Properties of Biodegradable Hydrogels Prepared by γ Irradiation of Microbial Poly(ε-lysine) Aqueous Solutions

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SYNOPSIS

Poly(ε-lysine) (PL) hydrogels have been prepared by means of γ irradiation of PL produced by *Streptomyces albulus* in aqueous solutions. When the dosage of γ irradiation was 70 kGy or more and the concentration of PL in water was 1–7 wt %, transparent hydrogels (opaque hydrogels for 1–3 wt % PL concentration) could be produced. In the case of 70 kGy of γ irradiation and 5 wt % PL concentration, the specific water content (wt of absorbed water/wt of dry hydrogel) of the PL hydrogel was approximately 160. Specific water contents of PL hydrogels decreased markedly with an increase in the dosage of γ irradiation. The specific water contents were increased with an increase in PL concentration in the irradiated solution. This result indicates the presence of a radical scavenger in the PL solution. Swelling equilibria of PL hydrogels were measured in water or in aqueous solutions of various pHs or concentrations of NaCl, Na_2SO_4, and $CaCl_2$. Under acid conditions, the PL hydrogel swelled due to the ionic repulsion of the protonated amino groups in the PL molecules. The degree of deswelling in electrolyte solution was smaller than that of other ionic hydrogels [poly(γ-glutamic acid), poly(acrylic acid) etc.]. In addition, the enzymatic degradations of PL hydrogel were studied at 40°C and pH 7.0 in an aqueous solution of the neutral protease [*Protease A (Amano)*] produced from *Aspergillus oryzae*. The rate of enzymatic degradation of the respective PL hydrogels was much faster than the rate of simple hydrolytic degradation. The rate of enzymatic degradation decreased with the increase in γ-irradiation dose during preparation of the PL hydrogel. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

Streptomyces albulus, an actinomycete, produces poly(ε-lysine) (PL) outside of the cell.[1-3] PL is water-soluble, biodegradable, and the molecular weight is approximately 4000. PL is an L-lysine homopolymer (25–30 residues) with a linkage between the α-carboxyl group and the ε-amino group (Scheme 1). PL was discovered as a result of screening for a Dragendorff-positive (alkaloid screening method) substance.[1] The production conditions were investigated.[2] The decline of pH during the fermentation process was an essential condition for the accumulation of PL. To enhance the productivity, a two-step cultivation was investigated and 4–5 g/L PL could be produced for 8 or 9 days.[4] PL showed antimicrobial activity against Gram-positive and -negative bacteria at concentrations of 1–8 μg/mL.[5] The activity of the antiphage of PL was also reported.[6]

As a modification of poly(amino acid), hydrogel preparation by γ irradiation was studied.[7,8] It was found that poly(γ-glutamic acid) (PGA), which is another poly(amino acid) produced by *Bacillus* strains,[9-11] converted to a transparent hydrogel with high water sorption ability on γ irradiation. When the dosage of γ irradiation was 20 kGy or more and the concentration of PGA in water was 2–5 wt %, transparent hydrogels could be produced. In the case of a 20 kGy γ-irradiation dose and 5 wt % PGA concentration, the obtained hydrogel was very weak, but it absorbed water equalling about 3500 times its weight. The hydrogel from a microbial PGA and PL mixture solution by γ irradiation was also prepared

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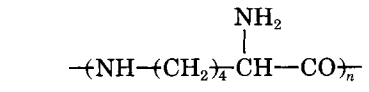

Scheme 1 Poly(ε-lysine) (PL).

to control the swelling properties.[12] The preparation of a hydrogel from a naturally occurring polymer is important because it is an environmental friendly material that is both biodegradable and independent of oil resources.

In this article, we are the first to report to the crosslinking reaction of PL by γ irradiation as a modification of PL. Second, we report the swelling properties of PL hydrogels in various solutions. Finally, the enzymatic degradation processes of PL hydrogels and the effects of γ-irradiation dose on the enzymatic degradation rates are discussed.

MATERIALS AND METHODS

Materials

PL fermented by *Streptomyces albulus* was obtained from Chisso Corp. (Japan). The number-average molecular weight, $\bar{M}_n$, of PL used here was approximately 4000. FTIR (KBr, cm^{-1}) 1529 (m) 1639 (m) 2936 (m) 3331 (m).

