

Poly(γ -benzyl *L*-glutamate)/Poly(ethylene oxide) 블록 공중합체의 합성과 항혈전성 : Poly(ethylene oxide) 분자량의 영향

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(1990년 9월 12일 접수)

Synthesis and Blood Compatibility of Poly(γ -benzyl *L*-glutamate)/ Poly(ethylene oxide) Triblock Copolymers : Effect of Molecular Weight of Poly(ethylene oxide)

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(Received September 12, 1990)

요 약 : poly(γ -benzyl *L*-glutamate)를 A성분으로, poly(ethylene oxide)를 B성분으로 구성된 ABA형의 블록공중합체가 PEO의 함량은 일정하게 하고 분자량을 다르게 하여 합성되었다. 용액상에서의 CD측정과 고체상에서의 IR측정으로 이 블록공중합체가 PBLG homopolymer에서와 같이 전형적인 α -helix 구조를 갖고 있음을 알았다. In vitro에서의 항혈전실험으로부터, 이 블록공중합 표면에서의 혈소판의 흡착정도는 블록공중합체의 PEO 분자량에 의존함을 알았고, 특히 PEO 분자량이 4000인 블록공중합체에서 혈소판의 점착이 가장 적음을 보였다.

Abstract : ABA type triblock copolymers consisting of poly(γ -benzyl *L*-glutamate)(PBLG) as the A component and poly(ethylene oxide)(PEO) as the B component were synthesized. Various molecular weight of PEO was utilized to study the effect of PEO molecular weight on blood compatibility. From circular dichroism measurements in solution as well as infrared spectra measurements in the solid state, it was found that the block copolymers exist in the α -helical conformation as in PBLG homopolymer. From the in vitro antithrombogenic test, it was found that antithrombogenicity of the block copolymer is dependant on the molecular weight of PEO in the block copolymer. The best blood compatibility was obtained at PEO molecular weight 4000.

INTRODUCTION

The problem of blood compatibility of the polymeric material is one of great concern in the field

of biomaterial science.¹ When in contact with blood, artificial polymeric surfaces generally induce platelet adhesion and subsequent activation, which can lead to thrombus formation accompa-

nied by the formation of an insoluble fibrin network.² Much research has been progressed to develop ideal antithrombogenic material, and one is a investigation on the synthesis of block copolymer. Block or graft copolymers containing two kinds of polymer chains usually undergo phase separation: upon casting from a solution, they may form a film whose surface is heterogeneous. It has been reported that the heterogeneity of the synthetic polymer surface, i. e., the microphase-separated structure plays an important role in blood compatibility.³

In practice, the normal vascular endothelium, which is the ideal non-thrombogenic material, is considered to have a microphase separated structure composed of hydrophilic and hydrophobic microdomains.⁴ Okano et al.⁵ proposed that the polymeric hydrophilic-hydrophobic microphase-separated structure is a key parameter for controlling antithrombogenic activation of polymers due to its apparent inhibition of platelet aggregation.

In previous stuies,^{6~9} we have synthesized ABA type triblock copolymers, having microphase-separated structure consisting of poly(γ -alkyl *L*-glutamate) as the A component and poly(ethylene oxide) (PEO) as the B component to study their blood compatibility depending on the content of PEO, and their blood compatibility has been studied. This result indicated that platelet adhesion on the block copolymer surfaces decreased with increasing content of PEO due to the highly dynamic mobility of PEO.

Nagaoka et al.¹⁰ investigated the effects of the poly(ethylene oxide) (PEO) chain length on adhesion of the platelets in the methoxy poly(ethylene oxide) monomethacrylates with PEO side chain. They reported that the amounts of adhered platelets decreased with increasing PEO chain length because of its very high chain mobility. They also studied the activity of immobilized heparin using PEO with various chain lengths as a spacer. The heparin activity increased with an increase in the chain length of PEO up to 22. However, when the PEO chain is too long, the

activity of the immobilized heparin decreased because heparin may become embedded in PEO chain.¹¹

Park et al.¹² reported that minimum platelet adhesion of heparin immobilized segmented polyurethaneurea surfaces with different PEO chains as spacers was achieved at PEO molecular weight 1,000 due to the optimum dynamic motion.

Takahara et al.¹³ reported that the platelet reactivity of segmented poly(etherurethaneureas) was influenced by the M_n of soft segment due to the glass transition.

Lee et al.¹⁴ studied that surface properties of aqueous PEO/PPO block copolymer surfactants were influenced by the PEO chain length.

In this study, we report the blood compatibility of poly(γ -benzyl *L*-glutamate)PBLG/PEO/PBLG block copolymers which have different molecular weight of PEO. It may be expected that the blood compatibility of the block copolymers is dependent on the molecular weight of PEO.

