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# Synthesis of Poly(γ-benzyl L-glutamate)/Poly(ethylene oxide) Diblock Copolymer Endcapped with Sugar Moiety for Cell-Specific Biomaterials

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AB block copolymers consisting of poly(γ-benzyl L-glutamate)(PBLG) as component A and poly(ethylene oxide) as component B were prepared containing [O-β-D-galactopyranosyl-(1→4)]-D-gluconamide or [O-α-D-glucopyranosyl-(1→4)]-D-gluconamide. From the circular dichroism measurements in solution state as well as infrared measurements in solid state, it was found that they had α-helical structure in both states as same as for the PBLG homopolymer. From adhesion of hepatocytes to the copolymer in vitro, it was found that lactose-carrying PBLG/PEO block copolymer had about 80% of adhesion within 2 h.

Several attempts have been proposed to obtain new types of drug delivery system using polymeric drugs.[1] Site-specific drug delivery targeted receptors is an area newly attracting considerable attention because of changing the body distribution of drugs and obviation of unwanted side-effects.[2] Among them, asialoglycoprotein receptor on the hepatocytes has been proposed as a useful means to target liver-specific chemotherapy[3] and sugar-containing conjugates have been used as a target permitting organ-specific therapy of liver diseases.[4] In previous studies, sugar-carrying polystyrene(PSt) was prepared[5] and especially, lactose-carrying PSt has the enhanced adhesion and survival efficiency of liver cells in culture dishes due to the recognition mechanisms for the sugar group.[6] Also, poly(γ-benzyl L-glutamate) (PBLG)/ poly(ethylene oxide) (PEO)/PBLG block copolymer was synthesized[7] and was studied as drug carriers.[8,9] The aim of this study is to synthesize PBLG/PEO diblock copolymers endcapped with sugar moiety for new cell-specific biomedical materials. It may be expected that the PBLG/PEO copolymer with sugar group will be tool for investigating biological recognition phenomena and applicable to the liver-targetable biodegradable drug-carriers.

Lactonolactone(LL)(1) was prepared according to the method proposed by Kobayashi et al..[5] Briefly , lactose-1-hydrate(12 g, 33 mmol) was dissolved in water(9 ml), diluted with methanol(25 ml), and added to an iodine(17.1 g) solution in methanol(240 ml) at 40 °C. At this temperature, a 4% potassium hydroxide solution in methanol(400 ml) was added dropwise for 35 min with magnetic stirring until the color of iodine disappeared. After cooling down of the solution in an ice-bath, the precipitated crystalline product was filtered, washed with cold methanol, and then cold ether, and recrystallized from a mixture(800 ml) of methanol and water(9/1:V/V). The resulting potassium lactonate was then converted to the free acid by passing the aqueous solution through a column of Amberlite IR-120B. The acidic eluate was collected and concentrated in a rotary evaporator. Repeated