ANAL. Calcd for $C_6H_{12}O_1N_2$: C, 56.2%; H, 9.4%; N, 21.9%. Found: C, 53.1%; H, 9.2%; N, 20.2%.

Preparation of PL Hydrogels

PL hydrogels were prepared by γ irradiation (1.6 kGy/h) of PL solutions (1–10 wt %) using an irradiation system with a ^{60}Co (110 TBq) source. The PL solution (2 mL) was contained in a 10-mL glass vial with a cap under nitrogen. The resultant hydrogels were swollen to equilibrium for 1 week. During this time, the uncrosslinked PL was removed by changing the swelling media daily.

Swelling of PL Hydrogel

The weights of the wet PL hydrogels (W_0) were measured after equilibrium in deionized water at 4°C. The PL hydrogels were then lyophilized and the weights of the dry PL hydrogels (W_d) were measured. The gel productivity for added PL could not be determined, because the PL hydrogel attached to the walls of the glass vial. The specific water content and the degree of swelling were calculated from the following expressions:

$$\text{specific water content} = (W_0 - W_d)/W_d \quad (1)$$

$$\text{degree of swelling} = W_s/W_0 \quad (2)$$

where W_s is the weight of the wet PL hydrogel at equilibrium in aqueous solutions of various pHs or NaCl, Na_2SO_4, or $CaCl_2$ concentrations, respectively. Repetitive swelling of the PL hydrogel was measured by swelling at equilibrium in deionized water at 4°C for 1 week and lyophilizing. The specific water content of the PL hydrogel was determined each time. The swelling of PL hydrogels in aqueous solutions of various pHs was measured in 25 m*M* McIlvaine buffer[13] (pH 3–8) and 25 m*M* NaOH/HCl (remaining pH). The swelling reversibility was also determined. The PL hydrogel was first swollen in 1 wt % NaCl solution at 4°C and the degree of swelling was measured each time. The PL hydrogel was then transferred to deionized water, and the degree of swelling was again determined each time.

Enzymatic Degradation of PL Hydrogel

The neutral protease, *Protease A* (Amano Pharmacy Ltd., Japan) produced from *Aspergillus oryzae* was used for enzymatic degradation of the PL hydrogel. The optimum pH and temperature are 7.0 and 50°C, respectively. The enzymatic degradation was carried out at 40°C in a 25 m*M* phosphate buffer (pH 7.0). PL hydrogels (initial wet weights, about 0.5 g) were placed in small bottles containing 20 mL of buffer with *Protease A* (6 mg). The reaction solution was incubated at 40°C with shaking. The total organic carbon (TOC) amounts in the filtered supernatant were periodically measured by a TOC analyzer (Shimadzu TOC 5000). The degradation ratio of the PL hydrogel was calculated from the ratio of the filtered TOC amount to the total amount of PL hydrogel.

RESULTS AND DISCUSSION

Effect of γ-Irradiation Dose on Preparation of PL Hydrogel

PL is a water-soluble and biodegradable polymer produced by a microorganism. Many water-soluble polymer solutions have been known to convert to a hydrogel with high water sorption ability by means of γ irradiation. Thus the preparation of a PL hydrogel by γ irradiation was studied as a modification of PL, indicating that a transparent PL hydrogel could be produced. The irradiation conditions for the PL solution were investigated. Figure 1 shows

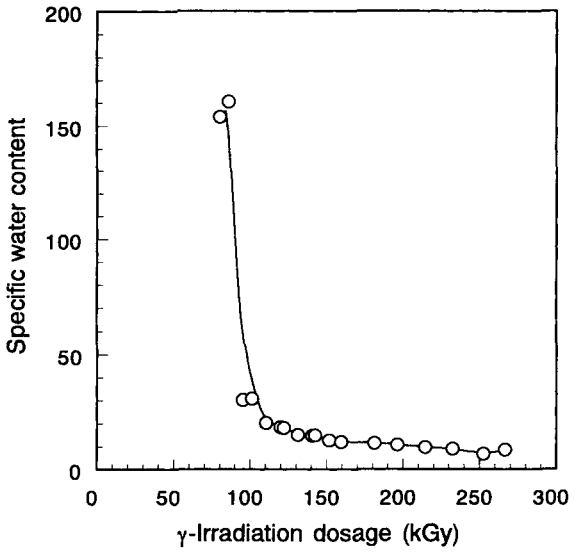

Figure 1 Change in specific water content (wt of absorbed water/wt of dry gel) of poly(ε-lysine) (PL) hydrogels during γ irradiation. PL concentration of irradiated aqueous solution was 5 wt %.