EXPERIMENTAL

Materials

Amine-terminated poly(ethylene oxide)(AT-PEO) : These PEO were supplied from Texaco Chem. Co., Ballaire, Texas. The molecular weights of the ATPEO were 900, 2000, 4000, and 6000, respectively.

Solvent : n-Hexane, tetrahydrofuran(THF), and dichloromethane(DCM) were purified by distillation.

Synthesis of γ -Benzyl *L*-Glutamate *N*-Carboxy-anhydride(γ -BLG NCA) : γ -BLG NCA was prepared according to the method proposed by Goodman, et al.¹⁵

Synthesis of PBLG/PEO/PBLG Block Copolymer (GEG)⁸ : The polymerization of γ -BLG NCA was carried out in DCM by ATPEO initiation at a total polymeric concentration of 3 wt%. The precipitated copolymer from diethyl ether was dried in vacuum.

Measurements

¹H-NMR Measurements : Nuclear Magnetic Resonance(NMR) spectra of the block copolymers were measured with Bruker WP 80 SY in chloroform-d.

CD measurements : The circular dichroism(CD) spectra were measured at room temperature on a JASCO J-500A spectropolarimeter equipped with a quartz cell having a path length of 1 mm.

FT-IR Measurements : Fourier Transform-Infrared(IR) spectra of the solid films cast from chloroform solution were measured with Mattson Polaris between 4,000 and 400 cm⁻¹.

Estimation of Platelet Adhesion(in vitro) : In vitro test was examined by microsphere column method. The beads(15~35 meshes, Sigma) pre-coated with polymer 1g prepared by solvent evaporation were closely packed in a poly(vinyl chloride) tube(diameter : 3mm, length : 10cm) equipped with a stop-cock. The packed column was primed with saline, and subjected to the following platelet adhesion test : 3.0 cm³ of fresh whole blood was collected from a healthy person with a disposable syringe without any anticoagulant. The bead was immediately passed through the column for 1 min at a flow rate of 1.5 cm³ min⁻¹ using syringe pump(Sage Instruments Model 351) to be collected in a sampling bottle containing

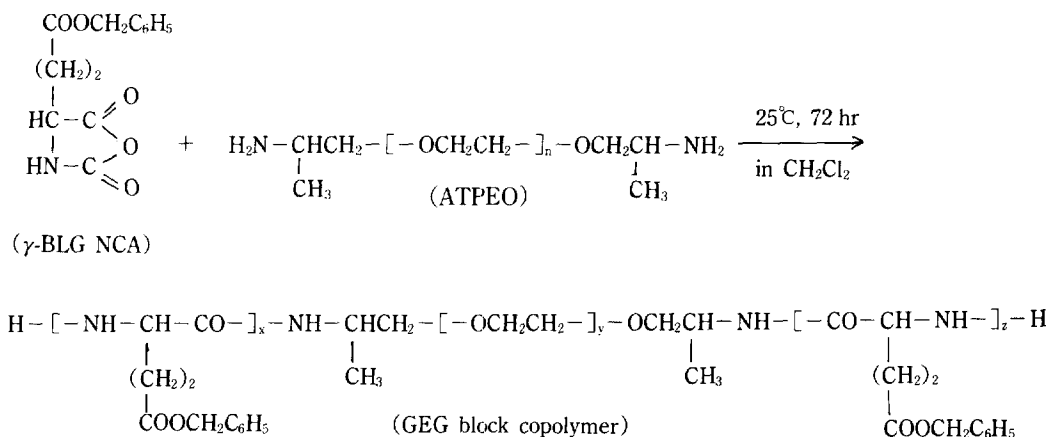
0.1 cm³ of ethylenediaminetetraacetic acid(EDTA) as an anticoagulant. Platelet in the eluted blood were counted at 3 times of a sample with platelet counter(Coulter Counter Model S-plus). The column was then washed with saline at a flow rate of 0.8 cm³ min⁻¹ for a period of 2 min. The beads in the upper part of the rinsed column were placed in a distilled water containing 1.25 wt% glutaraldehyde to fix the adhering platelets. The beads were rinsed with distilled water, freeze-dried, then coated with palladium-gold to be observed by a scanning electron microscope (SEM)(JEOL, Model TSM-35).

RESULTS AND DISCUSSION

Synthesis of the Block Copolymers

The PBLG/PEO/PBLG(GEG) block copolymers were synthesized by initiating the polymerization of γ -BLG NCA with PEO containing amino end groups(Scheme 1). It may be assumed that the polymerization mechanism is the primary-amine mechanism in which the initiator amine undergoes a nucleophilic addition to the C-5 carboxy group of the NCA.¹⁷

Fig. 1 shows ¹H-NMR spectrum of GEG-3 block copolymer in chloroform-d. Methylene peak of benzyl and phenyl peak are observed at 5 ppm and



Scheme 1. Synthesis of PBLG/PEO block copolymers

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7.2 ppm, respectively. The peak of poly (ethylene oxide) is observed at 3.6 ppm.

