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생체적합성과 생분해성을 갖는 Polypeptide Copolymers의 합성과 물성에 관한 연구 : 3. Polypeptide Hydrogels의 약물조절방출

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Synthesis and Physical Properties of Biocompatible and Biodegradable Polypeptide Copolymers : 3. Polypeptide Hydrogels and Their Controlled Drug Release

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요 약 : Poly(γ-benzyl L-glutamate) (PBLG)의 측쇄에 polyethylene glycol(PEG) 또는 ethanolamine (EA)을 반응시켜 적심성이 서로 다른 몇가지 폴리펩티드 공중합체를 합성하였고, 이들 공중합체의 약물방출특성을 살펴보았다. 합성된 폴리펩티드공중합체의 수분흡수율은 공중합체중의 PEG 또는 EA함량이 높아짐에 따라 증가하였다. PEG-PBLG-EA 공중합체로 부터의 5-fluorouracil의 방출속도는 PEG-PBLG 공중합체로 부터의 방출속도 보다 크게 나타났으며, 이러한 결과는 팽윤성의 폴리펩티드를 합성하고자 할때 사용되는 치환체로서는 PEG 보다도 EA가 더욱 효과적이라는 것을 암시한다. 한편, PEG를 가교시킨 PBLG 공중합체막상에서는 5-fluorouracil의 방출에 기인하여 작은 pores들이 명료하게 나타나고 있음을 알 수 있었다.

Abstract : Several copolypeptides having different swellabilities are synthesized by introducing polyethylene glycol (PEG) or ethanolamine (EA) to the side chains of poly (γ-benzyl L-glutamate) (PBLG) and their drug release characteristics are examined. The degree of swelling of copolypeptide is increased by increasing PEG or EA content in the polymer. The release rate of 5-fluorouracil from the PEG-PBLG-EA copolymers was higher than that of the PEG-PBLG copolymers. This result indicates that EA is more effective than PEG for the preparation of the swellable polypeptides. It was observed, from the morphological study by scanning electron microscope, the pores are generated on the PEG-crosslinked PBLG, but not on the PEG-grafted-PBLG.

INTRODUCTION

Polypeptides, which are components of the living body, have been used as biomedical materials such as artificial skin,[1] adhesive substrate,[2] etc. For the polypeptides having pendant amino or carboxyl groups, it is possible to introduce an organic moiety to the side chain by using the functional group of the peptides as a reaction site.[3]

Poly (γ-benzyl L-glutamate) (PBLG) is relatively hydrophobic and rigid. The hydrophobicity and rigidity of the PBLG films, however, can be controlled by combining them with polydimethylsiloxane (PDMS) or polyether. The block copolymers consisting of PBLG and PDMS or polypropyleneoxide (PPO) have been synthesized, and it has been found that the copolymers with a specific composition were highly antithrombogenic.[4,5] It has also been reported that the number of platelets adhered to the block copolymer films consisting of poly(2-hydroxyethyl methacrylate) and polyoxyethylene (PEG) or PPO was minimum for the copolymer with a specific composition.[6] Poly (N-hydroxyalkyl L-glutamate) hydrogels have been prepared by aminoalcoholysis reaction of PBLG and aminoalcohols.[7] Hayashi et al. proposed that the rate of degradation of the poly(N-hydroxyalkyl L-glutamate) hydrogels in vitro by pronase was highly dependent on the water absorption of the samples.[7] Similar work has been done by Anderson et al.[8] using polypeptide hydrogels consisting of PBLG and poly(N-hydroxyalkyl L-glutamate).

In our previous works[9,10], PEG grafted PBLG (PEG-g-PBLG) and PEG crosslinked PBLG (PEG-c-PBLG) was prepared by incorporating polyethylene glycol to the side chains of PBLG. The swelling of PEG-PBLG film was larger than that of the PBLG film. The swellability of PEG-PBLG film was increased by introducing ethanolamine(EA) to the film surfaces, resulting in a hydrated surface layer.

In this present work, the drug loaded polymer films composed of 5-fluorouracil and PEG-PBLG or PEG-PBLG-EA have been prepared, and their drug release behaviours have been investigated in relation to the water absorption of the polymer films.

