

Poly(γ -methyl *L*-glutamate)/Poly(propylene oxide)/ Poly(γ -methyl *L*-glutamate) 블록 공중합체의 합성, 구조연구 및 혈액 적합성

조 종 수 · 김 회 용 · 송 수 창 · 양 동 욱* · 김 주 오** · 김 상 수**

전남대학교 고분자공학과 · *전남대학교 임상병리과 · **원광대학교 정형외과

(1990년 8월 20일 접수)

Synthesis, Structural Studies of ABA Triblock Copolymers Composed of Poly(γ -methyl *L*-glutamate) as the A Component and Poly(propylene oxide) as the B Component and Their Blood Compatibility

Chong-Su Cho, Hee-Yong Kim, Soo-Chang Song, Dong-Wook Yang*, Ju-O Kim**, and Sang-Su Kim**

Dept. of Polymer Engineering, Chonnam National University, Kwangju 500-757, Korea

**Dept. of Clinical Pathology, Chonnam National University, Kwangju 500-757, Korea*

***Dept. of Orthopedics, Wonkwang University, Iri 570-749, Korea*

(Received August 20, 1990)

요 약 : poly(γ -methyl *L*-glutamate)(PMLG)를 A성분으로 하고 poly(propylene oxide)(PPO)를 B성분으로 구성된 ABA형 블록 공중합체가 poly(propylene oxide)의 양 말단에 있는 아미노기를 개시제로 γ -MLG NCA가 개환되는 친핵성첨가 mechanism에 의해 합성되었다. Trifluoroethanol 용액에서의 원이색성(CD)측정과 고체상에서의 IR측정으로부터 이 블록공중합체가 PMLG homopolymer와 같이 전형적인 α -helix구조를 가지고 있음을 알았다. 이 블록공중합체 표면에서의 혈소판 흡착실험은 platelet-rich plasma(PRP)를 사용하여 depositing 방법으로 행하였고, 혈소판의 흡착정도는 블록공중합체의 poly(propylene oxide)의 함량에 의존함을 알았다.

Abstract : ABA type triblock copolymers consisting of poly(γ -methyl *L*-glutamate) (PMLG) as outer blocks and poly(propylene oxide)(PPO) as the middle block were synthesized by polymerization of γ -methyl *L*-glutamate *N*-carboxyanhydride by amine terminated poly(propylene oxide), in which the terminal amine groups act as an initiator. 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 in the PMLG homopolymer. Platelets adhesion on the block copolymer surfaces was examined by depositing method using platelet-rich plasma(PRP). This result shows that platelet adhesion on the PMLG/PPO block copolymer surface was dependant on the content of PPO in the block copolymer.

INTRODUCTION

Recently, there has been much attention to anti-thrombogenic polymers with an increasing demand for artificial organs utilized in contact with blood to substitute for various functions. The blood compatibility of polymeric materials being used as cardiovascular prosthesis, artificial heart devices, and other similar objects with living tissues and particularly with blood is one of great concern in the field of biomaterial science.¹

Many researcher have typically taken one of two approaches in attempts to eliminate this problem : the surface modification approach,^{2,3} in which formed polymers are modified or new polymers synthesized with surface properties considered blood compatible, or the pharmaceutical approaches,^{4,5} in which antiplatelet agent and anticoagulants are employed along the polymer.

It has been reported that the heterogeneity of the synthetic polymer surface plays an important role in blood compatibility.^{6,7} In practice, the normal vascular endothelium, which is the ideal non-thrombogenic material, is considered to have a microdomains.⁸ Recently, Nakajima et al.⁹⁻¹⁴ synthesized ABA type triblock copolymers containing polypeptide blocks as the A component and polybutadiene or poly(oxytetramethylene) as the B component to characterize a microheterophase structure. Okano et al.¹⁵ proposed that the polymeric hydrophilic/hydrophobic microphase-separated structure is a key parameter for controlling the antithrombogenic activity of polymers due to its apparent inhibition of platelet aggregation. Kataoka et al.¹⁶ reported that selective cell adhesion behavior on PBLG/PEG block copolymers is attributable to the formation of a microphase separated structure. Also, synthetic polypeptides have been considered very useful for biomedical application, such as temporary artificial skin substitutes in burn therapy, and as temporary barriers to prevent adhesion between natural tissue planes that are damaged either by accident or as a result of surgery.