Characteristics of the Block Copolymers

The characteristics of PBLG homopolymer and GEG block copolymers are summarized in Table 1. The copolymer composition was estimated from peak intensities of the phenyl proton of the PBLG block and the methylene proton signal of the PEO block in the spectrum(Fig. 1). Also, the molecular weight of the block copolymers was estimated from the NMR spectroscopy.⁸

Chain Conformational Studies

The chain conformation of the block copolymers in the solution state was measured by CD spectroscopy. Fig. 2 shows the CD spectra of the block copolymers and PBLG homopolymer, where all have troughs at 222 nm, indicating the existence of α -helical conformations with different contents.¹⁹ The other troughs at 208 nm, typical of the α -helix, could not be recorded due to high absorption by the solvent (1,2-dichloroethane) used. Table 2 shows the experimental $[\theta]_{222}$ data for the samples in dichloroethane at 25°C. Assuming $[\theta]_{222}$ of 39,600 for

PBLG as a reference for 100 % helicity, the helical content of the block copolymers was estimated from the ratio of $[\theta]_{222}$ of the block copolymer to that of PBLG, $[\theta]_{222}^c/[\theta]_{222}^h$, is shown in the third column of Table 2. These results indicated that the

Table 1. Characteristics of Samples Prepared

Sample	M _w of PEO	Content of PEO(mol %)	M _n of Sample
PBLG ^{a)}	—	—	273000 ^{b)}
GEG-1	900	54.0	8300
GEG-2	2000	55.6	10000
GEG-3	4000	59.0	17900
GEG-4	6000	64.3	22600

^{a)} PBLG was obtained by polymerization of the corresponding NCA using triethylamine as the initiator.

^{b)} The molecular weight of PBLG was estimated from the $[\eta]$ -molecular weight relationship proposed by Doty et al.¹⁸

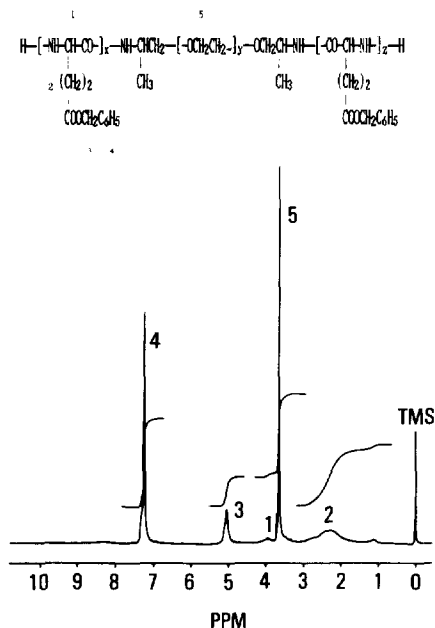


Fig. 1. ¹H-NMR spectrum of GEG-2 block copolymer in CDCl₃.

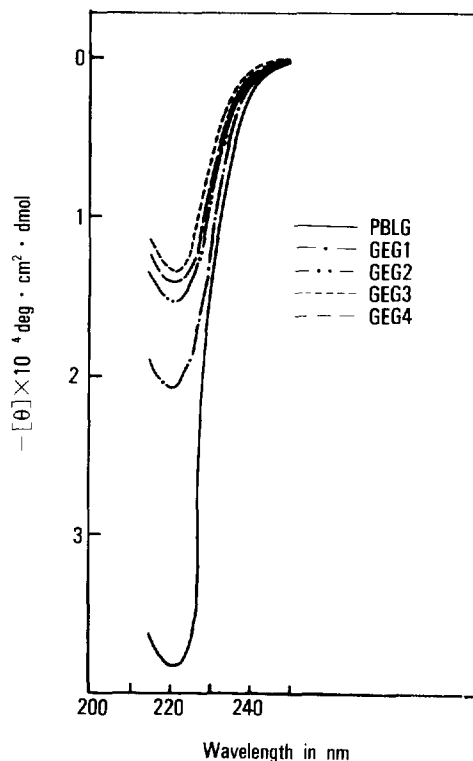


Fig. 2. CD spectra of the GEG block copolymers and PBLG homopolymer in 1,2-dichloroethane at 25°C.

helical content of block copolymers is consistent with PBLG content obtained by NMR.

