

Improvement of heat stability of poly(L-lactic acid) by radiation-induced crosslinking

Hiroshi Mitomo^{a,*}, Ayako Kaneda^a, Tran Minh Quynh^a, Naotsugu Nagasawa^b, Fumio Yoshii^b

^aDepartment of Biological and Chemical Engineering, Faculty of Engineering, Gunma University, Tenjin-cho 1-5-1, Kiryu, Gunma 376-8515, Japan

^bTakasaki Radiation Chemistry Research Establishment, Japan Atomic Energy Research Institute, Watanuki-machi, Takasaki, Gunma 370-1292, Japan

Received 19 October 2004; received in revised form 24 March 2005; accepted 28 March 2005

Available online 25 April 2005

Abstract

Poly(L-lactic acid) (PLLA) has poor heat stability above its glass transition temperature ($T_g \sim 60^\circ\text{C}$). To improve its softening above T_g , PLLA was mixed with small amount of crosslinking agents and irradiated with various irradiation doses to introduce crosslinking between polymer chains. The most effective agent for radiation crosslinking was triallyl isocyanurate (TAIC). For melt-quenched PLLA, it was found that the most optimal conditions to introduce crosslinking were around 3% of TAIC and the irradiation dose of 50 kGy. The typically crosslinked PLLA showed very low crystallinity because of wide formation of molecular chain network that inhibited molecular motion for crystallization. Notable heat stability above T_g was given by annealing of PLLA samples. Enzymatic degradation of PLLA was retarded with introduction of crosslinks.

© 2005 Elsevier Ltd. All rights reserved.

Keywords: Poly(L-lactic acid); Radiation-induced crosslinking; Thermomechanical analysis

1. Introduction

Poly(L-lactic acid) (PLLA) is a typical biodegradable plastic obtained by synthesis of lactic acid (or lactide), which produced by fermentation of corn starch [1,2]. Therefore, PLLA is a typical sustainable biodegradable thermoplastic that can be chemically synthesized from renewable resources, which is expected to be a suitable replacement of many nonbiodegradable engineering plastics in near future. PLLA is a polymer having high melting point $\sim 175^\circ\text{C}$, and high crystallizability [3]. It is transparent or translucent which is very suitable to bottles containing fresh drink or cosmetics, etc. However, it has some limits, e.g. poor gas barrier and brittle properties if crystallized completely, higher production cost and so on. It usually crystallized with very slow rate, so it almost remained amorphous after injection-molding. This makes a significant softening of PLLA above the glass transition temperature (T_g) [4].

There are many approaches to give heat stability of biodegradable plastics. Stereocomplex formation between PLLA and poly(D-lactic acid) (PDLA) resulted in extraordinary high T_m ($\sim 230^\circ\text{C}$) [5–7], annealing or crystallization with some nucleating agents [8,9] and formation nanocomposite by blending with some clays [10] also promoted heat stability of PLLA samples.

To improve heat stability, there are powerful methods to introduce crosslinking between polymer molecules by chemical and irradiation treatments. There were very few studies reported chemical crosslinking of PLLA [11]. While the radiation sterilization was usually applied to PLLA, the radiation-induced crosslinking of PLLA was widely investigated. PLLA showed a high sensitivity to γ -rays and electron beams [8,11–14]. There are recent reports concerning improvement of heat stability by introducing (γ -rays and electron beams) radiation-induced crosslinks into some biodegradable polymers [15–17]. Jin et al. reported that PLLA plate (3 mm thick) mixed with 3% of triallyl isocyanurate (TAIC) and irradiated at 10 kGy or mixed with 5% of TAIC and irradiated at 5 kGy gained 100% of gel fraction [18].

In this study, treatment conditions for the most effective introduction of crosslinking were investigated in detail, the

* Corresponding author. Tel.: +81 277 30 1494; fax: +81 277 30 1401.
E-mail address: mitomo@bce.gunma-u.ac.jp (H. Mitomo).

most optimum concentrations of crosslinking agents, irradiation doses and so on. The thermomechanical properties of the treated PLLA were also investigated to estimate thermal stability at the temperature ranges above T_g and even above melting point (T_m). PLLA was not usually degraded by enzymes but hydrolyzed in water or more preferentially in alkaline solution [19]. There are very few bacteria, which can degrade PLLA [20,21]. However, the proteinase K was used to degrade PLLA because of resemblance to protein molecules [22]. Therefore, enzymatic degradation was carried out using proteinase K in this study.

2. Experimental

2.1. Materials

Poly(L-lactic acid) (trade name: LACEA H-100J), with number-average (M_n) and weight-average molecular weights (M_w) were 9.9×10^4 and 20.8×10^4 , respectively, was kindly contributed from Mitsui Chemicals, Inc. Polydispersity (M_w/M_n) was 2.10. The melting point (T_m) and glass transition temperature (T_g) of PLLA were 159.2 and 59.3 °C, respectively. The specific rotation $[\alpha]_D^{20}$ of PLLA was -148.5° . Triallyl isocyanurate (TAIC) and trimethylallyl isocyanurate (TMAIC) were purchased from Nihon Chemicals Co. and other agents were purchased from Sigma-Aldrich Co. The toxicity rank of these agents is around 4–5 (innocuous: 0, the most toxicant: 10). TAIC, TMPTA and some others have been industrially used to improve the properties of polyolefins, rubbers, etc.

2.2. Preparation of samples and irradiations

Crosslinking agents were mixed with PLLA in a Labo Plastomill (Toyoseiki Co.) at 20 rpm, 180 °C for 10 min. Then the sample was hot-pressed at 180 °C for 3 min followed by cold-press (cooled with inner tubes filled with running water) at room temperature to form the film with thickness of 0.1 mm. For comparison, PLLA without any crosslinking agent was treated with the same procedure.

The film sample was heat-sealed in polyethylene bag in vacuum then irradiated by electron beams (dose energy: 2 MeV, dose rate: 10 kGy pass⁻¹) at the room temperature with various irradiation doses.