EXPERIMENTAL

Materials

Polyoxyethylene bis(6-aminohexyl) (POEA, m. w : 3500), methoxypolyoxyethyleneamine (MPOE, m. w. : 5000) and 5-fluorouracil were purchased from Sigma Chemical Co. L-glutamic acid, benzylalcohol and ethanolamine (Wako Chemical Co.) were used without further purification. Acetic acid and hexane were dried with anhydrous calcium chloride and distilled over calcium hydride. Triethylamine, dimethylformamide (DMF), and tetrahydrofuran (THF) were purified by distillation before use.

Measurements

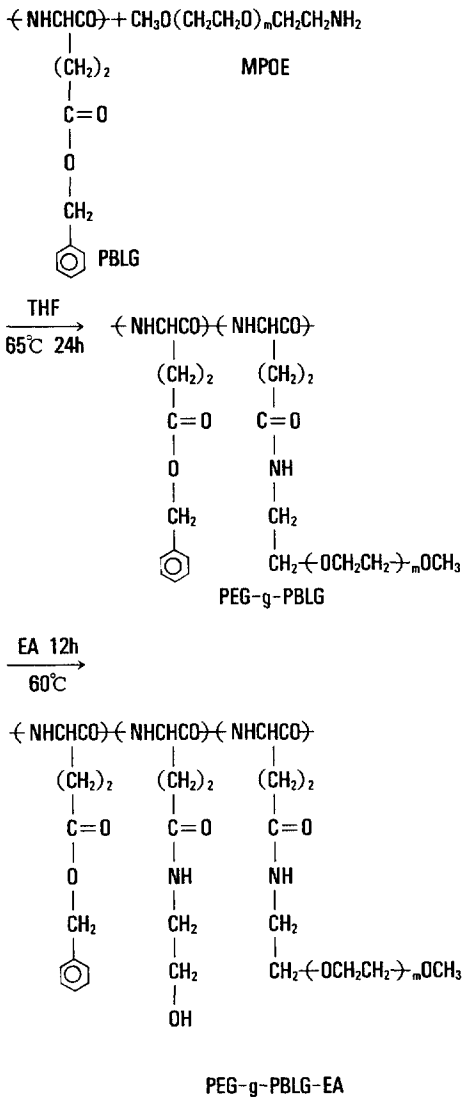
Infrared spectra were taken by a Jasco IR 0080 Spectrophotometer. Ultraviolet spectra were obtained by a Beckman DU 64 Spectrophotometer. Morphology of the film surface was observed with a Hitachi S-510 scanning electron microscope (SEM) after coating with gold using Eiko Model IR-3 Ion coater.