the specific water content (wt of absorbed water/wt of dry hydrogel) of a PL hydrogel prepared by γ irradiation (1.56 kGy/h) of the PL aqueous solution (5 wt %). After the induction period (70 kGy γ irradiation), a transparent hydrogel could be produced. In the case of 75 kGy, the specific water content of this gel was 160. The specific water content of the PL hydrogel decreased markedly with increasing dosage of γ irradiation. This may be due to the increase in the crosslink density of the PL hydrogel. The specific water content was kept almost constant at about 10 at over 100 kGy. In this region, the crosslink density may be saturated.

γ-Ray-induced cleavage of the C—H bonds may generate free radicals at the methyne carbons with the amino groups of PL. The subsequent intermolecular radical combination may lead to crosslinking. The detailed crosslinking mechanism of the PL hydrogel will be investigated in the future.

Effect of PL Concentration on Preparation of PL Hydrogel

The specific water content of the PL hydrogel was investigated at various PL concentrations in γ-irradiated aqueous solutions. Figure 2 shows the specific water content of PL hydrogels (43, 83, 124 kGy) at various PL concentrations. PL hydrogel could not be produced over 3 wt % (43 kGy), 7 wt % (83 kGy), or 8 wt % (124 kGy). Opaque hydrogels could be produced in the case of low PL concentrations (≤3 wt %). In these cases, the hydrogels shrank to a size smaller than the glass vial. PL polymer chains in the aqueous solution may be not close enough to each other for crosslinking of the entire solution. With intramolecular crosslinking, the polymer phase is also concentrated, bringing the aggregated polymer chains close to each other. The polymer aggregates are then crosslinked to produce a crosslinked heterogeneous opaque hydrogel. In addition, the specific water content increased with an increase in PL concentration. In general, the specific water content decreases with increased polymer concentration due to the increasing of the crosslink density. There was an induction period (70 kGy for 5 wt % PL concentration) for PL hydrogel preparation (Fig. 1) during γ irradiation. These results suggest the presence of a radical scavenger in γ-irradiated aqueous solutions. The concentration of the radical scavenger is thought to increase with PL concentration. The presence of a radical scavenger will be investigated and the purification methods for removing this radical scavenger will be developed in the future.

Repetitive Swelling of PL Hydrogel

The repetitive swellings of the PL hydrogels prepared with 90 kGy γ irradiation and 2 and 6 wt % PL concentration were investigated (Fig. 3). The PL hydrogel was first swollen in deionized water at 4°C for 1 week and then lyophilized. The specific water content was measured. The swelling of the PL

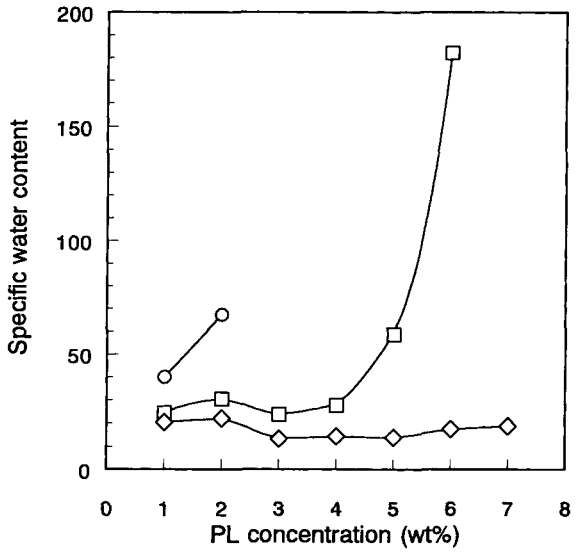

Figure 2 Specific water contents of PL hydrogel with (○) 43, (□) 83, and (◇) 124 kGy γ irradiation as a function of PL concentration.