2.3. Gel fraction measurement

Gel fraction was measured by the weight remaining after dissolved the sample in chloroform using the following equation,

$$\text{Gel fraction (\%)} = \left(\frac{W_g}{W_0} \right) \times 100 \quad (1)$$

where W_0 is weight (dry) of the crosslinked PLLA, W_g is the weight remaining (dry gel component) of the crosslinked PLLA after dissolved in chloroform at room temperature for 48 h.

Degree of swelling (volume ratio of absorbed solvent to dry gel sample) is expressed using the following equation,

$$\text{Degree of swelling (} q \text{)} = \left[\frac{(W_s - W_g)}{W_g} \right] \left(\frac{\rho_p}{\rho_{\text{CHCl}_3}} \right) \quad (2)$$

where W_g is the weight of dry gel component in the crosslinked PLLA sample, W_s is the weight of gel component swollen at room temperature for 48 h in chloroform. ρ_p and ρ_{CHCl_3} are densities of PLLA and chloroform, (i.e. $\rho_p/\rho_{\text{CHCl}_3} = 0.838$), respectively.

2.4. Enzymatic degradation

Enzymatic degradation of the crosslinked PLLA film was carried out in 5 ml of 0.1 mol-Tris/HCl buffer (pH 8.6) containing 1.0 mg of proteinase K and 1.0 mg of sodium azide [22]. Proteinase K (activity: 26 units mg⁻¹) was purchased from Wako Pure Chemical Industries, Ltd. About 14 mg of the film (10 × 10 mm², 0.1 mm) was immersed into the solution at 37 °C. At least three sheets of films were used for every one data point. After incubation, the film was taken out and washed with distilled water, methanol and freeze-dried to a constant weight in vacuum. Biodegradation was evaluated by measuring weight loss values (mg) per unit area (cm²) of the film sheets. For comparison, blank test was carried out using the same buffer solution without enzyme.

2.5. Analytical measurement

A thermomechanical analyzer (Shimadzu TMA-50) was used for evaluation of heat stability. A film 5 mm × 2 mm × 0.1 mm (thickness) in size was fixed to the sample holder under a constant load of 0.5 g (i.e. initial stress: 24.5 kPa), then heated up to 200 °C from 25 °C with a heating rate of 10 °C min⁻¹ in a nitrogen atmosphere. The elongation of film was recorded against the temperature.

Melting point (T_m), glass transition temperature (T_g) and enthalpy of melting (ΔH_m) of each crosslinked sample (ca. 3 mg) were measured using a differential scanning calorimeter (Shimadzu DSC-50) in a nitrogen flow with a heating rate of 10 °C min⁻¹. Degree of crystallinity was calculated using the following equation:

$$\text{Degree of crystallinity (\%)} = \left[\frac{(\Delta H_m + \Delta H_c)}{\Delta H_f} \right] \times 100 \quad (3)$$

where ΔH_m and ΔH_c are enthalpies of melting and crystallization, respectively. ΔH_f is the heat of fusion of PLLA crystal (93 J g⁻¹).

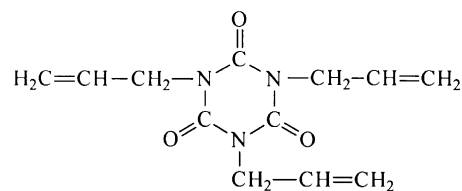
Dynamic viscoelastic properties were measured using a dynamic mechanical analyzer DMS 6100 C (Seiko

Instruments Inc.). The sample dimensions were 20 mm × 10 mm × 0.1 mm. The film was measured at a constant frequency of 0.5 Hz, temperature range of 0–200 °C and a heating rate of 2 °C min⁻¹ in nitrogen flow.

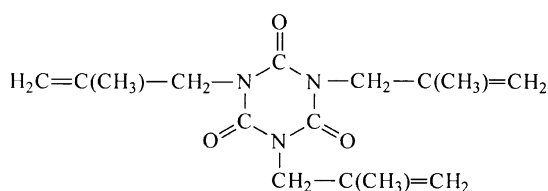
3. Result and discussion

3.1. Selection of the most effective crosslinking agent

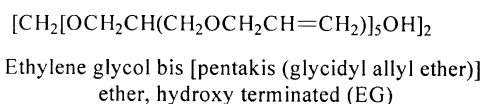
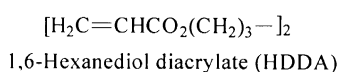
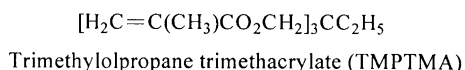
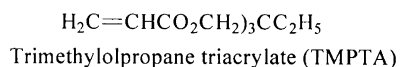
Scheme 1 lists structural formulae of prominent crosslinking agents. These agents all have multifunctional groups larger than diallyl and diacrylate, which can react with polymer chains to form crosslinks. Fig. 1 shows DSC heating curves of the crosslinked PLLA. The original PLLA (a) shows a glass transition temperature ($T_g \sim 60$ °C), then a broad exothermic peak of cold crystallization around 120 °C, followed by a melting peak ($T_m \sim 160$ °C), which is similar to the peaks of (c), (e) and (g). Curve (b) shows only a small peak around T_g , without any peak of cold crystallization and melting. This means that PLLA was tightly crosslinked when PLLA was mixed with 3% of TAIC and irradiated at 50 kGy. PLLA molecules were mostly fixed by crosslinks in amorphous state after melt-quenched, so that no crystallization or melting were permitted. Curves (d) and (f) are intermediate of the



Triallyl isocyanurate (TAIC)



Trimethylallyl isocyanurate (TMAIC)



Scheme 1. Structural formulae of multifunctional crosslinking agents.