Synthesis of Polypeptide Hydrogels

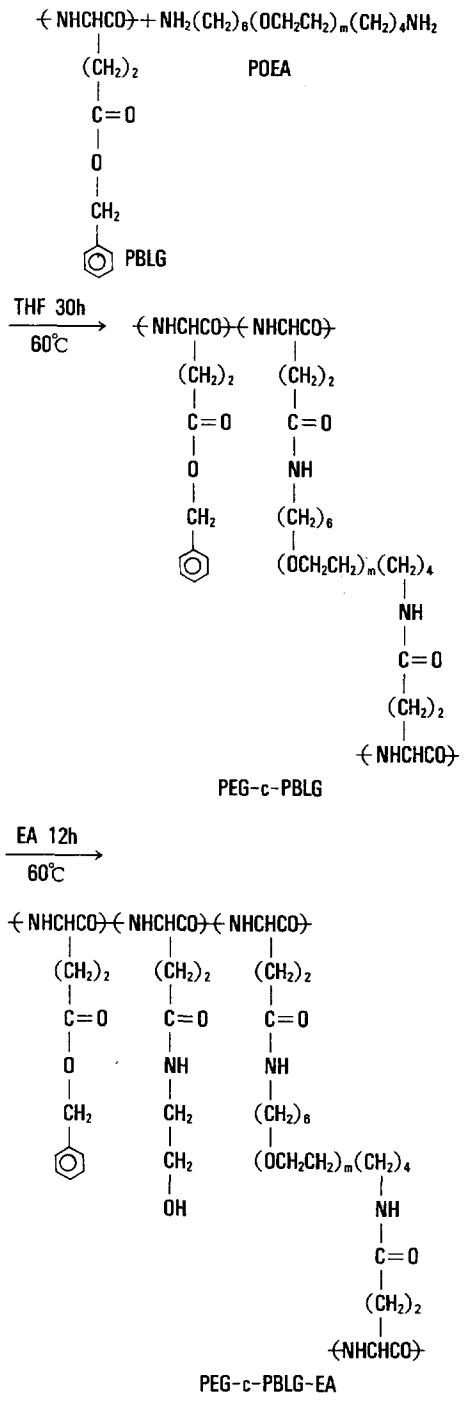
PBLG was synthesized by the polymerization of γ-benzyl L-glutamate (γ BLG)-N-carboxyanhydride (NCA) in dichloromethane.[11] Number average molecular weight of the PBLG was determined to be about 24,000 by vapor pressure osmometry of dimethylformamide solution. PEG-g-PBLG and PEG-c-PBLG were prepared by the substitution reaction of PBLG with PEG having primary amino groups at one and both ends, respectively. The PEG content of copolypeptides was determined by the integrated peak ratio of the methylene proton signal of the PEG segment(ca. 1.3 ppm) against the phenyl proton of the PBLG segment(ca. 7.2 ppm). It was found that about 2.5 % of the PBLG side chains were substituted with polyoxyethylene bis(6-aminohexyl). The details of

the synthetic procedure were reported previously.[9]

For enhancing water absorption of the copolypeptides, the reaction of PEG-g-PBLG or PEG-c-PBLG with ethanolamine(EA) was carried out as shown in Schemes 1 and 2. PEG-g-PBLG or PEG-c-PBLG (2 g) was dissolved in dioxane/chloroform (1 : 1 v/v, 80 ml) solution and refluxed for 12 h at 60℃ after addition of ethanolamine (12 g). The reaction product was concentrated and then



Scheme 1



Scheme 2

precipitated in acetone to remove unreacted ethanolamine. The resulting PEG-PBLG-EA polymer was dissolved in DMF/H_2O mixture. The solution was spread on a Teflon plate and the solvent was evaporated under infrared lamp (80℃) for 8 h. The Teflon plate was immersed in distilled water and the cast film was removed from the plate. Finally the film was dried in vacuum for 24 hrs and examined by IR spectroscopy.[12]

Water Absorption

To determine the water absorption rate of the polymer, the dried polymer film was weighed and then soaked in distilled water until the weight of the swollen film becomes constant. The degree of water absorption of the polymer film was calculated as follows.[13]

$$\text{Water absorption}(\%) = (W - W_0)/W_0 \times 100$$

where W is the weight of the swollen film and W_0 is the weight of the dried film.

Preparation of the Polymer-drug Matrix

PEG-g-PBLG or PEG-c-PBLG (30 mg) was dissolved in dimethylformamide (1 ml) and mixed with 5-fluorouracil (250 μg). The solution was spread on a Teflon plate to obtain the film having an area of 3.5 cm^2 and a thickness of 340~350 μm. On the other hand, the matrix consisting of PEG-PBLG-EA and fluorouracil was prepared in DMF /H_2O (9 : 1, v/v) mixed solution.

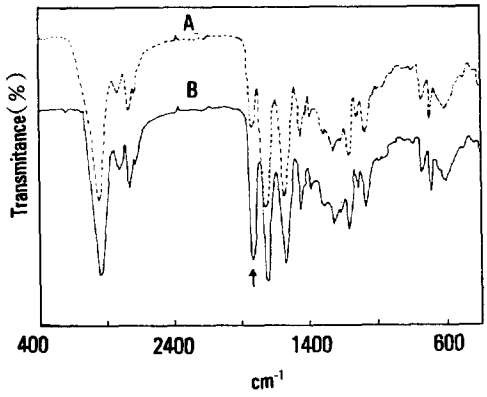


Fig. 1. Infrared spectra of PEG-c-PBLG (——) and PEG-c-PBLG-EA (----) polymers.

