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Preparation and Characterization of Poly(γ-benzyl L-glutamate)/Poly(ethylene oxide)-Lactoselactone Block Copolymers and Their Microspheres

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Poly(γ-benzyl L-glutamate)/Poly(ethylene oxide)-Lactoselactone 블록공중합체와 이들의 미립자 제조 및 특성

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A series of biodegradable block copolymers consisting of poly(γ-benzyl L-glutamate) (PBLG) and poly(ethylene oxide) (PEO)-lactoselactone were prepared by polymerization of PEO-lactoselactone and γ-benzyl L-glutamate-N-carboxyanhydride and characterized by IR and NMR. From circular dichroism measurements, it was found that the polymers exist in the α-helical conformation. Block copolymer microspheres were prepared by solvent-extraction-precipitation method for their primary evaluation for medical and biological applications.

Keywords—PBLG-PEO-lactoselactone, Microspheres, Polypeptide, Poly(ethylene oxide).

The saccharide moieties of glycolipids and glycoproteins on the cell surface are considered to play an important role in various intercellular recognition process.[1] Saccharide-terminating glycoprotein and neoglycoproteins accumulate in hepatocytes as a result of specific receptor-mediated pinocytic uptake mechanism.[2,3]

Synthetic polymers endowed with saccharide moieties as informational elements are of interest in connection with pharmacological and biomedical applications.[4]

In previous studies, we had synthesized and characterized different biodegradable block copolymers consisting of PBLG and polyether.[5-10] In this work, a series of biodegradable polypeptides carrying lactose moiety, poly(γ-benzyl L-glutamate)/poly(ethylene oxide)-lactoselactone (GEL) block copolymers were synthesized and characterized. In addition, microspheres of these block copolymers were prepared for their primary evaluation for medical and biological applications.

Experimental

Synthesis of Monomer and Block Copolymers

Synthesis of r-Benzyl L-Glutamate-N-Carboxyanh-

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ydride—In a 1000 m*l* three-necked round flask, 25g (0.1 mol) of γ-benzyl L-glutamate (γ-BLG, Sigma) was suspended in 300 m*l* of dry tetrahydrofuran and 9.12 m*l* (0.08 mol) of trichloromethyl chloroformate (TCF, phosgene dimer, Hodogaya Co.) was dropped until the mixture cleared. The solution was kept at 50℃ for 90 min., concentrated by a rotary evaporator and poured into a large amount of dry n-hexane. After overnight at −15 ℃, the precipitate was filtered and washed with n-hexane.

IR(KBr); 3400 cm^{-1}(N-H), 1840 and 1780 cm^{-1} (C=O), 930 cm^{-1} (ring peak), 740 cm^{-1} (aromatic ring).

^{1}H-NMR(DMSO-d_6); 9.2 ppm(-NH-), 7.3 ppm(-C_6H_5), 5.0 ppm(-CO-CH_2-), 4.4 ppm(-CH-) 2.0 and 2.5 ppm(-CH_2-CH_2-).

Synthesis of PEO-lactoselactone conjugate—Two grams (5.9 mol) of lactoselactone[11] was dissolved in refluxing methanol to which 3.7g (3.7 mol) of amine-terminated PEO (mol.wt. 2000, Texaco) solution in methanol was added. The mixed solution was refluxed for 5 hrs and allowed to stand at room temperature. The solvent was evaporated and the residue was dissolved in chloroform. The above solution was filtered to remove non-reactive lactoselactone. The solvent was evaporated and the residual oily material was freeze-dried after dissolving it in water.

^{13}C-NMR(DMSO-d_6); 60.3 ppm(-CH_2OH-, β-galactopyranosyl), 62.2 ppm(-CH_2OH-, β-D-glucopyranolactone), 104.9 ppm(-CH-O-, β-D-galactopyranosyl), 172.8 ppm(C=O).

Synthesis of PBLG-PEO-lactoselactone (GEL) block copolymer—GEL was prepared by polymerization of γ-benzyl L-glutamate-N-carboxyanhydride (γ-BLG-NCA) initiated with PEO-lactoselactone conjugate in methylene chloride at 25℃ for 72 hrs adjusting the ratio of r-BLG-NCA and PEO-lactoselactone contents. The reaction mixture was poured into a large excess of diethyl ether to precipitate the PBLG-PEO-lactoselactone block copolymer. The resulting block copolymers were washed with diethyl ether and then vacuum dried. The precipitate obtained was also washed with methyl alcohol to remove lactose-PEO-lactose and then vacuum dried again.