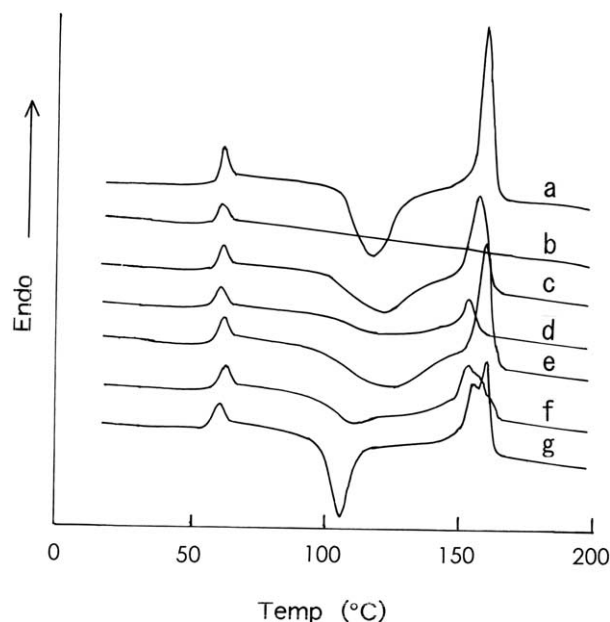


Fig. 1. DSC heating curves of PLLA mixed with 3% of various crosslinking agents and irradiated at 50 kGy (a) irradiated without any agents, (b) TAIC, (c) TMAIC, (d) TMPTA, (e) TMPTMA, (f) HDDA, (g) EG.

original (a) and the crosslinked samples (b), implying partial gel-formation of PLLA.

Table 1 lists values of gel fraction and degree of swelling of PLLA mixed with 3 wt% of 6 kind of crosslinking agents. Untreated PLLA showed no gelation even irradiation dose up to 100 kGy of γ -rays or electron beams [8,14]. Once PLLA was mixed with TAIC, 43% of gel fraction obtained at 10 kGy and increased to 85% at 50 kGy and 88% at 100 kGy. Degree of swelling of PLLA blended with TAIC and irradiated at 50 kGy was only 6, reflecting less dissolving but swelling into chloroform. The second effective crosslinking agent was TMPTA, which showed 72–78% of gel fraction at 50–100 kGy. The third one was HDDA, and only 28% of gel fraction was obtained at 50 kGy. Degree of swelling of PLLA blended with TMPTA and HDDA followed by irradiation at 50 kGy were 30 and 52, respectively, which were much looser than that crosslinked with TAIC ($q \sim 6$). Other crosslinking agents were ineffective showing almost no gelation even at the dose of 100 kGy.

Fig. 2 shows thermomechanical analysis (TMA) heating curves, i.e. elongation of PLLA film under the constant load or initial stress (24.5 kPa) against temperature. The original PLLA film (a) began to elongate at 50 °C and elongated significantly above 90 °C, reflecting the extreme softening of sample. If mixed with 3% of TAIC and irradiated at 50 kGy (b), the film began to elongate at ca. 50 °C and continued to elongate up to the higher temperatures. This film showed no significant softening above 90 °C and could withstand the stress even at the temperatures higher than 160 °C. Curves (c)–(g) shows intermediate variation between curves (a) and (b). They began to elongate at ca.

Table 1

Gel fraction and degree of swelling of PLLA mixed with 3% of crosslinking agents and irradiated with various doses

Crosslinking agent	Gel fraction					Degree of swelling (q)		
	Irradiation dose (kGy)					Irradiation dose (kGy)		
	0	10	20	50	100	0	20	50
None	0	0	0	0	0	— ^a	—	—
TAIC	0	43	73	85	88	—	8.4	6.4
TMAIC	0	0.5	0	0	0	—	—	—
TMPTA	0	35	48	78	72	—	48	30
TMPTMA	0	0.3	0.7	0	0	—	—	—
HDDA	0	11	16	28	25	—	87	52
EG	0	0.3	0.2	0.2	0.1	—	—	—

^a Dissolved completely.

50 °C, then elongated largely at ca. 100 °C and stopped to elongate up to 150 °C. Above 150 °C, they began to soften abruptly and elongate then broke at ca. 160 °C because of the sample melting. The films mixed with 3% of TMPTA and HDDA (curves (d) and (f)) showed the smallest elongations around 100 °C. Curves (c) and (g) were intermediate elongation and that mixed with TMPTMA (curve (e)) showed the largest elongation around 100 °C.

3.2. Optimum concentration of TAIC and irradiation dose

It was found that TAIC is the best crosslinking agent to give gel formation. Therefore, it needs to find the most optimum condition, i.e. concentration and irradiation dose to introduce crosslinks. Fig. 3 shows DSC thermograms of PLLA mixed with various concentrations of TAIC and irradiated at 50 kGy. Curves (b) and (c) are similar to curve (a) of original PLLA, except the melting peak shifts to the lower temperature as the concentration of TAIC increased. However, curve (d) (3% of TAIC) shows a typical thermogram of almost non-crystalline PLLA, scarcely

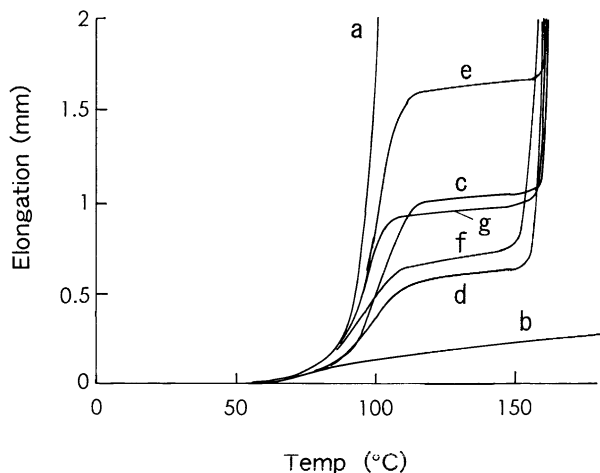


Fig. 2. TMA heating curves of PLLA mixed with 3% of various crosslinking agents and irradiated at 50 kGy (a) irradiated without any agents, (b) TAIC, (c) TMAIC, (d) TMPTA, (e) TMPTMA, (f) HDDA, (g) EG.

accompanied with crystallization and melting peaks, suggesting that molecular chains were almost crosslinked in the amorphous state. If PLLA is mixed with 5% of TAIC and irradiated 50 kGy, gel fraction of PLLA begins to decrease, reflecting the insufficient gel formation. Therefore, at the irradiation dose of 50 kGy, it can be said that the most optimal concentration of TAIC is around 3%. DSC curves of γ -ray crosslinked PLLA and chemically-crosslinked PLLA samples reported were very similar to Fig. 3 [11,18].

