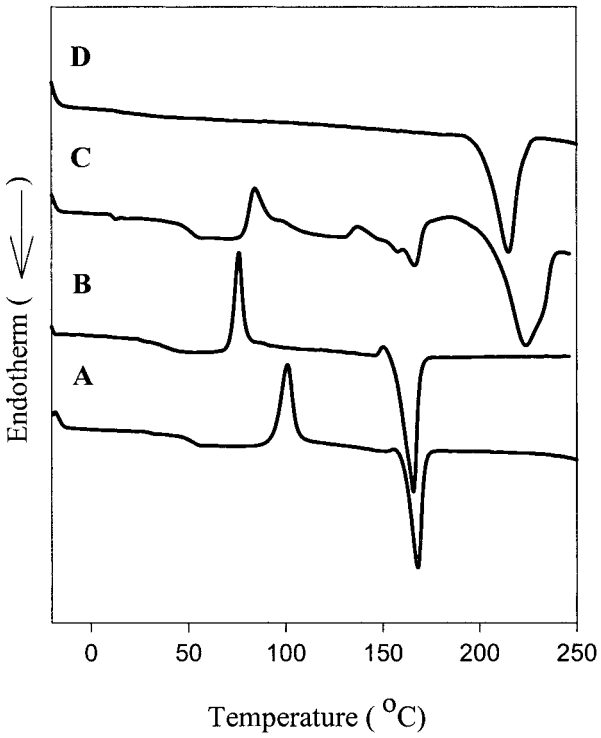

Figure 3 DSC thermograms of (A) PLLA–PEG (1000)–PLLA, (B) PLLA–PEG (3400)–PLLA, (C) PEG (1000) stereocomplex, and (D) PEG (3400) stereocomplex.

Table II Thermal Characteristics of the Polymers (T_g: Glass Transition Temperature; T_m: Melting Temperature, ΔH_f: Enthalpy of Fusion) as Measured from Second-Heating Procedure by DSC

Polymer	T_g (°C)	T_m (°C)	ΔH_g (J/g)
PLLA–PEG (3400)–PLLA	36.3	165.9	45.3
PDLA–PEG (3400)–PDLA	34.5	164.2	50.2
PLLA–PEG (1000)–PLLA	52.3	167.9	38.6
PDLA–PEG (1000)–PDLA	46.0	164.0	47.0
PEG (3400) stereocomplex[a]	ND[b]	214.9	49.2
PEG (1000) stereocomplex[a]	51.7	165.7	21.4
		223.4	63.3

[a] PEG stereocomplex obtained from the mixture of PLLA–PEG–PLLA and PDLA–PEG–PDLA.
[b] ND: no detection.

(46.0–52.3°C). PLLA homopolymers normally exhibit their glass transition temperature between 55 and 60°C. This suggests that the presence of the longer PEG chain length in the middle segment permits adjacent PL(D)LA polymer chains to be more mobile and flexible. It is of interest to note that the homopolymer crystallization temperature (T_{cr}) is more reduced for the PEG 3400 triblock copolymer than for the PEG 1000 triblock copolymer, while the crystalline melting temperatures are not changed. The decreased T_{cr} is related to the reduced glass transition temperature. The thermal properties of the triblock copolymers and their stereocomplexes are summarized in Table II.

Microspheres encapsulated with BSA were prepared using triblock copolymers and their stereocomplexes. The encapsulation of BSA within the microspheres was carried out by the double-emulsion solvent-evaporation method. It was reported that the microspheres prepared from PLLA–PEG–PLLA triblock copolymers were used to deliver BSA in a more sustained manner compared to those prepared from PLLA homopolymers.[22] To achieve a predictable controlled protein release from biodegradable microspheres over a desired period has been very difficult because severe protein stability problems such as aggregation and nonspecific adsorption play critical roles in protein release from microspheres. The use of triblock copolymers having hydrophilic PEG units resulted in better protein-release profiles, presumably due to minimizing the protein-stability problems in the presence of hydrophilic PEG domains in the microsphere matrix.[23] Table III lists characteristics of the various microspheres prepared. There are no apparent discrepancies in particle size, yield, drug-loading percent, and encapsulation efficiency, but water-uptake percents for the microspheres made of triblock copolymers and their stereocomplexes increase more than for the PLLA homopolymer microspheres due to the incorporation of PEG in the polymer structure. Morphological characteristics of surface and cross-sectioned microspheres were analyzed by SEM as shown in Figure 4. It can be seen that the microspheres prepared from the triblock copolymers have a relatively rough surface and porous internal structure as a result of the double-emulsion method used, while those from the stereocomplexes have a smoother surface with a more porous and hollow internal structure. The generation of the macroporous internal morphology in the stereocomplex microspheres could be caused by preferentially occur-

Table III Characteristics of Microspheres Containing BSA as a Model Protein Drug

Polymer	Average Particle Size (μm)[a]	Yield (%)[b]	Drug Loading (%)	Encapsulation Efficiency (%)[c]	Water Uptake (%)[d]
PLLA–PEG (3400)–PLLA	105.8	83.0	4.5	40.5	90.4
PLLA–PEG (1000)–PLLA	110.0	84.0	2.1	18.9	72.0
PEG (3400) stereocomplex	87.3	69.1	3.7	33.3	88.4
PEG (1000) stereocomplex	99.2	80.0	3.1	27.9	72.0
PLLA (MW: 50,000)	136.7	69.1	2.4	21.6	46.1

[a] Determined by SEM.
[b] 100 $W_{microsphere}/(W_{drug} + W_{polymer})$, measured gravimetrically.
[c] 100 actual/theoretical loading.
[d] 100 $(W_{wet} - W_{dry})/W_{dry}$, determined gravimetrically.