In our previous studies,¹⁷ we reported on the synthesis of ABA triblock copolymers having microphase separated structure consisting of poly(γ -benzyl *L*-glutamate)(PBLG) as the A component and polyether[poly(ethylene oxide), poly(propylene oxide)] as the B component according to the content of polyether to examine their blood compatibility through in vitro¹⁸ and in vivo test. This result indicated that platelet adhesion was suppressed on the surface of PBLG/polyether block copolymers which have microphase separated structure constructed hydrophilic-hydrophobic domains. Also, platelet adhesion was dependant on the content of polyether in the block copolymers.

Nagaoka, et al.¹⁹ investigated the effects of the poly(oxyethylene)(POE) chain length on adhesion of the platelets in the methoxy poly(ethylene glycol) monomethacrylates with POE side chain. They reported that the amounts of adhered platelets decreased with increasing POE 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 activity of it 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.²⁰

Park, et al.²¹ reported that minimum platelet adhesion of 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.²² reported that the platelet reactivity of segmented poly(etherurethaneureas) with soft segmented components was influenced by the M_n of soft segment due to the glass transition.

Lee, et al.²³ studied that surface properties of aqueous PEO/PPO block copolymer surfactants were influenced by the PEO chain length.

In the present study, our interest is focused on ABA type triblock copolymers composed of hydrophilic poly(propylene oxide)(PPO) having ran-

dom-coil conformation as middle block component and hydrophobic poly(γ -methyl *L*-glutamate) having α -helical conformation as component of the outer polypeptide blocks.

EXPERIMENTAL

Materials

Poly(γ -methyl *L*-glutamate)(PMLG) : The PMLG homopolymer ($M_w = 29,000$) was supplied by Ajinomoto Co. (Japan).

Amine-Terminated Poly(propylene oxide)(AT-PPO) : The ATPPO was supplied by Texaco Chemical Co., Ballaire, Texas ($M_w = 2,001$).

Solvents : n-Hexane, THF and dichloromethane (DCM) were dried and purified by distillation. Reagent grade of dichloroacetic acid(DCA) and trifluoroethanol(TFE) were used without purification.

Synthesis of γ -Methyl *L*-Glutamate *N*-Carboxy-anhydride(γ -MLG NCA) : The monomer, γ -MLG NCA was synthesized according to the method proposed by Goodman et al.²⁴

Synthesis of PMLG/PPO(MPM) Triblock Copolymer : The block copolymer was prepared by polymerization of γ -MLG NCA initiated by ATPPO in 3 wt% DCM at 25°C for 72 hr. After the characteristic absorption of anhydride (1785, 1860 cm^{-1})²⁵ had disappeared in the IR spectrum (72 hr), the reaction mixture was poured into a large excess of diethyl ether. The precipitated copolymer was dried in vacuum.

Measurements

Molecular Weight : The molecular weights of these block copolymers were estimated from the limiting viscosity number of the block copolymer in DCA and applying the $[\eta]$ -molecular weight relationship proposed by Doty et al.²⁶ for PBLG.

Composition of Copolymer : The molar content of polypeptide in each copolymers was determined¹⁷ by circular dichroism, Model JASCO J-500A.