In Vitro Drug Release Experiment

Drug release experiments were performed using the apparatus shown in Fig. 2. The drug loaded film was placed in a Teflon holder and incubated in phosphate buffered saline(PBS) at 37℃. The PBS solution was kept with stirring at 150rpm. Every 10~12 hrs, the fresh medium solution was replaced. Periodically PBS solution of 3 ml was taken and filtered through a green filter (pore size : 0.45 μm, Green Cross Medical Co., Korea) and the drug concentration in release medium was determined by measuring its absorbance at 267 nm with UV-Vis spectrophotometer.[14]

RESULTS AND DISCUSSION

Synthesis and Swelling Behaviours of Polypeptide Hydrogels

Synthesis and characterization of PEG-g-PBLG and PEG-c-PBLG have been reported previously.[9,10] In this study, PEG-PBLG copolymers were reacted with ethanolamine to obtain hydrogel polymers. The resulting copolypeptides are not easily soluble in organic solvent, but soluble in mixed solvent such as DMF/H_2O. A progress of the substitution reaction could be monitored by the decrease of ester carbonyl absorption. As shown in Fig. 1, the carbonyl absorption (1720 cm^{-1}) of the PEG-c-PBLG-EA is decreased as the benzyl ester of the PEG-c-PBLG is reacted with ethanolamine. However, the amide II absorption generated by the substitution reaction could not be monitored because of their overlapping with the backbone

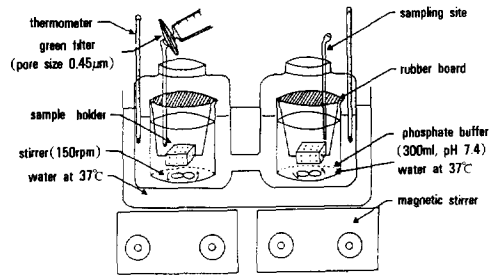


Fig. 2. Apparatus for drug release experiments.

amide absorption (1650 cm^{-1}). The degree of substitution reaction of PEG-PBLG with EA was proportional to the reaction time. When the PEG-g-PBLG reacted with EA over 24 hrs, the resulting copolypeptides became soluble in water. In this study, the reaction time of PEG-PBLG with EA was fixed at 12 hrs. As this reaction time(12 hrs), the degree of substitution reaction of EA with PEG-g-PBLG was higher than that with PEG-c-PBLG. This is confirmed by comparing the decreased ester carbonyl absorption of both polymers.

The water content of the various polypeptides is shown in Table 1. The degree of water absorption of PBLG was very low and was not significantly different from that of PEG-g-PBLG. However, with the increase of PEG content, the degree of water absorption was enhanced(PEG-c-PBLG). This indicates that the degree of water absorption is mainly determined by the hydrophilicity of the polymer.[15] The degree of water absorption of PEG-PBLG-EA(71~170%) was larger than that of PEG-c-PBLG(16%). In particular, PEG-g-PBLG-EA showed a high water content(170%). This may be attributed to that a great number of hydroxyl groups are introduced to the side chain of PEG-g-PBLG, thus leading to a strong interactions of hydroxyl groups with water.[16]

The kinetics of water absorption of PEG-g-PBLG-EA and PEG-c-PBLG-EA is shown in Fig. 3. It was found that the equilibrium swelling is reached within a few hours.

Table 1. Degree of Water Absorption of Various Copolypeptide Films

Sample	BLG Unit (mol%)	PEG Unit (mol%)	Degree of Water Absorption(%)
PBLG	100	0	3± 2
PEG-g-PBLG	96	4	5± 2
PEG-c-PBLG	37	63	16± 3
PEG-g-PBLG-EA	–	4	170± 2
PEG-c-PBLG-EA	–	63	71± 3

*BLG Unit of PEG-g-PBLG-EA and PEG-c-PBLG-EA can not be determined because of their low solubility.