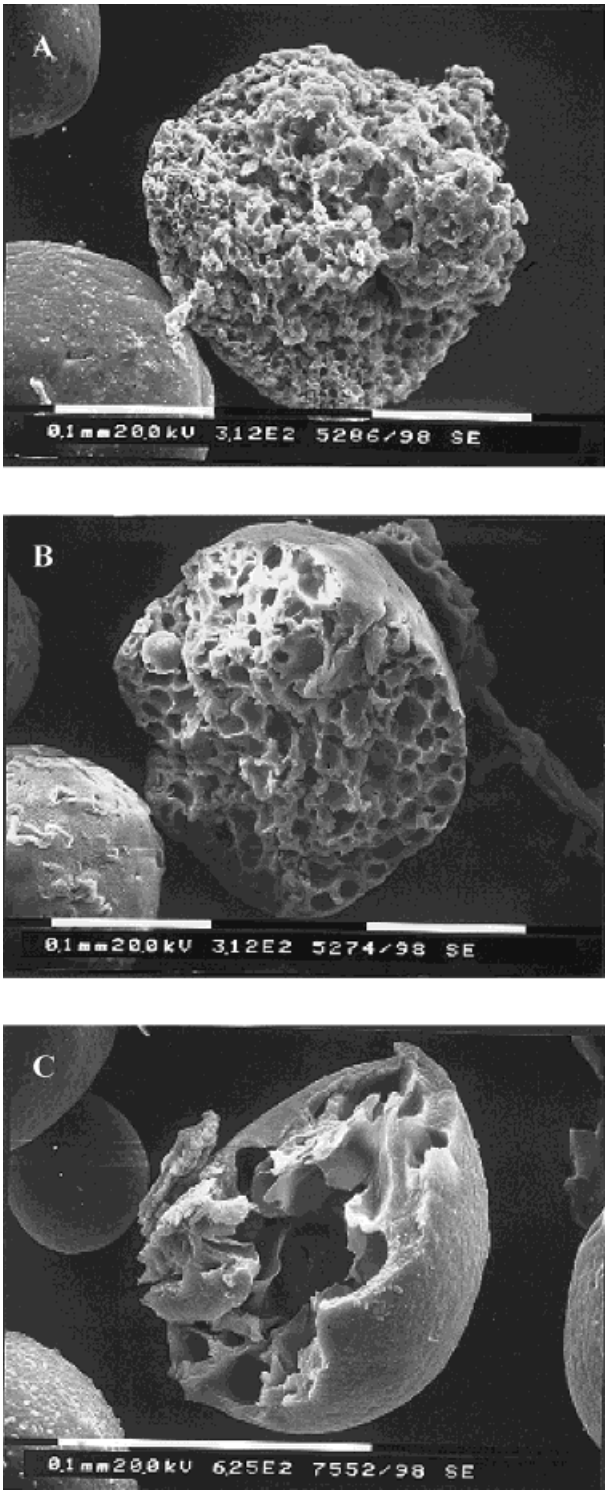

Figure 4 Cross-sectional morphology of BSA microspheres from (A) PLLA, (B) PLLA–PEG (3400)–PLLA, and (C) PEG (3400) stereocomplex observed by SEM.

ring racemic crystallization upon blending a pair of enantiomeric triblock copolymers in methylene chloride. As methylene chloride was slowly removed from the second emulsion droplets, a progressively developing viscous and highly concentrated polymer blend solution in the droplets transformed into a gel state. The nucleation and growth of preferentially formed racemic crystallites might act as physical crosslinking points for the gel state in the embryonic microsphere droplets, resulting in the very heterogeneous microsphere structure composed of polymer-rich domains and solvent-rich domains. These gelation and subsequent racemic crystallization events took place primarily in the water-contacting surface of the droplets, making large voids in the internal core region. This is the most plausible reason for the formation of a denser surface and more macroporous internal structure.[21]

Figure 5 shows BSA release profiles from three different microspheres prepared by using the PEG 3400 triblock copolymer, the PEG 3400 stereocomplex, and the PLLA homopolymer. The BSA-release profiles from the microspheres exhibit an initial burst effect and then sustained-release patterns over 50 days. The stereocomplex and triblock copolymer microspheres show a slightly larger burst effect compared to the homopolymer microspheres. This is likely to be caused by a higher water-uptake capacity of the microspheres made of PEG-containing triblock copolymers. The greater hydration extent of the microspheres by the incorporated PEG presumably developed more water-filled microporous