IR(KBr); 1645, 1548 and 615 cm^{-1} (amide I, II, V).

^{13}C-NMR($CDCl_3$); 172 ppm(C=O), 105 ppm(-CH-O-, β-D-galactopyranosyl), 71 ppm(-NH-CH_2-CH_2-O-NH-), 25 and 31 ppm(-CH_2-CH_2-), 136 ppm(-C_6H_5), 175.5 ppm(-COO-).

Spectroscopic Measurement

IR spectra were measured on a Nicolet 5-MX FT-IR spectrophotometer by KBr disk method and NMR spectra were measured using Bruker WP 80 SY FT-NMR spectrometer. Circular dichroism spectra were recorded at room temperature on a JASCO J-500A spectropolarimeter equipped with a quartz cell having a path length of 1 mm.

Preparation of Microspheres

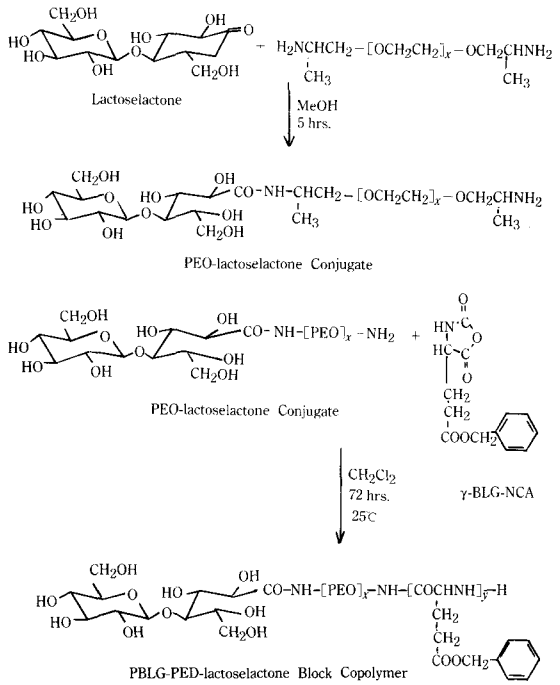
Microspheres were prepared by the solvent-extraction-precipitation method. Briefly, 10% GEL block copolymer solution in methylene chloride was pured into 15 m*l* of 1% aqueous solution of poly(vinyl alcohol). The mixture was emulsified by sonication at 100W for 3 min (VS300, Nihoseiki). The mixture was rapidly added to 10% isopropyl alcohol and stirred for another 10 min to precipitate microspheres. Microspheres were washed and vacuum dried. Size and morphological observation of micropsheres produced were perfomed using Jeol JSM 840A scanning electron microscope.

Results and Discussion

γ-BLG-NCA was prepared by the method proposed by Goodman *et al.*[12] The initial reaction involving protection of r-carboxyl group of L-glutamic acid by esterification with a benzyl alcohol, followed by treatment of TCF gave rise to the γ-BLG-NCA in 90% yield.

Three kinds of GEL block copolymers consisting of different ratio of PBLG and PEO-lactoselactone contents were prepared by polymerization of γ-benzyl L-glutamate-N-carboxy-anhydride (γ-BLG-NCA) utilizing the amino group in modified PEO-lactoselactone as an initiator as shown in Scheme I.

The samples prepared and their molecular data



Scheme I—Synthetic pathway of PBLG-PEO-lactoselactone block copolymers

Table I—*Characteristics of PBLG-PEO-lactoselactone Block Copolymers*

Polymers	Contents of monomeric units in mole%		Molecular weight
	PEO	PBLG	
GEL-1	14.3	85.7	55,000
GEL-2	21.6	78.4	34,000
GEL-3	35.4	65.5	19,000

PEO: polyethylene oxide, PBLG: poly (γ-benzyl L-glutamate)

are summarized in Table 1. The molecular weight and composition of block copolymers were estimated by proton NMR.[5]

The chain conformation of the polymers in solution state was measured by circular dichroism (CD). Fig. 1 shows the CD spectra of the block copolymers in 1,2-dichloroethane. All of these spectra show negative Cotton effects,[13] characteristic of an α-helical conformation with a band at 222 nm assigned to the n-π* transition. Table II shows the experimental data, $[\theta]_{222}$ of 39,600 as a reference for 100% helicity, the helical content of the block copolymer corresponds to that of PBLG.