CD Measurements : The circular dichroism(CD) spectra were measured at room temperature on a

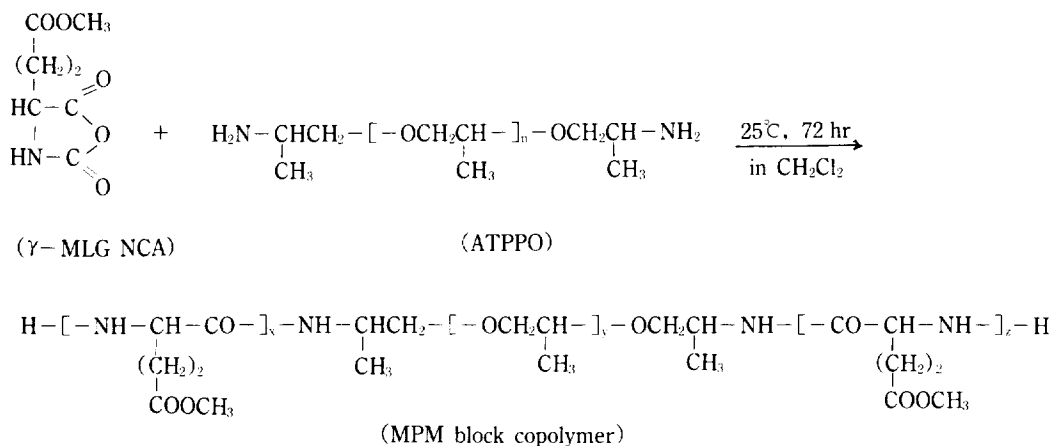
JASCO J-500A spectropolarimeter equipped with a quartz cell having a path length of 1 mm.

IR measurements : Infrared (IR) spectra of the solid films cast from TFE solution were measured with a Shimadzu Model-43 IR spectrophotometer between 4,000 and 400 cm^{-1} .

X-ray Diffraction Measurements : Wide angle X-ray diffraction diagrams of solid films of the sample cast from TFA solution were obtained with a Rigaku Geigerflex using Ni-filtered CuK_α radiation.

Estimation of Platelet Adhesion in vitro : The polymers were coated on glass beads(15~30 meshes ; Sigma) by evaporation technique. Platelet-rich plasma(PRP) was prepared from the citrated blood of a healthy person. 100 ml of fresh blood was collected in a disposable syringe containing 10 cc of 3.8 wt% aqueous of sodium citrate. The citrated blood was centrifuged at 4°C for 10 min at 1000 rpm to obtain PRP. 1 gram of copolymer-precoated glass beads were incubated at 37°C for 4 hr.

Antithrombogenicity of Polymer Surfaces in Vivo : The catheter of silastic tubing (outer diameter : 2 mm, length : 10 mm) was coated on its internal and external surface with the MPM-1 block copolymer by solvent evaporation technique with 2 wt% polymer solution in chloroform and trifluoroethanol mixture (9/1 in v/v). Optical microscopy observation showed uniform coating of copolymer on the silastic catheter. The catheter was implanted in mongrel dogs of mixed sex (10~15 kg) anesthetized with pentobarbital. The catheter was rinsed with PBS and positioned in external jugular and femoral veins in the direction of blood flow.⁵ Bare silastic tubing was used as contralateral control. The animal was systematically heparinized (200~300 V/kg) during 1 hr of implantation and terminated (conc. KCl) by syringe. Vessels were exposed, ligated, and remaining blood was gently flushed with PBS, followed by in situ fixation of thrombi with 1.25% glutaraldehyde in PBS solution(pH 7.4). After removal and fixation in fresh solution, vessels were opened

**Scheme 1.** Synthesis of PMLG/PPO block copolymers

longitudinally. The difference of weight between 3 cm section of implanted and nonimplanted catheters determined the thrombus weight.

Synthesis of the Block Copolymers

The PMLG/PPO(MPM) block copolymers were synthesized by initiating the polymerization of MLG-NCA with PPO containing amino end groups (Scheme 1). It may be assumed that the initiator amine undergoes a nucleophilic addition to the C-5 carboxy group of the NCA to yield a polymerization on both directions.

Characteristics of the Block Copolymers

The characteristics of PMLG homopolymer and MPM block copolymers are summarized in Table 1. As shown in Table 1, intrinsic viscosity and molecular weight decreased with increasing content of PPO in the block copolymers.