evaporation of methanol and ethanol solution converted the lactonic acid into LL(yield:90%). $^{13}C$ NMR($Me_2SO$-$d_6$, TMS. Primes designate the terminal β-D-galactopyranosyl residue): δ 172.0 C=O, 138.9 and 135.2 phenyl(ipso), 136.1 -CH=$CH_2$, 127 and 125.6 phenyl(meta and ortho), 113.3 =$CH_2$, 104.3 C(1'), 82.7 C(4), 68.0 C(4'), 62.2 C(6), 60.5 C(6'), 41.5 benzyl methylene, 75.5, 73.1, 71.9, 71.3, 70.9 and 70.5 ppm the remaining pyranose carbons. Maltono-lactone was synthesized in the same way. Diamine-terminated PEO(ATPEO=$H_2N$-PEO-$NH_2$)(2) was supplied by Texaco Chem.Co., Ballaire, Texas. It has a number-average molecular weight of 2000. $H_2N$-PEO-[O-β-D-galactopyranosyl-(1→4)]-D-gluconamide($H_2N$-PEO-LA)(3) was synthesized as follows: 1(2 g, 5.9 mM) was dissolved in refluxing methanol (30 ml) and 2(3.7 g, 3.7 mM) in methanol solution(20 ml) was added. The mixed solution was refluxed for 5 h with magnetic stirring 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 unreacted LL. The solvent was evaporated and the residual oily material was freeze-dried after dissolving it in water. $^1H$ NMR($CDCl_3$, TMS): δ=3.6 ppm(-OC$H_2$C$H_2$). But the NMR signals of the LA in the $H_2N$-PEO-LA could not be identified. $H_2N$-PEO-[O-α-D-glucopyranosyl-(1→4)]-D-gluconamide(PEO-MA) was synthesized in the same way. γ-Benzyl L-glutamate N-carboxyanhydride(BLG-NCA)(4) was prepared according to the method proposed by Goodman et al.(yield:88%).[10] PBLG/PEO-LA block copolymer(5) was prepared by polymerization of 4 initiated with 3 in methylene chloride at a total concentration of BLG-NCA and $H_2N$-PEO-LA of 3%(W/V). After the IR absorption at 1785 $cm^{-1}$ and 1860 $cm^{-1}$, which is characteristic of BLG-NCA, had disappeared in the course of polymerization(up to 72 h), the reaction mixture was poured into a large excess of diethyl ether to precipitate the PBLG/PEO-LA copolymer. The resulting copolymer was washed with diethyl ether and then dried in vacuo. The precipitate obtained was also washed with methanol to remove LA-PEO-LA and then dried in vacuo. The precipitate obtained did not contain unreacted $H_2N$-PEO-LA, oligohomopolymer formed by autopolymerization[11,12] as well as LA-PEO-LA.The yields of the block copolymers were 59, 57 and 53% for GEL-1, GEL-2 and GEL-3, respectively. $^1H$ NMR($CDCl_3$, TMS): δ=7.2(phenyl); 5.0(benzyl); 4.0(CHNH); 3.6(-OC$H_2$C$H_2$); 2.3(γ-CH) and 2.2 ppm(β-CH). But the NMR signal of the LA in the PBLG/PEO-LA could not be identified. PBLG/PEO-MA block copolymers were synthesized in the same way. The homopolymer PBLG was obtained by polymerization of 4 initiated by triethylamine in 1,4-dioxane with a monomer-to-initiator mole ratio of 25(yield:84%). Hexane,tetrahydrofuran(THF), and 1,4-dioxane were dried and purified by distillation. $^1H$ NMR of the copolymers were measured in $CDCl_3$ to estimate the copolymer composition and the molecular weights of PBLG blocks,using a JEOL Datum 270 MHZ NMR spectrometer. As the number-average molecular weight(2000) of PEO is known, one can estimate the number-average molecular weights of the PBLG block and of the copolymer from the copolymer composition calculated from the peak intensities in the spectrum assigned to both polymers. Circular dichroism(CD) spectra were recorded at room temperature on a JASCO J-600 spectropolarimeter equipped with a quartz cell having a path length of 5 mm. Infrared(IR) spectra of solid films of the sample cast from chloroform solution were measured on a Shimadzu Model-43 IR spectrophotometer. Hepatocytes were isolated from the livers of rats weighing between 150 and 250 g using the 2-step collagenase perfusion technique of Seglen[13] following the procedure described previously.[13] Culture of hepatocytes:A 0.2 wt.-% solution of PBLG/PEO-LA copolymer(abbreviated as GEL) was prepared in chloroform. The polymer solution was casted onto a 35 mm-diameter glass dish and then dried. A suspension of hepatocytes (1.5 ml) was poured into the polymer-cast dishes and maintained at 37 °C in a humidified air/$CO_2$ incubator(95/5 vol.-%) for a prescribed time. The suspension was then decanted and the dish was washed with 1 ml of a PBS(-) solution. The non-