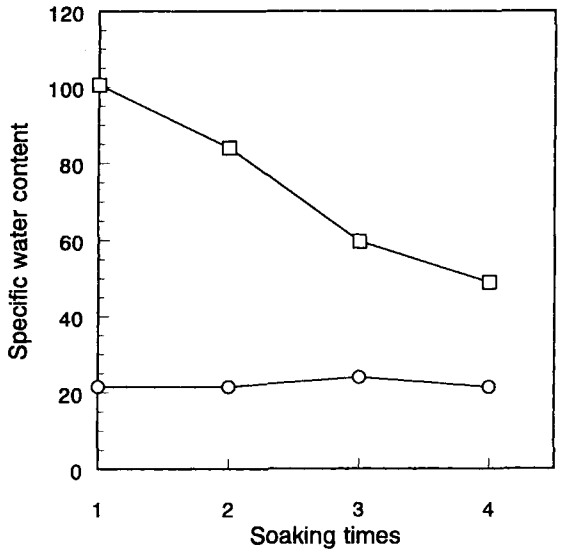

Figure 3 Swelling of PL hydrogel by repetitive soaking with deionized water. PL hydrogels were irradiated with 90 kGy γ irradiation at (○) 2 and (□) 6 wt % PL concentration.

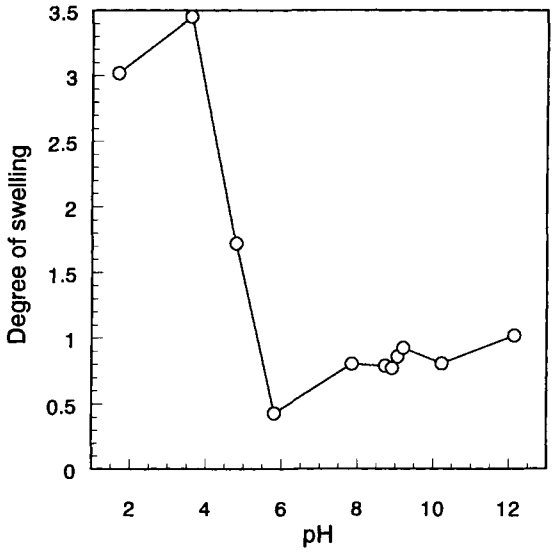

Figure 4 Swelling of PL hydrogels in aqueous solutions of various pHs. PL hydrogels were irradiated with 90 kGy γ irradiation at 5 wt % PL concentration.

hydrogel was repeated. The specific water content was kept almost constant during four repetitive swellings in the case of 2 wt % PL concentration. However, the specific water content decreased during repetitive swelling in the case of 6 wt %. It was found that PL hydrogel with a low capability for water sorption could be used repetitively and that PL hydrogel with a high capability for water sorption could not be used repetitively.

Effect of pH on PL Hydrogel Swelling

The influence of the various pHs or the presence of salts in the swelling medium of a hydrogel is of importance in agricultural and biomedical applications such as diapers, water reservoirs in agriculture, and hydrogels as implants for drug release applications. Swelling equilibria of PL hydrogels prepared with 90 kGy γ irradiation and 5 wt % PL concentration were measured in aqueous solutions of various pHs. Figure 4 shows the degree of swelling of PL hydrogels in aqueous solutions of various pHs. It can be seen that the degree of swelling of PL hydrogel was strongly dependent on pH. The PL hydrogel swelled in the low pH region (< 4.0). Because of their positive charge, the amino groups ($-NH_3^+$) are incorporated into the polymer network; the gel swells in the low pH region due to the ionic repulsion of the protonated amino groups and collapses at high pH values because of unprotonated amino groups as schematically depicted in Figure 5. The degree of swelling was decreased gradually with the decrease in pH from 12 to 6. This may be due to the increase in protonated amino groups. The net charge of the PL hydrogel increased gradually, thereby increasing the osmotic pressure, and the PL hydrogel deswells. Thus, it was found that the PL hydrogel was a pH-

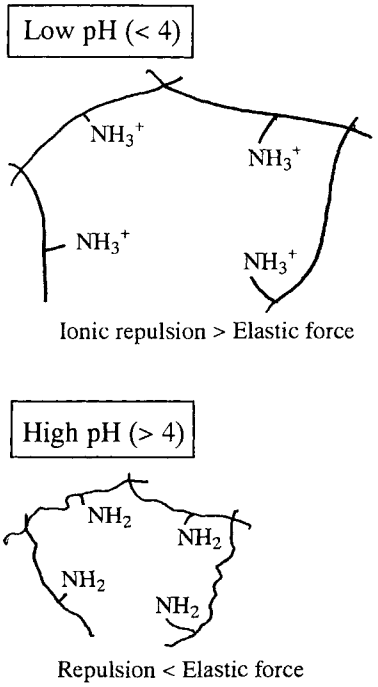

Figure 5 Hypothetical sketch of swelling–deswelling molecular structure of PL hydrogel in aqueous solutions in low pH region (< 4.0) and high pH region (> 4.0).