Chain Conformational Studies

The chain conformation of the block copolymers in the solution state was investigated by CD spectroscopy. Fig. 1 shows the CD spectra of PMLG homopolymer and MPM block copolymers in TFE. All these spectra show negative Cotton effects characteristic of an α -helical conformation, with a band at 222 nm assigned to the $n\text{-}\pi^*$ transi-

Table 1. Characteristics of Samples Prepared

Sample	$[\eta]/(\text{dl g}^{-1})$	$M_n \times 10^{-4}$	Content of Propylene Oxide	
			mol %	wt %
PMLG	—	2.9	0.0	0.0
MPM-1	0.91	10.1	17.5	7.9
MPM-2	0.65	6.4	30.0	14.8
MPM-3	0.41	3.4	42.5	23.1

tion, and a second peak at 208 nm due to the $\pi\text{-}\pi^*$ transition.²⁷ Table 2 shows the experimental data, $[\theta]_{222}$, for the samples in TFE at 25°C. The ratio of the $[\theta]_{222}$ values of MPM block copolymers to that of PMLG homopolymer, $[\theta]_{222}^c/[\theta]_{222}^h$, is shown in the third column of Table 2.

Infrared(IR) spectra of solid films of MPM block copolymers and PMLG homopolymer cast from TEE in the region of 2000~400 cm^{-1} are shown in Fig. 2. The amide I, II and V bands of MPM block copolymers appear at 1650, 1550 and 615 cm^{-1} , respectively, at the same wavenumbers as for the PMLG homopolymer.

The wide-angle X-ray diffraction patterns for the MPM block copolymers and the PMLG homopolymer are shown Fig. 3. The main reflection corresponds to an intermolecular spacing of α -helical chains and is 10.3 Å for the film cast from TFE.

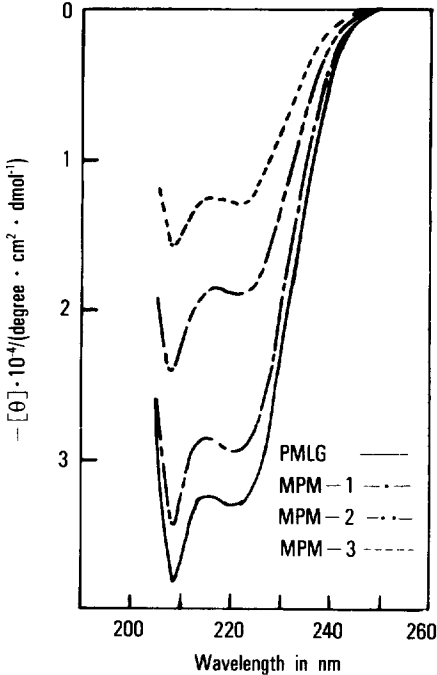


Fig. 1. CD spectra of MPM block copolymers and PMLG homopolymer in trifluoroethanol at 25°C.

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

Sample	M ^a in mol%	$[-\theta]_{222}$	$[\theta]_{222}^c/[\theta]_{222}^b$
PMLG	100.0	33,000	1.00
MPM-1	82.5	29,300	0.89
MPM-2	70.0	19,000	0.58
MPM-3	57.5	12,800	0.39

^a M: content of γ -methyl *L*-glutamate units in copolymer samples.

^b $[\theta]_{222}^c$: ellipticity of the block copolymers.
 $[\theta]_{222}^b$: ellipticity of the PMLG homopolymer.

Also, crystallinities of the block copolymers decreased with increasing PPO content in the block copolymer.