Table 2 shows gel fraction and degree of swelling of PLLA mixed with various concentration of TAIC and irradiated with various doses. Fig. 4 shows gel fraction and reciprocal degree of swelling ($1/q$) against the irradiation dose. PLLA/(1% TAIC) began to form gel at irradiation dose above 10 kGy. Gel fraction and $1/q$ of PLLA/(1% TAIC) increased at 20 kGy then leveled off $\sim 65\%$ at 50 kGy. Gel fraction and $1/q$ of PLLA mixed with 2 or 3% of TAIC increased steeply with increasing the content of TAIC and the irradiation dose up to 20 kGy. Above 20 kGy, both values became gradually levelled off. However, gel fraction and $1/q$ of PLLA/(5% TAIC) were slightly lower

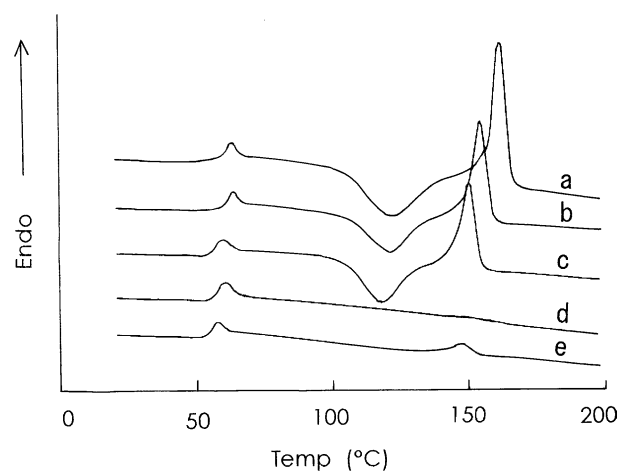


Fig. 3. DSC heating curves of PLLA mixed with various concentration of TAIC and irradiated at 50 kGy (a) 0%, (b) 1%, (c) 2%, (d) 3%, (e) 5%.

Table 2
Gel fraction and degree of swelling of PLLA mixed with various concentration of TAIC and irradiated with various doses

Conc. of TAIC (%)	Gel fraction (%)				Degree of swelling (q)			
	Irradiation dose (kGy)				Irradiation dose (kGy)			
	0	10	20	50	0	10	20	50
0	0	0	0	0	–	– ^a	–	–
0.5	0	0	0	0	–	–	–	–
1	0	10	38	64	–	163	59	18
2	0	23	57	75	–	101	57	16
3	0	43	73	84	–	47	7	6
5	0	31	67	83	–	58	9	8

^a Dissolved completely.

than 3%, implying insufficient dose for higher TAIC content.

Crosslinking of PLLA/TAIC by γ -rays irradiation was also reported by Jin et al. [18]. Their results were essentially similar to this study, although the gelation conditions were slightly different. They reported that PLLA/(3% TAIC) irradiated at 10 kGy or PLLA/(5% TAIC) irradiated at 5 kGy resulted in 100% of gel fraction. The differences might be caused from PLLA sample difference, radiation sources of γ -rays and EB, dispersion of TAIC during the blending, etc.

Fig. 5 shows TMA thermograms of PLLA film mixed with various concentration of TAIC and irradiated at

50 kGy. The melt-quenched PLLA film began to elongate at 50 °C and elongated significantly above 90 °C (a). PLLA/(1% TAIC) film started to elongate at 50 °C, however it elongated gradually above 90 °C (b), followed by rapid elongation at 160 °C. PLLA film mixed with 2% TAIC showed heat resistance till 200 °C, though it elongated with the steepest slope above 50 °C except for curves (a) and (b). PLLA film mixed with 3% TAIC showed the best heat resistance (d), and that mixed with 5% TAIC began to softening again (e).

3.3. Radiation-induced crosslinking of annealed PLLA/TAIC

PLLA film obtained from melt-quenching shows almost amorphous, and very slow crystallization rate even at temperatures around T_g (60 °C) [23]. If melt-quenched PLLA film mixed with 3% TAIC is irradiated at 50 kGy, typically-crosslinked PLLA is obtained, however, the sample softened above T_g . The crosslinking was introduced in the amorphous state of PLLA sample, in other words, fixing the whole sample almost amorphous. Heat stability above the temperatures of T_g was obtained for the PLLA

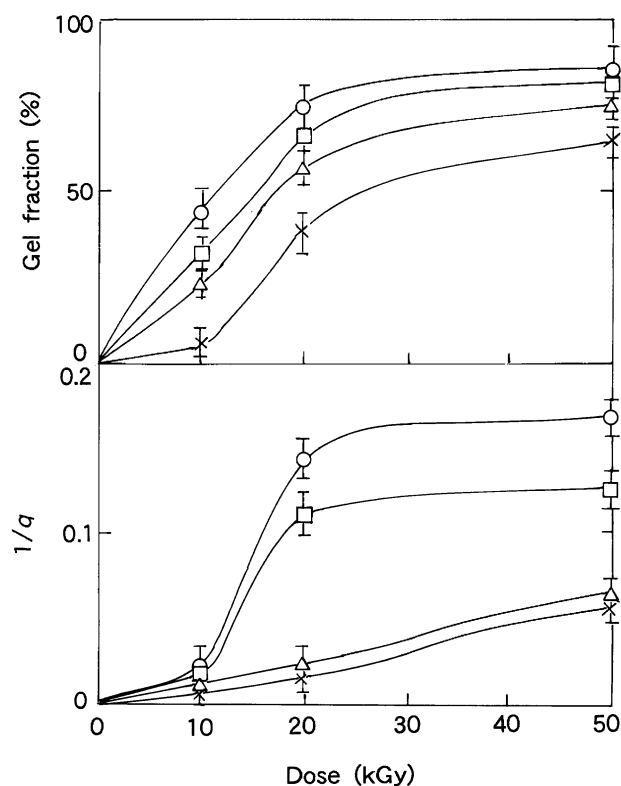


Fig. 4. Gel fraction and reciprocal of degree of swelling ($1/q$) of PLLA film mixed with various concentrations of TAIC and irradiated at 50 kGy (x) 1 wt%, (Δ) 2%, (\circ) 3%, (\square) 5%.