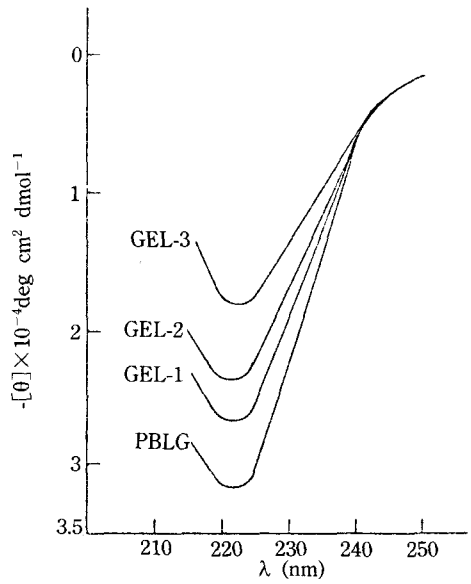


Figure 1—CD spectra of PBLG-PEO-lactoselactone block copolymers in 1,2-dichloroethane at 25℃

Table II—*Negative Ellipticity at 222 nm, $-[\theta]_{222}$ of PBLG Homopolymer and GEL Block Copolymers in 1,2-Dichloroethane at Room Temperature*

Polymers	G[a]	$-[\theta]_{222}$	$[\theta]^c_{222}/[\theta]^o_{222}$[b]
PBLG	100	39,600	1.00
GEL-1	85.7	32,500	0.88
GEL-2	78.4	29,900	0.81
GEL-3	65.5	24,700	0.67

[a] G: content of γ-BLG units in the block copolymers
[b] $[\theta]^c_{222}$: ellipticity of the block copolymers
$[\theta]^o_{222}$: ellipticity of PBLG homopolymer

Polymeric microspheres have been extensively applied as a tool for the characterization of synthetic biodegradable polymers in their biodegradability and biocompatibility test as well as their medical and biological applications.[14-16] For this purpose, we tried to prepare the microspheres of GEL block copolymers and the solvent-extraction-precipitation process described in the experimental was confirmed to be suitable for these newly synthesized GEL block copolymers. Fig. 2 shows scanning electron microphotographs of 3 kinds of

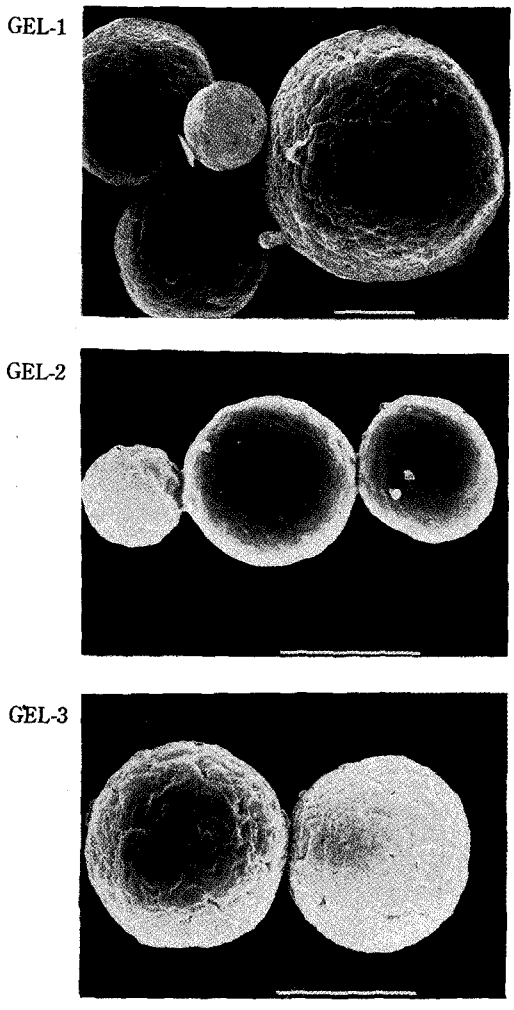


Figure 2—Scanning electron micrograph of PBLG-PEO-lactoselactone (GEL) block copolymers

GEL block copolymer microspheres prepared using 1% poly(vinyl alcohol) solution as an emulsifying agent. The shape of the polymeric microspheres was spherical and their size was ranged from 0.5 μm to 3 μm in diameter, which are suitable for injection. Further studies to characterize the GEL block copolymers as a drug delivery carrier using these microspheres are currently in progress.

Acknowledgements

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