Adhesion Behavior of Platelet on the MPM Block Copolymer Surface in vitro

Adhesion behavior of blood platelets on the surface of the block copolymers was examined by depositing method. Fig. 4 shows platelet adhesion from PRP on the polymer surfaces. As shown in

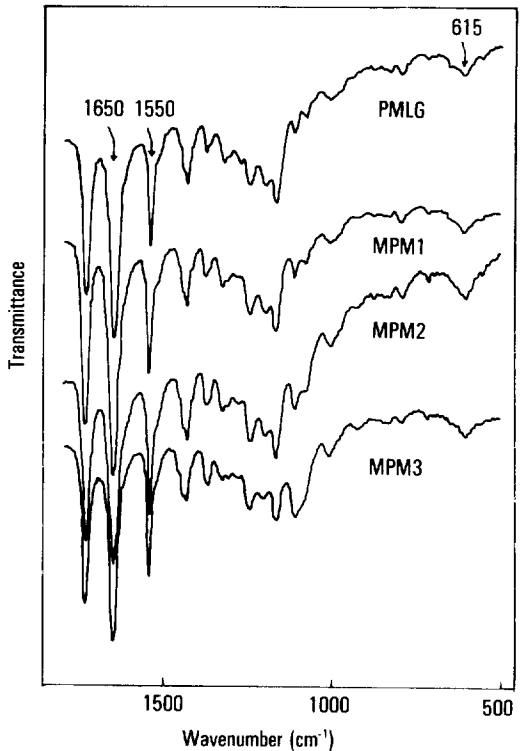


Fig. 2. IR spectra of MPM block copolymers and PMLG homopolymer.

Fig. 4, less platelets adhered on the block copolymer surfaces than on PMLG homopolymer and bare glass. Fig. 5 shows the relationship between the platelet adhesion and the PPO content in the block copolymers. As shown in Fig. 5, platelet adhesion onto the MPM block copolymers was dependant on the content of PPO in the block copolymers. Also, platelet adhesion decreased with increasing PPO content in the block copolymers. These results may be attributed to the mobility of PPO in the block copolymers. But the relationship between microstructure of the block copolymer and platelet adhesion should be clarified in more detail.

Scanning electron micrographs of platelets adhered on the surfaces of the samples are shown in Fig. 6. Many platelets are adhered on the surface of the glass and the PMLG homopolymer. Also, the adhered platelets were found to form

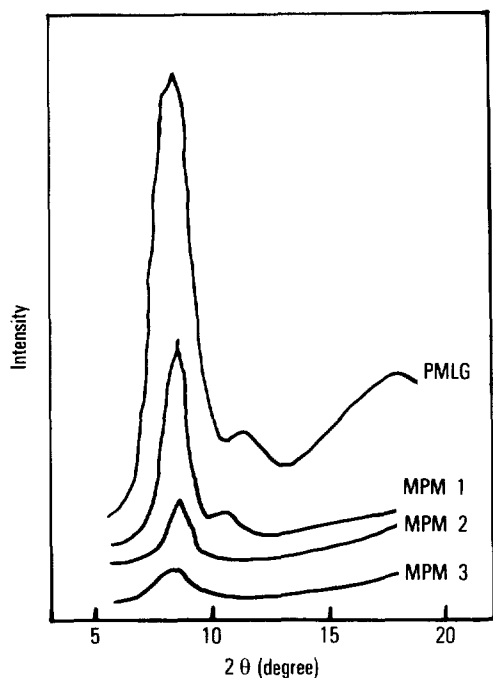


Fig. 3. Wide-angle X-ray diffraction patterns of MPM block copolymers and PMLG homopolymer.

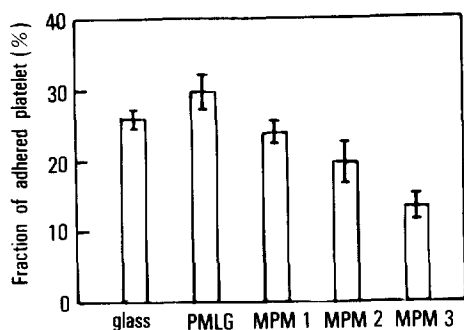


Fig. 4. Platelet adhesion on the surfaces of MPM block copolymers, PMLG homopolymer and bare glass deposited for 4 hours in platelet-rich plasma (PRP) solution.

elongated pseudopods followed by a process of spreading. On the contrary, less platelets are adhered on the surface of MPM-3 block copolymer. Platelets attached on the MPM-3 block copolymer surface form pseudopods, but do not spread.