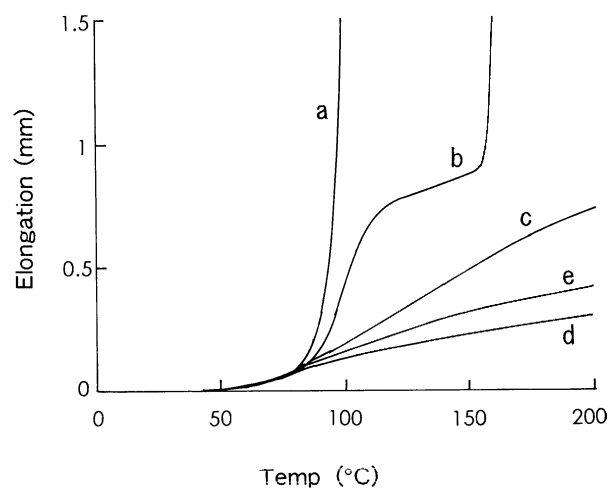


Fig. 5. TMA heating curves of PLLA film mixed with various concentration of TAIC and irradiated at 50 kGy (a) 0%, (b) 1%, (c) 2%, (d) 3%, (e) 5%.

sample annealed and fully crystallized as reported already [8].

Fig. 6 shows DSC heating curves of PLLA samples annealed at 90 °C for 1 h and irradiated at 50 kGy. Melt-quenched sample (a) showed a crystallization peak at around 110 °C and a melting peak at around 160 °C. However, annealed PLLA (b) and annealed and irradiated PLLA (c) showed no crystallization peak and showed larger melting peaks at 145–150 °C, corresponding to melting of the crystals formed during annealing at 90 °C.

Table 3 showed gel fraction and degree of swelling of PLLA film mixed with various concentration of TAIC and annealed at 90 °C for 1 h followed by irradiation at various irradiation doses. Fig. 7 shows gel fraction and 1/q of PLLA samples annealed and irradiated. Gel fraction and 1/q increased as the TAIC concentration increased from 1 to 5%.

Table 4 showed gel fraction and degree of swelling (q) of PLLA mixed with 3% of TAIC and annealed at 90 °C for various periods of time followed by irradiation at various doses. Gel fraction and 1/q decreased when the annealing time increased as inferred from Fig. 7, suggesting that TAIC was squeezed out from crystalline regions with increasing crystallinity. It is clear that crosslinking of TAIC was mainly introduced into amorphous regions.

Fig. 8 shows TMA heating curves of PLLA mixed with various concentration of TAIC and annealed at 90 °C for 1 h followed by irradiation at 50 kGy. Original PLLA (curve (a)) became slightly soft around 50 °C and extremely softened at ca. 90 °C. While all annealed PLLA samples with or without TAIC showed slight softening at ca. 50 °C and continued to elongate to a small extent up to the melting point. The elongations of all annealed PLLA samples just

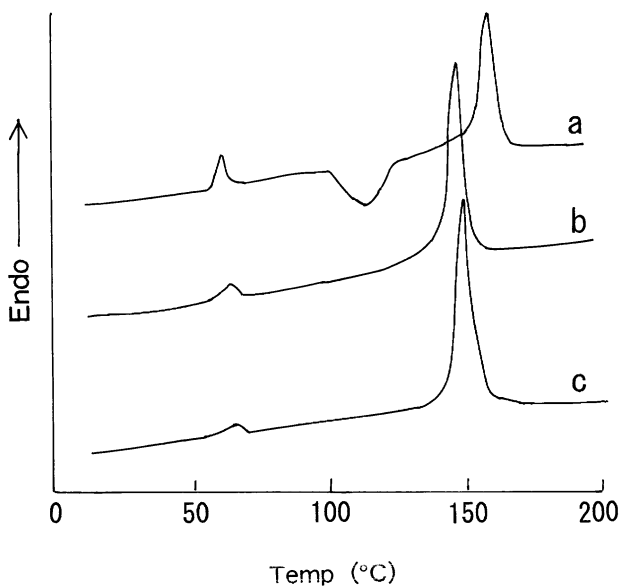


Fig. 6. DSC heating curves of melt-quenched PLLA film (a) and PLLA films mixed with 3% TAIC and annealed at 90 °C for 1 h (b) and 4 h (c) followed by irradiation 50 kGy.

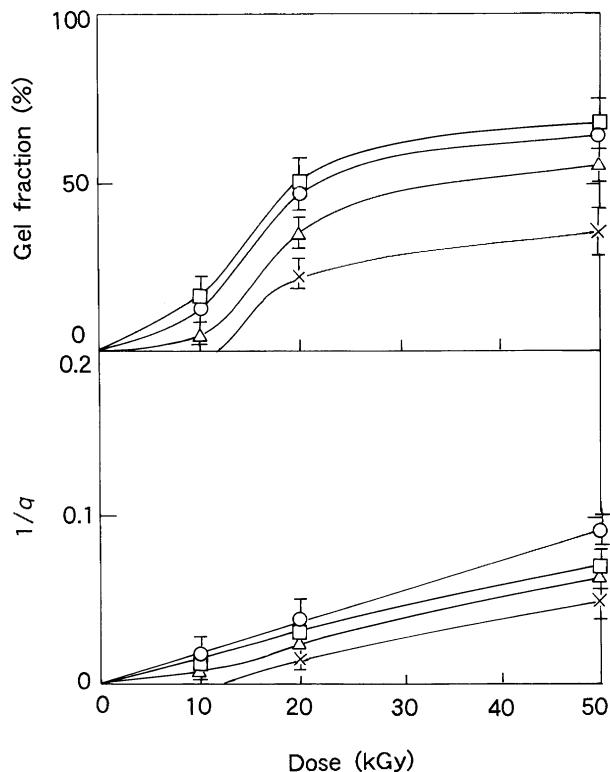


Fig. 7. Gel fraction and 1/q of PLLA film mixed with TAIC and annealed at 90 °C for 1 h followed by irradiation at various doses. Conc. of TAIC, (x) 1%, (Δ) 2%, (\circ) 3%, (\square) 5%.