Drug release

To prepare the samples for drug release experiment, the copolypeptides were impregnated with 5-fluorouracil. This allowed the study of the effect of polymer swelling on the diffusion of 5-fluorouracil. Release of 5-fluorouracil from the drug loa-

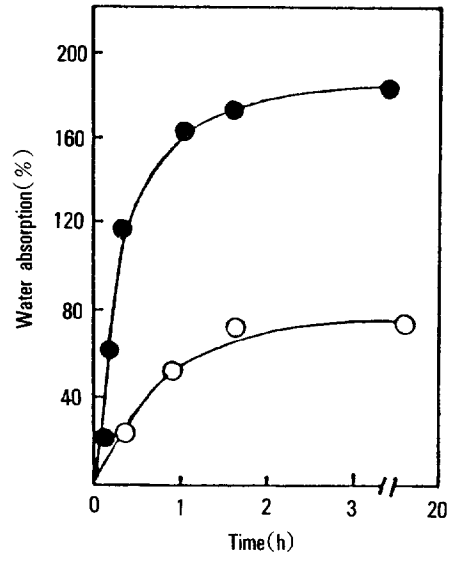


Fig. 3. Dynamic water absorption of hydrophilic copolypeptides as a function of incubation time : (○) PEG-c-PBLG-EA, (●) PEG-g-PBLG-EA.

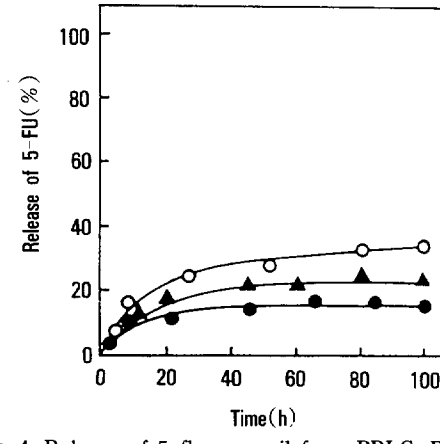


Fig. 4. Release of 5-fluorouracil from PBLG, PEG-g-PBLG and PEG-c-PBLG polymers vs. real time : (●) PBLG, (▲) PEG-g-PBLG, (○) PEG-c-PBLG.

ded polymers is shown in Fig. 4. The drug release was dependent on PEG content of the polymers. For example, a PEG-g-PBLG having 4 mol% of PEG exhibited a slow release with about 25% of the 5-fluorouracil released in 80 hrs, while a PEG-c-PBLG having 63 mol% of PEG showed a fast release with about 35% in the same period.

Fig. 5 shows the release rate of 5-fluorouracil from PEG-g-PBLG-EA and PEG-c-PBLG-EA membranes. Both membranes showed a fast release rate of 5-fluorouracil. This may be attributed to that the membranes are swollen in a short period as shown in Fig. 3. When a polymer is placed in an aqueous medium, the water penetrates the polymer, forming a swollen phase in the wetted region. The formation of the swollen phase is accompanied by reduction in mechanical strength and increment in permeability in the swollen region.[17]

The relationship between the amount of drug released and the water absorption of the polymers is shown in Fig. 6. The result indicates that the release rate of 5-fluorouracil is proportional to the degree of water absorption of the polymers. When a dry hydrophilic polymer is placed in an aqueous medium, it begins to imbibe water and possibly other components of the environment such as low molecular weight solutes. This can lead to considerable swelling of the polymer. It results in the water penetration front into the polymer and separates the glassy from the rubbery state of the polymeric material. Under these conditions, the macromolecular relaxation of the polymer influence the mechanism of diffusion of the drug through the rubbery state.

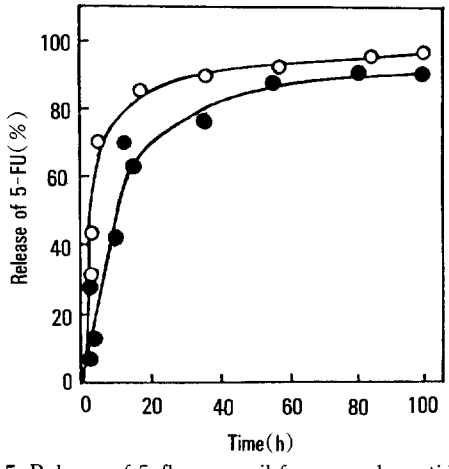


Fig. 5. Release of 5-fluorouracil from copolypeptides containing hydroxyl groups vs. real time : (●) PEG-c-PBLG-EA, (○) PEG-g-PBLG-EA.