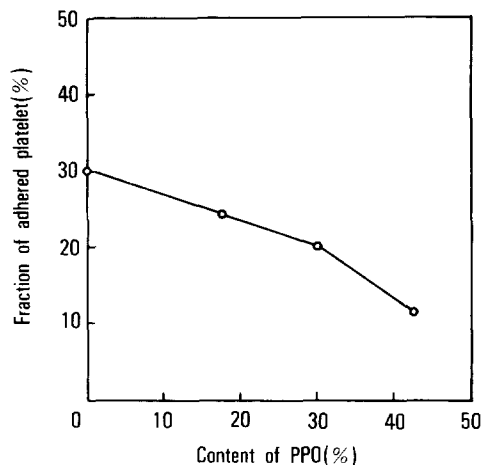


Fig. 5. Platelet adhesion on the surface of MPM block copolymer according to the content of PPO.

Table 3. Thrombus Weight on MPM-1 Coated Silastic and Silastic Catheter Implanted in Canine Veins for one Hour

Sample	Site	venous thrombus (mg/3cm)			
		1st.	2nd.	3rd.	average
MPM-1	jugular	5.89	4.57	12.8	7.75
silastic	"	6.13	4.21	10.7	7.01
MPM-1	femoral	2.98	5.75	5.24	4.65
silastic	"	1.87	6.17	8.60	5.55

In vivo Evaluation of the Antithrombogenicity of Polymer Surface

The antithrombogenicity of the synthesized block copolymers was examined by implanting the block copolymer-coated catheter of 2 mm outer diameter into the canine external jugular and femoral veins, where the antithrombogenicity was evaluated by mean thrombus weights of 3 cm sections. Table 3 shows thrombus weights on 3 cm sections of the catheter.

Three experiments were run for 1 hr. These results indicated that the thrombus formation on the MPM-1 block copolymer surface is almost similar to that of silastic tubing. Blood compatibility was expected to be superior in jugular veins, with their consistently greater diameters. However, significant differences in thrombus formation at the two

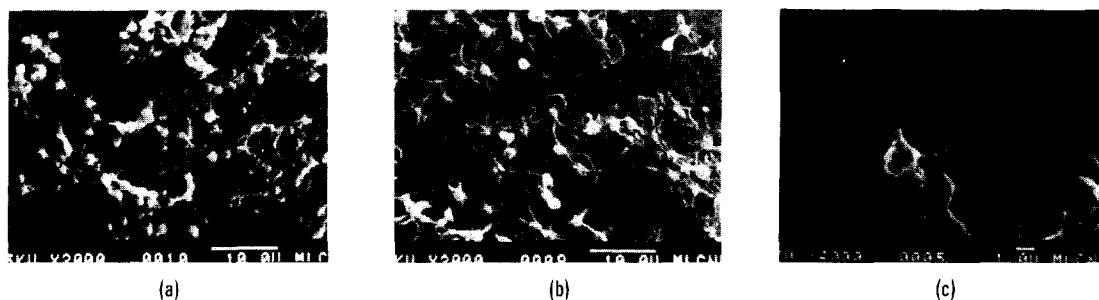


Fig. 6. Scanning electron micrographs of platelets adhered on the surfaces of bare glass(a), PMLG homopolymer(b) and MPM-3 block copolymer(c).

sites were not observed.

