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Poly(γ-methyl *L*-glutamate)와 Poly(propylene oxide)로 된 Diblock 공중합체의 합성, 구조 연구 및 혈액적합성에 관한 연구

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Synthesis, Structural Studies and Blood Compatibility of AB Block Copolymers Consisting of Poly(γ-methyl *L*-glutamate) as the A Component and Poly (propylene oxide) as the B Component

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요 약 : PMLG를 A성분으로 하고 PPO를 B성분으로 하는 A-B형 블록공중합체는 PPO의 말단에 있는 아미노기가 γ-MLG NCA 에의 친핵성첨가 메카니즘에 의해 합성되었다. TFE 용매에서의 원이색성 분광측정과 고체상태에서의 적외선 분광측정으로부터 이 중합체가 PMLG homopolymers와 같은 전형적인 γ-helix 구조를 가지고 있음을 알 수 있었다. Wide angle X-ray diffraction pattern으로 부터는 이 공중합체가 PMLG homopolymer와 같은 결정구조를 가지고 있음을 알수 있었다. 블록 공중합체표면에서의 혈소판의 점착거동은 사람의 혈액을 사용한 microsphere column method에 의해 검토되었는데 47mol% 이하의 PPO를 갖는 블록 공중합체표면에서의 혈소판의 점착은 PMLG homopolymer 나 glass 표면에서 보다 적었음을 알수 있었다. 그리고 PMLG homopolymer 표면에 점착된 혈소판의 형태는 블록 공중합체에 비해 많은 의족과 형태변화가 관찰되었다.

Abstract : A-B type block copolymer composed of poly(γ-methyl *L*-glutamate) (PMLG) as the A component and poly(propylene oxide) (PPO) as the B component was obtained by polymerization of γ-methyl *L*-glutamate N-carboxy anhydride, initiated by

triamine- terminated poly(propylene oxide). From circular dichroism measurements of the block copolymer in trifluoroethanol solution as well as from infrared spectra in the solid state, it was found that the polypeptide block exists in the α-helical conformation, as that in PMLG homopolymer. Wide-angle X-ray diffraction patterns for the block copolymers show basically similar reflection to the PMLG homopolymer. Platelets adhesion on the PMLG / PPO block copolymer surfaces was examined by a microsphere column method using whole blood. The number of platelets adhered from whole blood was smaller for the block copolymer surfaces than for the homopolymer and for the bare glass. The shape of the platelets adhered on the polymer surfaces was observed with a scanning electron microscope.

Introduction

Recently, there has been much attention to antithrombogenic polymers with an increasing demand for artificial organs utilized in contact with blood to substitute for various body functions such as cardiovascular prostheses, artificial hearts, and other devices. The problem of blood compatibility of polymeric materials is one of great concern in the field of biomaterial science.[1] When in contact with blood, artificial surfaces generally induce platelet adhesion and subsequent activation, which can lead to thrombus formation accompanied by the formation of an insoluble fibrin network.[2] Therefore, biomaterials cannot perform their own functions. Much research work has been carried out to develop ideal artificial organs, and one is a study 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, which surface is heterogeneity. It has been reported the heterogeneity of the synthetic polymer surface plays an important role in blood compatibility due to its apparent inhibition of platelet aggregation.

In previous studies,[4~8] we reported the synthesis of ABA block copolymers consisting of poly(γ-benzyl *L*-glutamate) as the A component and poly(ethylene glycol) or poly(propylene glycol) as the B component, and their antithrombogenicity. Those results showed that platelet adhesion was suppressed on the surface of PBLG / PEG block copolymers which have microphase-separated structure constructed of hydrophilic and hydrophobic microdomains. Furthermore, it was found out that the good antithrombogenicity of the block copolymer was due to its mild disruption of albumin structure after adsorption onto the microdomain structure.[8]

However, the relationship between surface structure with hydrophilic-hydrophobic microdomain surface and its antithrombogenicity is not clear until now. In this study, we have synthesized and characterized AB block copolymers consisting of poly(γ-methyl *L*-glutamate) (PMLG) as the A component and poly (propylene oxide) (PPO) as the B component, and also examined platelet adhesion on the surface of these block copolymers in vitro.

It may be expected that the surface structure of the block copolymer affects protein adsorption and platelet adhesion due to the dynamic motions of PPO chains.[9]

Experimental

Materials

Triamine - terminated poly(propylene oxide) (TATPPO) : The PPO block terminated with amine groups was supplied by Texaco Chemical Co., Ballaire, Texas. The average molecuar weight of TATPPO was 440.