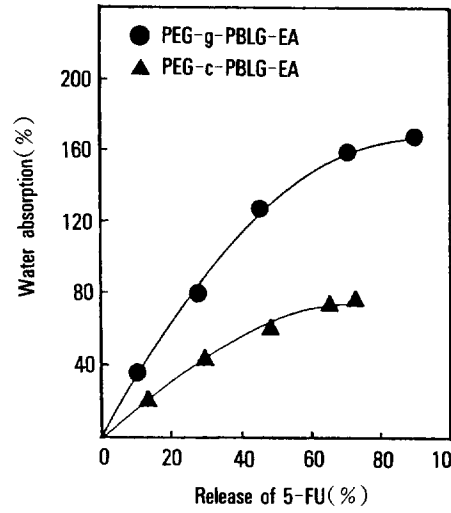


Fig. 6. Dependence of the water absorption on the drug release : (▲) PEG-c-PBLG-EA, (●) PEG-g-PBLG-EA.

If the dry hydrogel contains a water soluble drug, the drug is essentially immobile in a glassy phase, but begins to diffuse out through the amorphous phase as the polymer swells with water. Drug release thus depends on two simultaneous rate processes, water migration into the polymer, which results in increased pore generation and drug diffusion outward through the swollen phase.[18]

In an attempt to observe the surface morphology of the copolypeptide membranes before and after drug release, the PEG-c-PBLG and PEG-c-PBLG-EA membranes were examined by scanning electron microscopy(Fig. 7). Pores could not be observed on the surface of PEG-g-PBLG and PEG-g-PBLG-EA after drug release, but found on the surface of the PEG-c-PBLG and PEG-c-

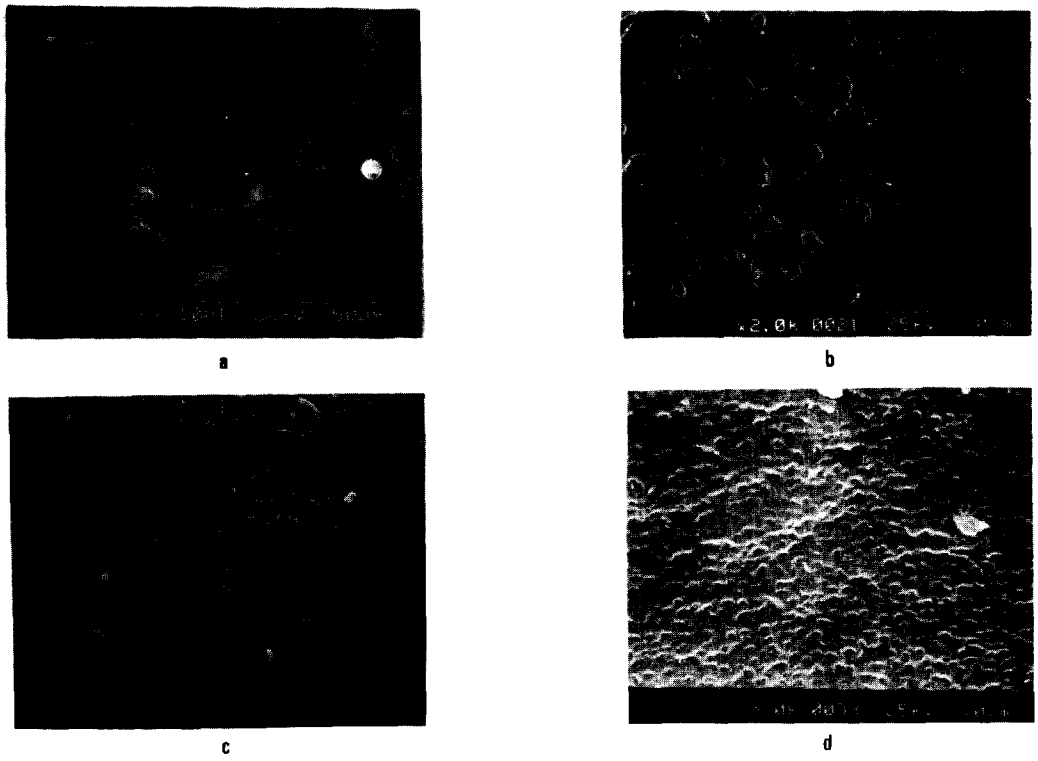


Fig. 7. Scanning electron micrographs of PEG-c-PBLG (a, before release ; b, after release) and PEG-c-PBLG-EA (c, before release ; d, after release) surfaces.