REFERENCES

1. Y. Sakurai, T. Akaike, K. Kataoka, and T. Okano, "Interfacial Phenomena in Biomaterial Chemistry", A. Nakajima and E. Goldberg, Eds., Academic, New York, 1980, pp. 335-379.
2. J. D. Andrade, *Med. Instrum.*, **7**, 110 (1973).
3. T. Okano, S. Nishiyama, I. Shinohara, T. Akaike, Y. Sakurai, K. Kataoka, T. Tsuruta, K. Kuwana, and S. Miyata, *Artificial organs*, **3**(Suppl.), 253 (1979).
4. S. W. Kim, C. D. Ebert, J. Y. Lin, and J. C. McRea, *ASAIO*, **6**, 76 (1983).
5. P. W. Heyman, C. S. Cho, J. C. McRea, D. W. Olsen, and S. W. Kim, *J. Biomed. Mater. Res.*, **19**, 419 (1985).
6. D. J. Lyman, D. W. Hill, R. K. Stirk, C. Adamson, and B. R. Mooney, *Trans. Am. Soc. Artif. Intern. Organs*, **18**, 19 (1972).
7. T. Okano, S. Nishiyama, I. Shinohara, T. Akaike, Y. Sakurai, K. Kataoka, and T. Tsuruta, *J. Biomed. Mater. Res.*, **15**, 393 (1981).
8. P. N. Sawyer, C. Burrowes, J. Ogoniak, A. O. Smith, and S. A. Wesolowski, *Trans. Am. Soc. Artif. Intern. Organs*, **10**, 316 (1964).
9. A. Nakajima, T. Hayashi, K. Kugo, and K. Shinoda, *Macromolecules*, **12**, 840 (1979).
10. A. Nakajima, K. Kugo, and T. Hayashi, *Macromolecules*, **12**, 844 (1979).
11. A. Nakajima, K. Kugo, and T. Hayashi, *Polym. J.*, **11**, 995 (1979).
12. G. W. Chen, T. Hayashi, and A. Nakajima, *Polym. J.*, **13**, 433 (1981).
13. K. Kugo, T. Hayashi, and A. Nakajima, *Polym. J.*, **14**, 401 (1982).
14. K. Kugo, M. Murashima, T. Hayashi, and A. Nakajima, *Polym. J.*, **15**, 267 (1983).
15. T. Okano, M. Uruno, N. Sugiyama, M. Shimada, I. Shinohara, K. Kataoka, and Y. Sakurai, *J. Biomed. Mater. Res.*, **20**, 1035 (1986).
16. T. Nishimura, Y. Sato, M. Yokoyama, M. Okuya, S. Inoue, K. Kataoka, T. Okano, and Y. Sakurai, *Makromol. Chem.*, **185**, 2709 (1984).
17. C. S. Cho, S. W. Kim, Y. K. Sung, and K. Y. Kim, *Makromol. Chem.*, **189**, 1505 (1988).
18. C. S. Cho, S. C. Song, S. W. Kim, D. W. Ryang, Y. K. Sung, and K. Y. Kim, *J. of Korea Society of Medical and Biological Engineering*, **8**, 199 (1987).
19. S. Nagaoka, Y. Mori, H. Takiuchi, K. Yokota, H. Tanzawa, and S. Nishu "Polymers as Biomaterials", S. W. Shalaby, A. S. Hoffman, B. D. Ratner and T. A. Horbett, ed., pp. 361-374, Plenum Press (1984).
20. S. Nagaoka, H. Kurumatani, Y. Mori, and H. Tanzawa, *J. of Bioactive and Compatible Polymers*, **4**, 323 (1989).
21. K. D. Park, T. Okano, C. Nojiri, and S. W. Kim, *J. Biomed. Mater. Res.*, **22**, 977 (1988).
22. A. Takahara, N. J. Jo, and T. Kajiyama, *J. Biomater. Sci. Polymer Edn.*, **1**, 17 (1989).
23. J. H. Lee and J. D. Andrade, "Surface Properties of Aqueous PEO/PPO Block Copolymer Surfacta-

- nts", in *Polymer Surface Dynamics*, J. D. Andrade (Ed.), Plenum Press, New York, 1988, pp. 119-136.
24. W. D. Fuller, M. S. Verlander, and M. Goodman, *Biopolymers*, **15**, 869 (1976).
25. J. E. Alter, G. T. Taylor, and H. A. Scheraga, *Macromolecules*, **5**, 739 (1972).
26. P. Doty, J. H. Bradbury, and A. M. Holtzer, *J. Am. Chem. Soc.*, **78**, 947 (1956).
27. G. Holtzwarth and P. Doty, *J. Am. Chem. Soc.*, **87**, 218 (1965).