Solvents: *n*-hexane, tetrahydrofuran(THE), and dichloromethane were dried and purfid by dist-

illation. Reagent grade of dichloroacetic acid (DCA) and trifluoroethanol(THE) were used without purificaion.

Poly(γ-methyl L-glutamate): The homopolymer PMLG(M.W.:29,000) was supplied by Ajinomoto Co.(Japan)

(γ-methly L-glutamate N-carboxyanhydride(γ-MLG-NCA): The monomer, γ-methyl *L*-glutamate N-carboxy anhydride was prepared according to the method proposed by Goodman et al.[10]

PMLG/PPO(MP) block copolymer : The block copolymer was prepared by polymerization of γ-MLG-NCA initiated by TATPPO in dichloromethane at a total concentration of γ-MLG--NCA and TATPPO of 3%. The reaction mixture was poured into a large excess of diethyl ether, and then the precipitated copolymer was dried in vacuum.

Measurements

Molecular Weight : The molecular welghts of these block copolymers were estimated from the limiting viscosity number of the block copolymer in DCA measured using Ubbelohde type viscometer and applying the $[\eta]$-molecular weight relationship proposed by Doty et al.[11] for PMLG.

Composition of Copolymer: The molar content of polypeptide in each copolymers was determined[4] by circular dichroism spectropolarimeter, Moder Jasco J-500A.

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 1mm.

IR Measurements: Infrared(IR) spectra of the solid films cast from TFE solution were measured with a Shimazu Model-43 IR spectrophotometer between 4000 and 400 cm^{-1}.

X-Ray Diffraction Measurements : Wide angle X-ray diffraction diagrams of solid films of the sample cast from trifluoroacetic acid soultion were obtained with a Rigaku Geigerflex usinmg Ni-filtered CuKα radiation.

Estimation of Platelet Adhesion : 1g of copolymer precoated glass beads(15-35 meshes: Sigma) 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 was subjected to the following platelet adhesion test: 3.0㎤ of fresh whole blood was collected from a healthy person with a disposable syringe without using any anticoagulant and was immediately passed through the column for 1 min at a flow rate of 1.5 cm^3 min^{-1} using syringe pump(Sage Instruments Model 351). The eluted blood was collected in a sampling bottle containing 0.1 cm^3 of ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Platelet counts in the eluted blood were done at 5 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 cm^3 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 palladium-gold. The beads were observed by a scanning electron microscope(SEM) (JEOL, Model TSM-35).

RESULTS AND DISCUSSION

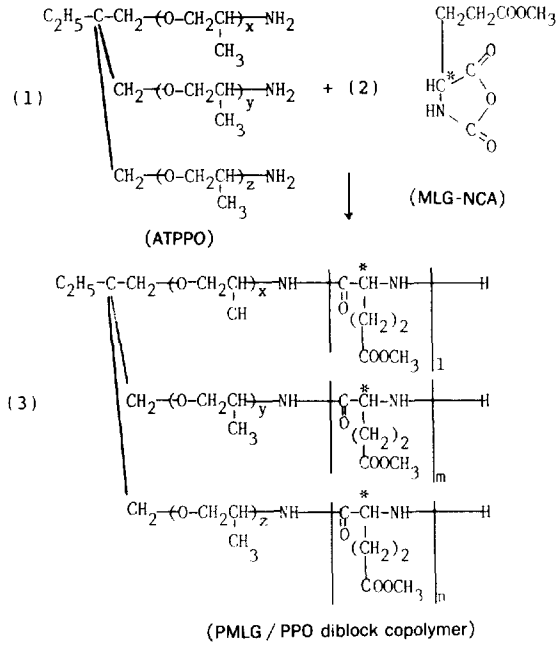
Synthesis of PMLG/PPO Block Copolymers

The PMLG / PPO block copolymers(3) were synthesized by initiating the polymerizaton of MLG-NCA(2) with PPO containing amino end groups(1). (Eq.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 carboxyl group of NCA, as suggested by Hashimoto et al.[12] PPO chains which did not initiate the polymerization of MLG-NCA could be removed by

precipitation of the product in diethyl ether. A series of A-B block copolymer consisting of PMLG as the A component and PPO as the B component are hereafter designated as MP block copolymer.

The prepared samples and their characteristics are summarized in Table 1. As shown in Table 1, intrinsic viscosity and molecualr weights of the block copolymers decreased with increasing content of propylene oxide in the MP block copolymers.