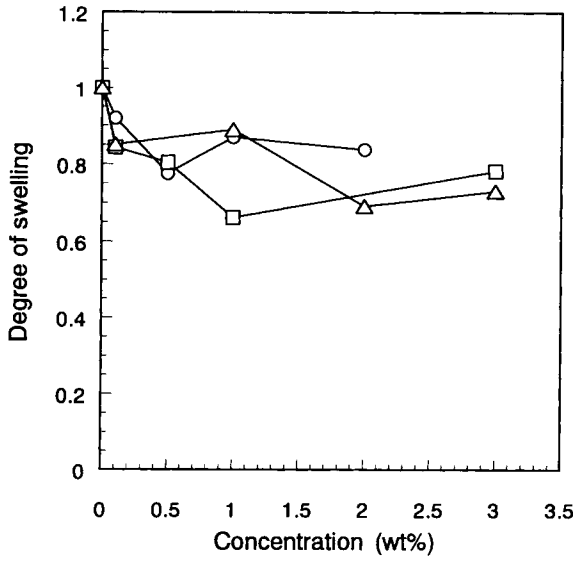

Figure 6 Swelling of PL hydrogels in aqueous solutions of various (○) NaCl, (□) Na_2SO_4, and (△) $CaCl_2$ concentrations. PL hydrogels were prepared with 90 kGy γ irradiation at 5 wt % PL concentration.

sensitive hydrogel and is expected to be used as an artificial muscle or a drug carrier in response to an input signal.

Effect of NaCl, Na_2SO_4, or $CaCl_2$ Concentration on PL Hydrogel Swelling

Figure 6 shows the degree of swelling of a PL hydrogel (90 kGy, 5 wt % PL concentration) in aqueous solutions of various NaCl, Na_2SO_4, or $CaCl_2$ concentrations. The degree of swelling decreased gradually with an increase in the NaCl, Na_2SO_4, or $CaCl_2$ concentrations. It was found that the ratio of decrease in a PL hydrogel in solute solutions is smaller than that of a general ionic hydrogel. In the case of a PGA hydrogel, the degree of swelling decreased markedly, 0.07 at 0.1 wt % $CaCl_2$ solution.[7] The PL hydrogel does not deswell as does a PGA hydrogel in electrolyte solutions because the amino group is unprotonated in deionized water (neutral pH region) and the PL hydrogel is scarcely influenced by electrolytes.

Reversibility of Swelling–Deswelling of PL Hydrogel

Reversible swelling–deswelling behavior of a hydrogel upon exposure to external solution is desirable for repetitive usage as an agricultural water reservoir and a drug carrier in response to an input signal. The reversibility of swelling–deswelling of a PL hydrogel (90 kGy, 5 wt % PL concentration) was investigated (Fig. 7). The PL hydrogel was first swollen in aqueous solution. The degree of swelling in the case of 1 wt % NaCl was 0.43 at 26 h. This hydrogel was then swollen in deionized water. The degree of swelling in deionized water was 4.14 at 28 h. This hydrogel swelled to four times its initial weight. In the case of the $CaCl_2$ solution, the swelling–deswelling behavior was the same as that in the NaCl solution. It was found that the swelling–deswelling of the PL hydrogel in the aqueous solution was not reversible. The degree of swelling (4.14) in deionized water from 1 wt % NaCl solution was similar to that at pH 3.5 (Fig. 4). It appeared that Na^+ or Cl^- was absorbed by the unprotonated amino groups in the PL hydrogel and the PL hydrogel swelled in deionized water due to the ionic repulsion of the absorbed Na^+ or Cl^- ion. The possibility of degradation is also present in the swelling of a PL hydrogel. The mechanism of irreversible swelling–deswelling must be clarified.

Enzymatic Degradation of PL Hydrogel

A PL hydrogel from a naturally occurring polymer may be used in an environment or a human body. In these cases, the biodegradability of the gel is an important property. It was known that uncrosslinked PL can be degraded by a neutral protease, *Protease A* (*Amano*), produced from a microorganism (*Aspergillus oryzae*). Therefore, biodegradation of the PL hydrogel by this enzyme was investigated.