below the melting points were less than 3%, while those of unannealed and crosslinked PLLA were above 5% (Fig. 5). High crystallinity by annealing resulted in hardening of PLLA at temperatures between T_g and T_m . For TAIC concentration above 2% (curves (d)–(f)), all samples sustain the load up to ca. 200 °C, though they showed partial heat stability above the melting point and the smaller elongation for the sample of higher gel fraction. Steep elongation at the melting point reflected the melting of crystalline regions and

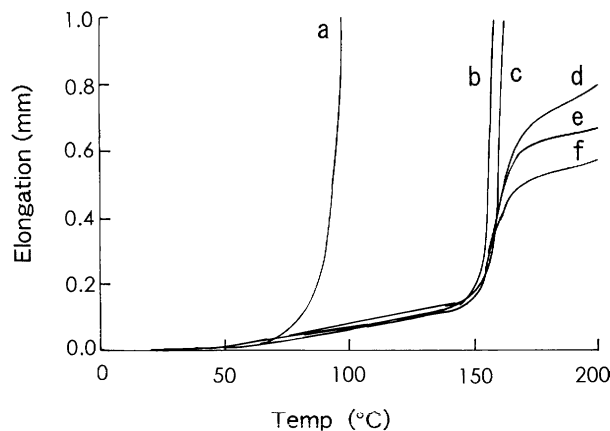


Fig. 8. TMA heating curves of PLLA mixed with various concentrations of TAIC and annealed at 90 °C for 1 h and irradiated at 50 kGy (a) PLLA film (melt-quenched), (b) mixed with 0% of TAIC and annealed and irradiated, (c) 1% of TAIC, (d) 2%, (e) 3%, (f) 5%.

Table 3
Gel fraction and degree of swelling of PLLA mixed with 3% of TAIC and annealed at 90 °C for 1 h

Conc. of TAIC (%)	Gel fraction (%)				Degree of swelling (<i>q</i>)			
	Irradiation dose (kGy)				Irradiation dose (kGy)			
	0	10	20	50	0	10	20	50
0	0	0	0	0	– ^b	–	–	–
1	0	0	23	25	–	–	61	20
2	0	10	35	55	–	102	49	16
3	0	25	46	64	–	52	26	11
5	0	29	50	68	–	85	34	15
3 ^a	0	43	73	85	–	49	8.4	6

^a Unannealed.

^b Dissolved completely.

then gel networks in the amorphous regions sustained the load up to 200 °C.

Table 5 listed thermal properties of PLLA samples mixed with 3% TAIC and annealed at 90 °C for various periods of time followed by irradiation at 50 kGy. Crystallinity of PLLA increased largely by annealing 90 °C for 1 h and slightly increasing for longer annealing time. T_g and T_m of PLLA mixed with TAIC and annealed at 90 °C followed by irradiation increased slightly as the annealing time was lasted [24].

Fig. 9 shows dynamic mechanical properties, i.e. storage modulus (E') and $\tan \delta$ of PLLA, PLLA/(3% TAIC) irradiated and annealed followed by irradiated at 50 kGy against the temperature. Melt-quenched PLLA films showed steep drop of E' at ca. 60 °C due to softening of the glass transition temperature of PLLA. The film of PLLA/(3% TAIC) irradiated at 50 kGy showed steep drop of E' and a sharp peak of $\tan \delta$ at around 60 °C, then rose up again with the temperatures up to ca. 130 °C because of cold crystallization of the sample. Similar rising curve of E' was observed in the other literature [18]. From DSC study in Fig. 3(d), the crosslinked PLLA/(3% TAIC) sample hardly crystallized above T_g , however, it showed recrystallization above 80 °C. This might be explained that the film sample in DMS measurement was recrystallized in some extents under the heavy load and high temperatures. Then the film became extremely soft above 130 °C, E' and $\tan \delta$ of the irradiated film decreased steeply as the temperature increased. The

film became rubbery and soft, however it never broke even far above T_m . Once PLLA/(3% TAIC) was annealed at 90 °C for 1 h, then irradiated at 50 kGy, the sample showed gentler drop in E' and broader peak of $\tan \delta$ at ca. 70 °C. The film of PLLA/(3% TAIC) annealed and irradiated did not show any rise in E' caused by cold crystallization above T_g , because the crystallites formed by annealing and radiation-induced crosslinks prevented the molecular motion or rearrangement. The film of PLLA/(3% TAIC) annealed and irradiated showed typical heat stability up to T_m , however, the sample became too soft to support the initial tension of DMS above T_m .

Jin et al. reported the similar dynamic mechanical analysis data, where E' of original PLLA or PLLA/(1% TAIC) did not break after the steep drop at around T_g , but increased again up to ca. 120 °C then decreased slightly followed by steep drop above 160 °C [18]. Crosslinked PLLA/(3% TAIC) showed the steep drop at T_g , followed by slight increase up to ca. 120 °C then decreased with a gentle curve up to 200 °C. The difference might be caused by the crystallinity of the original PLLA, i.e. our sample was obtained by melt-quenching and that of Jin et al. was molded to a 3 mm thick plate, which might be crystallized to some extent.

Fig. 10 shows enzymatic degradation curves of PLLA treated variously. The enzyme used was proteinase K. Weight loss of melt-quenched PLLA treated for 24 h was ca. 1.5 mg cm⁻² and that for 48 h was ca. 2.8 mg cm⁻².