PBLG-EA. The pore size of PEG-c-PBLG (diameter ; 1.5~2.5μm) was larger than that of PEG-c-PBLG-EA (0.8~1.7μm). This seems to be attributed to the difference of water swelling between PEG-c-PBLG (16%) and PEG-c-PBLG-EA (170 %). It is considered that during drug release PEG-c-PBLG-EA film fully swells with water and the pendant hydroxyl groups of the polymers give an intermolecular hydrogen bonding between the peptide amide bonds.

By incorporating polyethylene glycol or hydroxyalkyl to the side chains of poly(γ-benzyl L-glutamate), the degree of water absorption of the polymers is increased. It is found, in the drug release experiments, that the release rate of 5-fluorouracil from the hydrophilic copolypeptides can be controlled by changing the water absorption of the polymers.

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REFERENCES

1. T. Hayashi, Y. Tabata, and A. Nakajima, *Biomaterials, Prepr. Jpn.*, **5**, 23 (1983).
2. K. Kugo, T. Uno, H. Yamano, J. Nishino, and H. Masuda, *Kobunshi Ronbunshu Jpn.*, **11**, 731 (1985).
3. Y. Imanishi, *Adv. Polym. Sci.*, **20**, 1 (1985).
4. I. -K. Kang, Y. Ito, M. Sisido, and Y. Imanishi, *Polym. J.*, **19**, 1329 (1987).
5. I. -K. Kang, Y. Ito, M. Sisido, and Y. Imanishi, *Biomaterials*, **9**, 138 (1988).
6. T. Okano, M. Uruno, N. Sugiyama, M. Shimada, I. Shinohara, K. Kataoka, and Y. Sakurai, *J. Biomed.*

Mater. Res., **20**, 1035 (1986).
7. T. Hayashi, K. Takeshima, E. Kobatake, and A. Nakajima, *Kobunshi Ronbunshu Jpn.*, **11**, 777 (1985).
8. R. Marchant, A. Hilton, C. Hamlin, A. Rabinovitch, R. Slobodkin, and J. M. Anderson, *J. Biomed. Mater. Res.*, **17**, 301 (1983).
9. I. -K. Kang, D. -R. Kwon, C. -S. Cho, and Y. K. Sung, *J. Korean Chem. Soc.*, **34**, 199 (1990).
10. I. -K. Kang, D. -R. Kwon, C. -S. Cho, and Y. K. Sung, *J. KOSOBME*, **10**, 237 (1989).
11. I. -K. Kang, Y. Ito, M. Sisido, and Y. Imanishi, *Int. J. Biol. Macromol.*, **10**, 169 (1988).
12. I. -K. Kang, Y. Ito, M. Sisido, and Y. Imanishi, *Biomaterials*, **9**, 349 (1988).
13. J. F. Rabek, "Experimental Methods in Polymer Chemistry", John Wiley and Sons Ltd, p. 55 (1980).
14. R. W. Korsmeyer and N. A. Peppas, *J. Controlled Release*, **1**, 89 (1984).
15. J. Kost and R. Langer, "Hydrogels in Medicine and Pharmacy", CRC Press, vol. 1, p. 1-25 (1986).
16. N. A. Peppas and A. G. Mikos, "Hydrogels in Medicine and Pharmacy", CRC Press, vol. **3**, p. 95-108 (1987).
17. R. Langer and N. A. Peppas, *Biomaterials*, **2**, 201 (1981).
18. N. A. Peppas, "Release of bioactive agents from swellable polymers : theory and experiments", in Recent Advances in Drug Delivery Systems, J. M. Anderson and S. W. Kim, Eds., Plenum press, New York, p. 279 (1984).