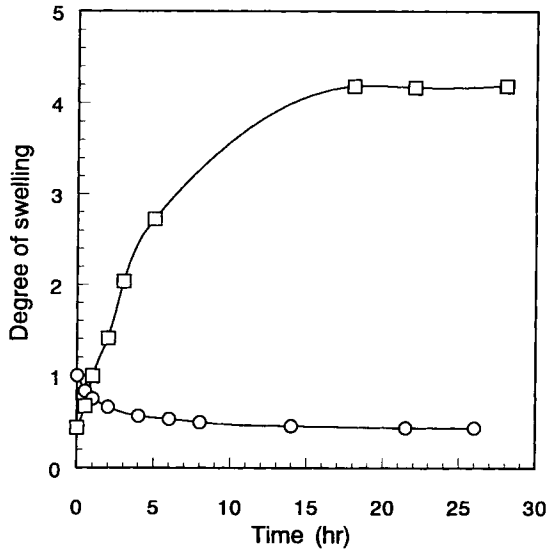

Figure 7 (○) Degree of swelling changes in swollen gels in 1 wt % NaCl solution, and (□) degree of swelling of deswollen gels in deionized water as a function of time.

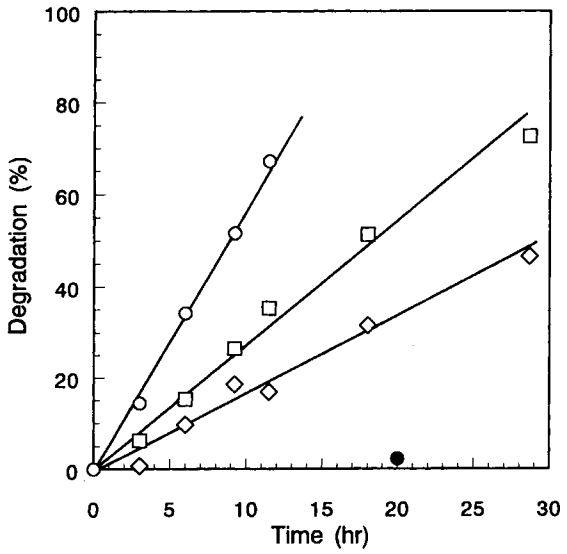

Figure 8 Enzymatic degradation profiles on PL hydrogels in the aqueous solution (20 mL) of *Protease A* (6 mg) at 40°C and pH 7.0. PL hydrogels were prepared with (○) 101, (□) 147, and (◇) 203 kGy γ irradiation at 5 wt % PL concentration. (●) PL hydrogel prepared with 101 kGy immersed in the aqueous solution without enzyme at 40°C and pH 7.0 for 20 h.

Figure 8 shows the enzymatic degradation profiles of PL hydrogels (101, 147, 203 kGy and 5 wt % PL concentration) as a function of degradation time. The degradation reaction of the PL hydrogel (ca. 0.5 g, wet) was carried out at 40°C and 3 mg/10 mL enzyme concentration in 20 mL phosphate buffer. The degradation ratio using 25 mg/10 mL enzyme concentration was almost the same as that using the 3 mg/10 mL enzyme. The degradation ratio was calculated from the TOC amount in filtered supernatant of the reaction mixture (gel, enzyme, and buffer). The PL hydrogel was not degraded in 20 h without enzymes (Fig. 8) at 40°C. The rate of PL hydrogel degradation by *Protease A* is strongly dependent upon the γ-irradiation dose during the crosslinking reaction and decreases with an increase in the γ-irradiation dose. This may be due to the increase in the crosslink density of the PL hydrogel. A PL hydrogel with a higher crosslink density needs more chain scission at the amide bonds by the protease for degradation. The increase in crosslink density can be presumed because the specific water content decreases with the increase in the γ-irradiation dose (21.9 for 101 kGy, 11.5 for 147 kGy, and 6.9 for 203 kGy). Thus, the PL hydrogel is biodegradable and the biodegradation rate of the PL hydrogel can be controlled by the γ-irradiation dose during the crosslinking reaction.

The PL hydrogel obtained in this study will be beneficial for use in the environment due to its biodegradability.

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