Table 4
Gel fraction and degree of swelling of PLLA mixed with 3% of TAIC and annealed at 90 °C for various periods of time followed by irradiation at various doses

Annealing time (h)	Gel fraction (%)				Degree of swelling (<i>q</i>)			
	Irradiation dose (kGy)				Irradiation dose (kGy)			
	0	10	20	50	0	10	20	50
0	0	42	69	87	– ^a	47	4	5
1	0	27	46	64	–	51	24	8
2	0	25	45	54	–	47	26	14
4	0	24	42	56	–	58	27	15
24	0	20	40	46	–	71	34	19

^a Dissolved completely.

Table 5
Thermal properties of PLLA mixed with 3% of TAIC and annealed at 90 °C for various periods of time and irradiated at 50 kGy

Time (h)	T_g (°C)	T_m (°C)	ΔH_m (J/g)	Crystallinity (%)
0	57.6	148.7	1.8	1.7
1	62.8	150.3	37.7	40.6
2	63.3	151.8	42.3	45.5
4	63.8	152.1	43.1	46.4
24	64.7	154.4	44.3	47.6

Weight loss of PLLA blended with 3% TAIC was almost same to that of melt-quenched one. Once PLLA/(3%TAIC) irradiated at 50 kGy, weight loss was suddenly decreased, i.e. that treated for 48 h was ca. 0.6 mg cm^{-2} . Weight loss of PLLA/(3%TAIC) annealed at 90 °C for 1 h was ca. 0.5 mg cm^{-2} . Weight loss of PLLA/(3%TAIC) annealed at 90 °C for 1 h followed by irradiation at 50 kGy were lowest, i.e. that treated for 48 h was ca. 0.2 mg cm^{-2} . For comparison, blank test (buffer without enzyme) was also shown in the figure, indicated that any weight loss was observed for degradation time of 48 h. Crosslinking between molecular chains retarded degradation significantly, because the enzyme could hardly enter into the polymer gels. Higher crystallinity by annealing resulted in lower weight loss, implying that the enzyme could hardly enter into crystalline regions but easily enter into the amorphous regions [25].

3.4. Heat stability and chemical resistance of crosslinked or annealed PLLA/TAIC samples

The melt-quenched PLLA film showed very poor heat stability above T_g . As reported in the preceding paper,

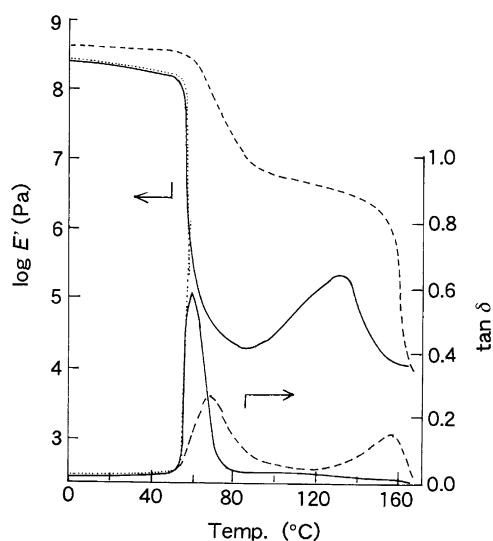


Fig. 9. Storage modulus (E') and $\tan \delta$ of PLLA or PLLA/TAIC films variously treated vs. temperature obtained by DMS. (---): PLLA (melt-quenched); (—): PLLA/3%TAIC irradiated at 50 kGy; (-·-·): PLLA/3%TAIC annealed at 90 °C for 1 h and irradiated at 50 kGy.

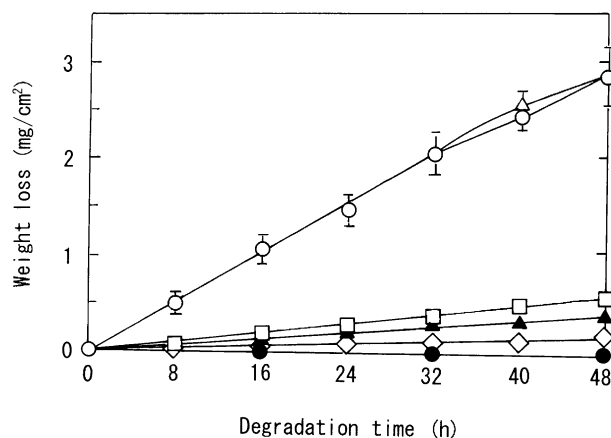
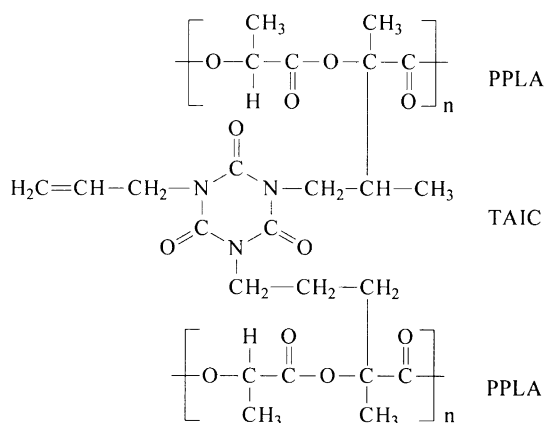


Fig. 10. Enzymatic degradation curves of PLLA and PLLA mixed with 3% of TAIC (○) melt-quenched PLLA film, (△) PLLA blended with TAIC, (□) PLLA/TAIC irradiated at 50 kGy, (▲) PLLA annealed at 90 °C for 1 h, (◇) PLLA/TAIC annealed at 90 °C for 1 h and irradiated at 50 kGy, (●) blank test.