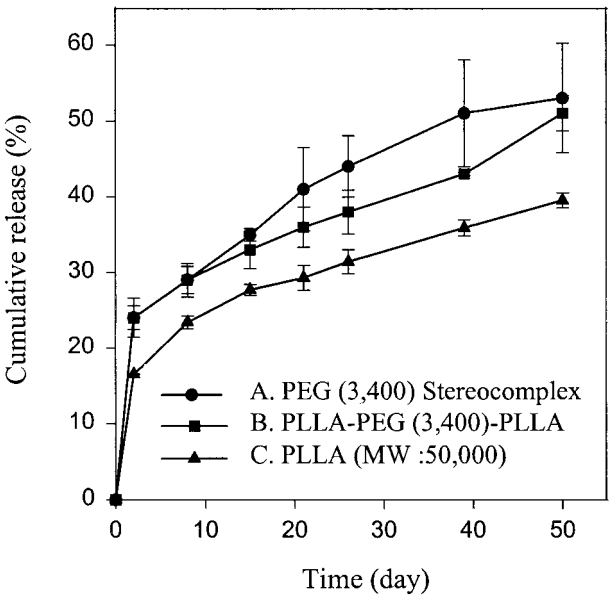

Figure 5 *In vitro* release profiles of BSA from various biodegradable microspheres.

Table IV Characteristics of *In Vitro* Degradation of Various Microspheres

Polymer	LA/EG[a] (0 day)	LA/EG[a] (50 days)
PLLA–PEG (3400)–PLLA	5.18	5.24
PDLA–PEG (3400)–PDLA	5.39	N[b]
PLLA–PEG (1000)–PLLA	21.82	22.35
PDLA–PEG (1000)–PDLA	22.80	N[b]
PEG (3400) stereocomplex	5.28	4.69
PEG (1000) stereocomplex	22.31	17.08

[a] LA/EG unit ratio measured by ^{1}H-NMR spectroscopy.
[b] N: no measurement.

channels within the polymer matrix, through which the entrapped BSA was more readily released out. The slow release of BSA after the initial burst seems to take place in a diffusion-controlled mechanism through the water-filled micropores. SEM observation of the microspheres after a 50-day incubation showed a partially degraded surface morphology for the triblock copolymer microspheres, but demonstrated intact surface morphology for the stereocomplex microspheres, supporting a more degradation-resistant stereocomplex matrix (data not shown). However, the relative molar ratio of lactyl and ethylene glycol (LA/EG) in the triblock copolymer structure assessed from the ^{1}H-NMR results before and after the degradation reveals that the LA/EG ratio of the triblock copolymer microspheres slightly increased from 5.18 to 5.24 for PLLA–PEG (3400)–PLLA, whereas that of the stereocomplex microspheres significantly decreased from 5.28 to 4.69.

The PLLA–PEG (1000)–PLLA triblock copolymer and its stereocomplex also exhibit a similar trend. This is shown in Table IV. These data suggest that, although the morphological polymer degradation occurred marginally, chemical degradation gradually took place during the incubation period. Since it is expected that the amorphous region of these microspheres was preferably degraded due to greater water accessibility, it is conceivable that the hydrolytic cleavage site of ester linkage in the triblock copolymers occurred primarily in the conjunction site between PLLA and PEG located in the vicinity of the PEG segment. This would lead to that the water-solubilized PEG segment was preferentially released, resulting in the slightly increased LA/EG ratio, in good agreement with the degradation pattern of the PLLA–PEG–PLLA triblock copolymers as previously reported.[22] In the case of the stereocomplex, the decreased LA/EG ratio after a 50-day incubation was unexpected. This might be caused by the presence of different cleavage sites in polymer chains of the stereocomplex from those of triblock copolymers. The stereocomplex might be cleaved in the middle of the amorphous PL(D)LA chain prior to the linkage site of PLLA–PEG with concomitant releasing of water-soluble PL(D)LA short-chain oligomers, thus showing the significantly reduced LA/EG ratio. However, it is not clearly understood why such different degradation sites exist for triblock copolymers and stereocomplexes. Their different microenvironments for the hydrolytic cleavage of ester linkage in the polymer backbone might play a role in exhibiting such degradation behaviors.

CONCLUSIONS

It has been shown that enantiomeric triblock copolymers having the PL(D)LA–PEG–PL(D)LA structure were synthesized and their stereocomplexes were formed upon blending them by a solvent-casting method. The presence of PEG in the triblock copolymer structure varied the glass transition and crystallization temperatures without changing the crystalline melting temperature. The stereocomplexes exhibited a higher melting temperature than that of the optically pure homopolymers. The triblock copolymers and the resultant stereocomplexes were used to prepare different morphological microspheres for a sustained BSA release. They demonstrated slightly better BSA release characteristics than those of the homopolymer microspheres due to increased water-uptake capacity.

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