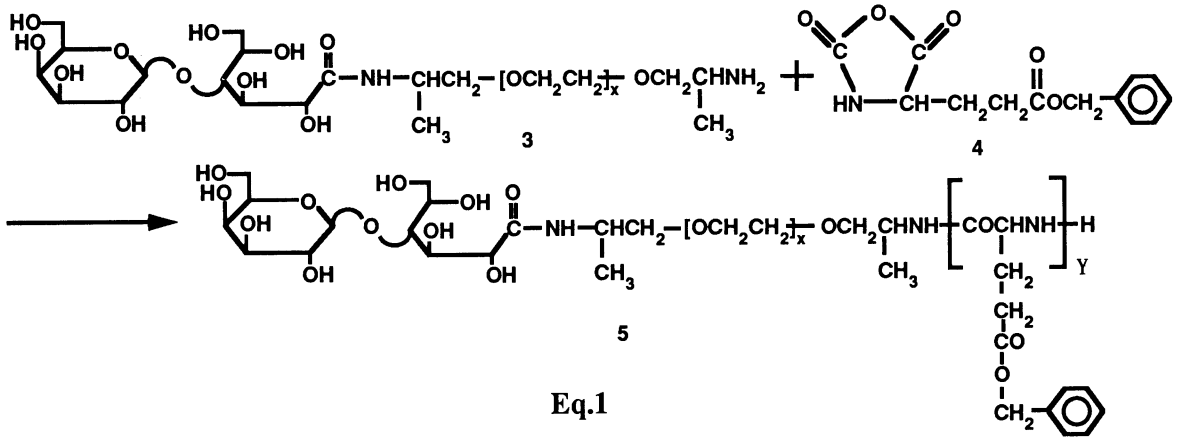
adhering cells were collected and counted by a hemocytometer.

The PBLG/PEO-LA block copolymers(GEL) were prepared by polymerization of BLG-NCA(4) initiated by the PEO-LA containing amino end groups (3) (Eq.1) in methylenechloride. Since excess LL was added into $H_2N$-PEO-$NH_2$ solution, unreacted $H_2N$-PEO-$H_2N$ was not expected to exist in the product of $H_2N$-PEO-LA. Also, it may be expected that the primary amine of $H_2N$-PEO-LA can dominantly initiate polymerization of BLG-NCA than the hydroxy groups of the sugar moiety.[11,12] The samples prepared and their characteristics are summarized in Table 1. The copolymer composition and the molecular weight were estimated by $^1$H-NMR. The PEO content in the block copolymer was 14.3, 21.6 and 34.5 mol-% for GEL-1, GEL-2 and GEL-3, respectively.

From the CD results of the copolymers and PBLG, all have maxima of [θ] at ca.222 nm,indicating the existence of α-helical conformation(not shown here).[14] The helical content of the copolymers was estimated from the ratio of the copolymer to that of PBLG. The helical content thus estimated was 88, 81 and 67% for GEL-1, GEL-2 and GEL-3, respectively. These helical contents are in good agreement with the content (86, 78 and 66 mol-% resp., see Table 1) of PBLG in the copolymer chains, as evaluated from $^1$H NMR measurements.

From IR measurements in the solid state(not shown in Fig.), it was found that the amide I, II, and V bands of these GEL block copolymers appear 1650, 1550, 615 $cm^{-1}$, respectively, at the same wavenumbers as for the PBLG homopolymer.

From adhesion of hepatocytes to GEL-1-casted dishes in vitro(not shown in Fig.), adhesion occurred gradually within 1 h, and reaches 82% whereas adhesion to PS(Falcon 3001) as control was 48% after 2 h. Previously, it was demonstrated that lactose-carrying polystyrene has 80% of adhesion efficiency within 3 h.[6] The marked adhesion for the polymer can be explained on the basis of the participation of galactose-specific lectins which are located at the surface of rat hepatocyte.[15] Therefore, the high adhesion of hepatocytes to the GEL-1-casted dish can be explained by the positive galactose-dependent interaction. More detail on adhesion of hepatocytes onto the series of block copolymers will be reported.



Eq.1

Table 1. Characterization of PBLG/PEO-LA block copolymer

| Sample | Content of monomeric units in mol-%[a)] | | Mn[a)] | DPn | |
|---|---|---|---|---|---|
| | PBLG | PEO | | copolymer | block PBLG |
| PBLG[b)] | 100 | 0 | 273000 | - | 1247 |
| GEL-1 | 85,7 | 14,3 | 54900 | 287 | 242 |
| GEL-2 | 78,4 | 21,6 | 34200 | 192 | 147 |
| GEL-3 | 65,5 | 34,5 | 19000 | 123 | 78 |

a) Composition and Mn of the copolymer was calculated from NMR measurement.

b) The average molecular weight was estimated from the [η]-molecular weight relationship as previously reported.[7)]

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