PLLA film annealed at 90 °C and crystallized completely showed typical heat stability above temperatures of T_g [9]. The annealed PLLA film showed very low elongation at temperatures between T_g and T_m . However, once the sample was melted or dissolved by a solvent, it showed no heat stability because it became amorphous. On the contrary, PLLA film crosslinked with TAIC became soft and elongated to some extent, but it still showed durable heat stability even above T_m . Moreover, the crosslinked PLLA showed strong chemical stability to any solvents or resistance to recrystallization. Therefore, if TAIC crosslinks can be introduced into annealed PLLA film, the crosslinked PLLA film shows high heat stability at temperatures from T_g to T_m . Above T_m , it became typical rubbery and elongated to some extents. The sample became amorphous again unless the sample was annealed and crystallized.

Once PLLA sample was irradiated by electron beams, H abstraction occurred predominantly from $-\text{CH}<$ groups in PLLA chains (i.e. $-\dot{\text{C}}<$) as reported in the preceding paper [8,14]. At the same time, if TAIC molecules coexisted with PLLA, the double bonds of allyl groups in TAIC were broken to form a pair of radicals $-\dot{\text{C}}<$ and $-\dot{\text{C}}<$. One recombined with abstracted $\dot{\text{H}}$, the other combined with $-\dot{\text{C}}<$ groups. Consequently, two (or three) allyl groups of TAIC combined with $-\dot{\text{C}}<$ groups in PLLA chains, and crosslinks between PLLA molecules were performed as shown in Scheme 2. Two (or three) allyl groups combined with two (or three) PLLA molecules. Two kinds of combinations were illustrated, i.e. $-\dot{\text{C}}<$ group in TAIC recombined with abstracted $\dot{\text{H}}$ resulting in $-\text{CH}-\text{CH}_3$ and the remnant radical combined with $-\dot{\text{C}}<$ group in PLLA (upper, usual case) and the other case, resulting in $-\text{CH}_2-\dot{\text{C}}<$ and the remnant radical combined with PLLA (bottom, relatively rare case). These two cases usually occurred at the radiation-induced crosslinking, though there are very a few possibility of other combination styles considerable.



Scheme 2. One possibility of radiation-induced crosslinking of TAIC between two PLLA molecules (the third allyl group was not activated).

4. Conclusion

PLLA film obtained by melt-quenching was transparent but low crystallinity, therefore, it became extremely soft above glass transition temperature. PLLA mixed with 3% of TAIC and irradiated at 50 kGy showed 84% of gel fraction, however, it showed softening above glass transition temperature even if introduction of crosslinking. PLLA showed typical heat stability until its melting point if it was annealed and crystallized completely. Once PLLA/(3% TAIC) sample was annealed at 90 °C for 1 h and irradiated at 50 kGy, it showed typical heat stability above glass transition temperature. It also held the shape above the melting point even though it elongated to some extents due to the partial crosslinking. Other advantages in properties of PLLA by introducing crosslinks or gel formation were improvement of undissolving to any solvents or preventing further crystallization and so on.

Enzymatic degradation rate of PLLA sample decreased significantly by introduction of crosslinking or crystallization at around 90 °C, because the enzyme could hardly attack into polymer gels or crystallites.

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific

Research (C) No. 15655078 (2001) from the Ministry of Education, Culture, Sports, Science and Technology (Japan).

References

- [1] Nijenhuis AJ, Grijsma DW, Pennings AJ. *Macromolecules* 1992;25:6419.
- [2] Ozeki E. *Kagaku-to-Kougyo* (Japanese) 1996;49:33.
- [3] Garlotta D. *J Polym Environ* 2002;9:63.
- [4] Celli A, Scandola M. *Polymer* 1992;33:2699.
- [5] Tsuji H, Hyon SH, Ikada Y. *Macromolecules* 1991;24:5651.
- [6] Li S, Vert M. *Macromolecules* 1994;27:3107.
- [7] Cartier L, Okihara T, Ikada Y, Tsuji H, Puiggali J, Lotz B. *Polymer* 2000;41:8909.
- [8] Nugroho P, Mitomo H, Yoshii F, Kume T. *Polym Degrad Stab* 2001;72:337.
- [9] Urayama H, Kanamori T, Fukushima K, Kimura Y. *Polymer* 2003;44:5635.
- [10] Trimaille T, Pichot C, Elaissari A, Fessi H, Briancon S, Delair T. *Colloid Polym Sci* 2003;281:1184.
- [11] Nijenhuis AJ, Grijsma DW, Pennings AJ. *Polymer* 1996;37:2783.
- [12] Babanalbandi A, Hill DST, Whittaker AK. *Polym Degrad Stab* 1997;72:203.
- [13] Burdick JA, Frankel D, Dernal S, Anseth KS. *Biomaterials* 2003;24:1613.
- [14] Babanalbandi A, Hill DJT, O'Donnell JH, Pomery PJ, Whittaker AK. *Polym Degrad Stab* 1995;50:297.
- [15] Suhartini M, Mitomo H, Nagasawa N, Yoshii F, Kume T. *J Appl Polym Sci* 2003;88:2238.
- [16] Nugroho P, Mitomo H, Yoshii F, Kume T, Nishimura K. *Macromol Mater Eng* 2001;286:316.
- [17] Mitomo H, Sasada K, Nishimura K, Nagasawa N, Yoshii F. *J Polym Environ* 2004;12:95.
- [18] Jin F, Hyon SH, Iwata H, Tsutsumi S. *Macromol Rapid Commun* 2002;23:909.
- [19] Cam D, Hyon SH, Ikada Y. *Biomaterials* 1995;16:833.
- [20] Jarerat A, Tokiwa Y, Tanaka H. *J Polym Environ* 2004;12:139.
- [21] Pranamuda H, Tokiwa Y, Tanaka Y. *Appl Environ Microbiol* 1997;63:1637.
- [22] Reeve MS, McCarthy SP, Downey M, Gross RA. *Macromolecules* 1994;27:825.
- [23] Nugroho P. Doctoral Thesis. Degradation and crosslinking of biodegradable synthetic polymers to improve processability and heat resistance by irradiation. Gunma University; 2001.
- [24] Tsuji H, Ikada Y. *Polymer* 1995;36:2709.
- [25] Abe H, Doi Y. *Biol Macromol* 1999;25:185.