Eq. 1 Synthesis of PMLG / PPO diblock copolymer

Circular Dichroism Properties

The CD Spectra, expressd by the mean ellipticity$[\theta]$ per residue of MP block copolymers and PMLG homopolymer in TFE, are shown in Fig.1. All these spectra show negative Cotton

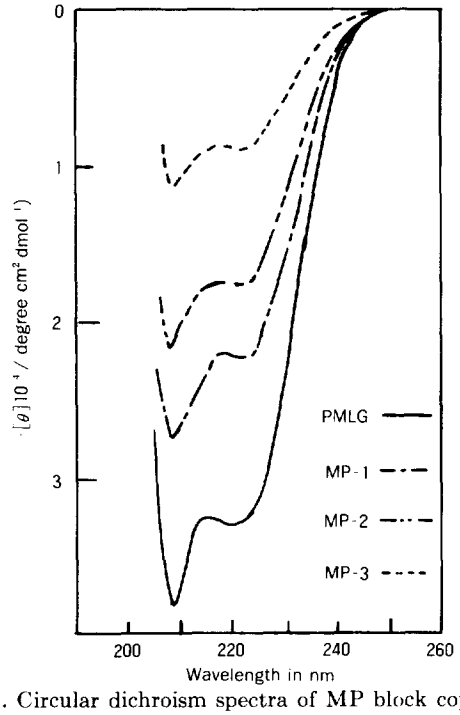


Fig.1. Circular dichroism spectra of MP block copolymers and PMLG homopolymer in TFE solution.

Table 1. Characteristics of Prepared Samples

Smple	Mole Ratio of Reactant (ATPPO / γ-MLG-NCA)	$[\eta]$ / (dl · g^{-1})	$\overline{M}_w \cdot 10^{-4}$	PPO Mole Percent in Block Copolymer	
				mol %	wt %
PMLG	–	–	2.9	0.0	0.0
MP-1	20.7	0.33	2.5	33.0	16.7
MP-2	51.1	0.19	1.2	47.0	26.5
MP-3	80.7	0.14	0.8	70.0	48.6

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

Sample	M,mol %	$-[\theta]_{222}$	$[\theta]^c_{222} / [\theta]^0_{222}$
PMLG	100.0	33,000	1.00
MP-1	67.0	22,000	0.67
MP-2	53.0	17,000	0.53
MP-3	30.0	9,900	0.30

effects characteristic of an α-helical conformation, with a band at 222 nm assigned to the n-π^* transition, and a second peak, due to the π-π * transition, appering at 208nm.[13] Table 2 shows the experimental data $[\theta]_{222}$ for the samples in TFE at room temperature. The ratio of the $[\theta]_{222}$ values of MP block copolymers to that of PMLG homopolymer, $[\theta]^{c}_{222}$ / $[\theta]^{o}_{222}$, is shown in the third column of Table 2.

Chin Conformation of the Block Copolymers in the Solid State

IR spectra of solid films of MP block copolymers and PMLG homopolymer cast from TFE in the region of 1800-500 cm^{-1} are shown in Fig. 2. The amide I, II and V bands of these MP block copolymers appear at 1650, 1550 and 615cm^{-1}, respectively, at the same wavenumbers as for the PMLG homopolymer. This implies that the M-block component in the MP copolymers assumes an α-helical conformation.

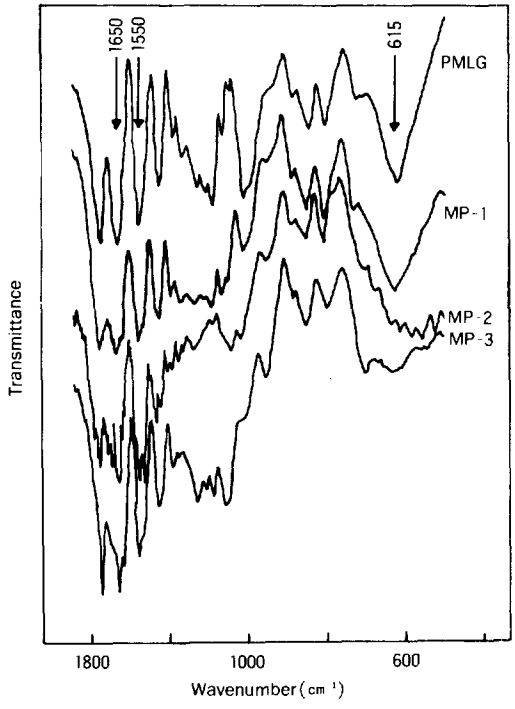


Fig.2. Infrared spectra of MP block copolymers and PMLG homopolymer films cast from TFE solution.