Infrared(IR) spectra of solid films of GEG block

Table 2. Negative Ellipticity at 222 nm, $-[\theta]_{222}$, of Samples

Sample	Content of PBLG	$-[\theta]_{222}$	$[\theta]_{222}^c/[\theta]_{222}^h$ ^{a)}
PBLG	100.0	39,600	1.00
GEG-1	46.0	21,118	0.53
GEG-2	44.4	15,068	0.38
GEG-3	41.0	14,000	0.35
GEG-4	35.7	14,100	0.36

^{a)} $[\theta]_{222}^c$: ellipticity of the block copolymers.

$[\theta]_{222}^h$: ellipticity of the PBLG homopolymer.

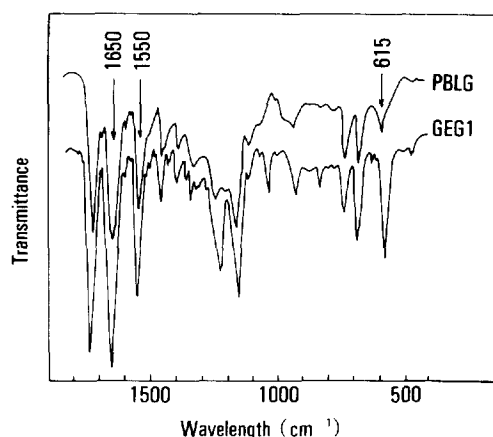


Fig. 3. IR spectra of the GEG block copolymers and PBLG homopolymer.

copolymers and PBLG homopolymer cast from chloroform in the region of $2000\sim 4000\text{ cm}^{-1}$ are shown in Fig 3. The amide I, II and V bands of GEG block copolymers are appeared at 1650, 1550 and 615 cm^{-1} , respectively, at the same wavenumbers as for the PBLG homopolymer.

Effect of PEO Molecular Weight in the Block Copolymers on Platelet Adhesion

Fig. 4 shows that correlations between platelet adhesion and molecular weight of PEO in the block copolymer. They indicated that platelet adhesion decreased with increasing molecular weight of PEO in the block copolymer. However, the very higher molecular weight of PEO effected in an opposite way. Minimum platelet adhesion

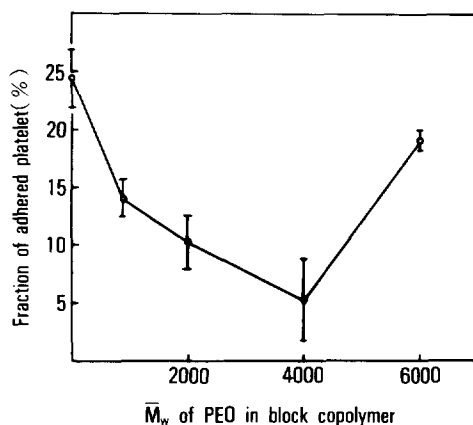


Fig. 4. Platelet adhesion on the surface of GEG block copolymer according to the molecular weight of PEO.

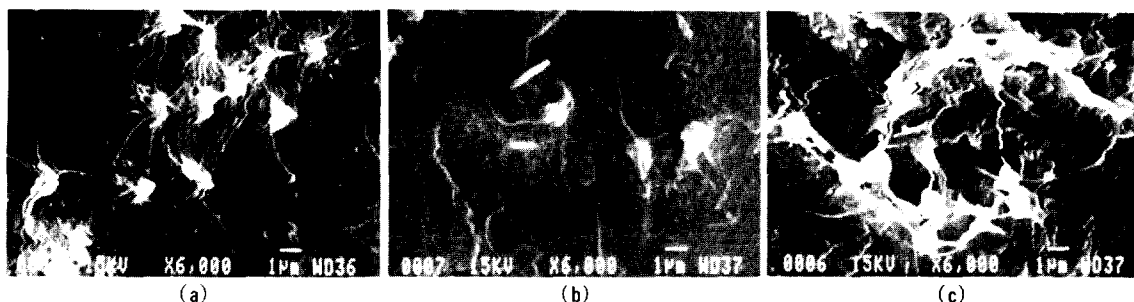


Fig. 5. Scanning electron micrographs of platelets adhered on the surfaces of GEG-2(a), GEG-3(b), and GEG-4(c).

was achieved at PEO molecular weight 4,000. This aspect cannot be explained adequately and should be studied in more detail. However, this suggests PEO 4,000 might show optimum dynamic motion to prevent platelet adhesion.¹²

Scanning electron micrographs of platelets adhered on the surfaces of the samples are shown in Fig. 5. As shown in Fig. 5, the degree of adhered platelet on the surface of the block copolymers was decreased with increasing molecular weight of PEO up to 4,000. On the contrary, more platelets are adhered on the surface of GEG-4 copolymer with 6,000 molecular weight of PEO. Also, the adhered platelets were found to form elongated pseudopods followed by the process of spreading.

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