Wide-Angle X-Ray Diffraction

The wide-angle X-ray diffraction(WAXD) patterns for the MP block copolymers and PMLG homopolymer are shown in Fig. 3. In Fig. 3, the main reflections correspond to an intermolecular spacing of α-helical chains and diffraction patterns of MP block copolymers showed basically similar reflections to those of the corresponding PMLG homopolymer.[14] The intensity of the diffraction peaks for the MP block copolymer becomes low with increase of PPO components in the block copolymers. This indicates that the crystallinities of block copolymers decrease with increase of PPO component.

Adhesion Behavior of Blood Platelets on the MP Block Copolymer Surfaces

Adhesion behavior of blood platelets on the block copolymer surfaces was examined by a microsphere column method. Fig. 4 shows adhesion of platelets from whole blood on the block copolymer surfaces. These results indicate

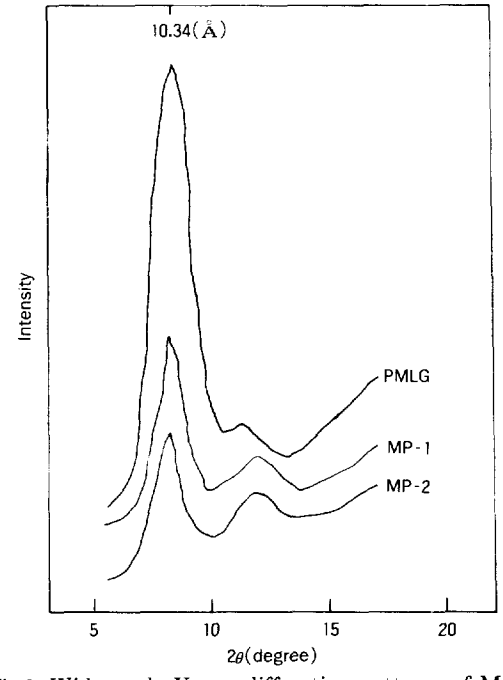


Fig.3. Wide angle X-ray diffraction patterns of MP block copolymer and PMLG homopolymer films.

that less platelets are adhered on the block copolymer surfaces(MP-1 and MP-2) than on PMLG homopolymer and on glass.

Fig. 5 shows the relationship between platelet adhesion and PPO content in the block copolymer. Percent of platelet adhesion on the surface of the MP block copolymers was highly dependent on the PPO content. Namely, platelet adhesion increased with increasing PPO content in the MP block copolymers as the same tendency of the PBLG / PEO block copolymer systems.[6] These results suggest that the conformation of the block copolymer at the surface is dependent on the content of polyether. One of the initial events occurring as blood comes in contact with a polymer is the adsorption of a protein layer at the blood-material interface.[15] This layer modifies the original surface and has an important influence on subsequent phenomena such as platelet adhesion and blood coagulation.[16] The concentration of adsorbed proteins determine the degree of platelet adhesion. Also, the hydrophilic or hydrophobic nature of the polymer surfaces plays an important role in the adsorption of proteins.[17] It may be regarded that the molecule of the block copolymer has a nearly flat conformatin at low content of PPO(MP-1 and MP-2) while that is folded out at the higher content PPO(MP-3).[18] Therefore, it was suggested that the protein will weakly bind on the surface of block copolymer with low content of PPO due to the smaller number of binding sites for hydrophobic interaction, while the protein will result in strong binding on the surface of block copolymer with higher content of PPO.

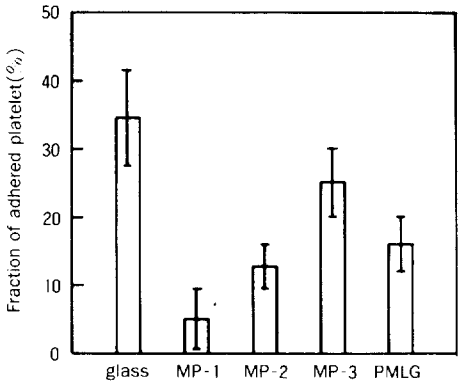


Fig.4. Fraction of platelet adhered to the surface through polymer-coated glass bead columns.

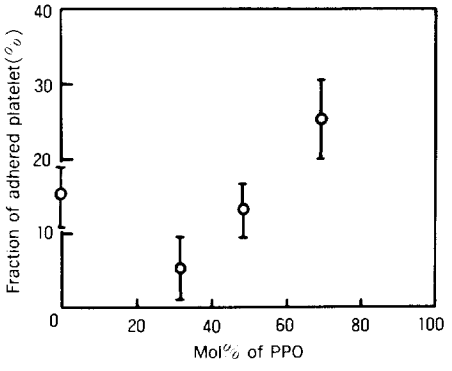


Fig.5. Platelet adhesion on the surface of MP block copolymer in relation to the content of PPO.

These results suggest that a mild disruption of the adsorbed protein takes place on the MP-1 and MP-2 surfaces after the weak binding of proteins on the MP-1 and MP-2 surfaces while strong conformational change of adsorbed one occurs after the strong binding of proteins on that of MP-3. It has been found that a conformational change of the proteins may contribute significantly to the subsequent reactions after binding of the proteins with platelets.

But in the case of the homopolymer, PMLG forms a homogeneous structure. It may be expected that homogeneous surfaces do not form an organized structure corresponding to that of the surface microdomain. Therefore, the number of adhered platelets was larger for the PMLG surface than for any block copolymer surfaces.[19] In previous study,[8] a milder disruption of albumin structure takes place on the antithrombogenic system. These results suggest that the conformation of the block copolymer at the surface is one of the determining factors in suppressing platelet adhesion and activation

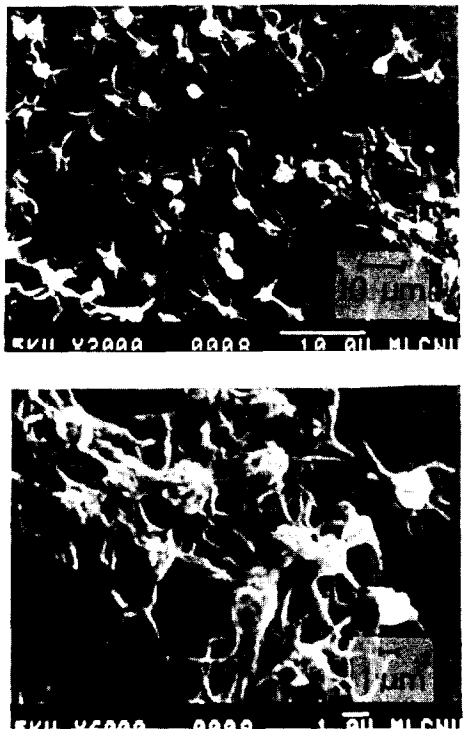


Fig.6. Scanning electron micrographs of appearances of the adhered platelet on the PMLG homopolymer surface.

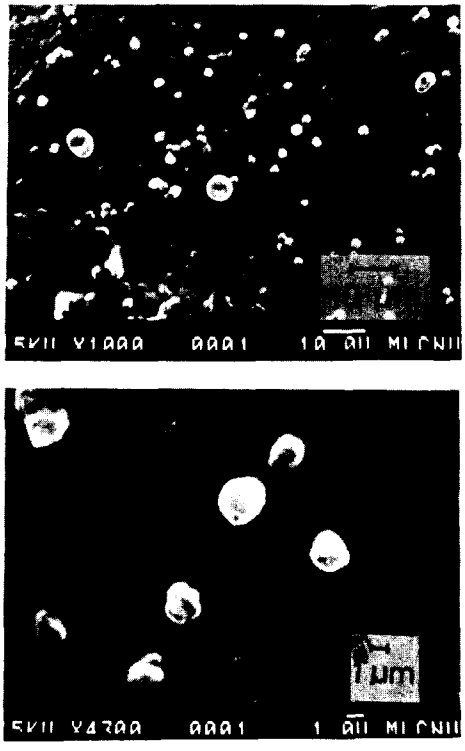


Fig.7. Scanning electron micrographs of appearances of the adhered platelet on the MP-1 block copolymer surface.

on the polymer surfaces. But the relationship between antithrombogenicity and surface structure should be clarified in more detail.

Comparison of the physical appearances of the adhered platelets yields significant differences between the homopolymer and the block copolymer surfaces, as shown in Fig. 6 and Fig. 7. On the PMLG homopolymer surfaces, adhered platelets were deformed and extended many pseudopods and aggregated with one another. In contrast, shape changes and aggregation of adhered platelets were suppressed effectively on the MP-1 block copolymer surfaces.

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