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Microbial Synthesis and Physical Properties of Ultra-High-Molecular-Weight Poly[(*R*)-3-Hydroxybutyrate]

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MICROBIAL SYNTHESIS AND PHYSICAL PROPERTIES OF ULTRA-HIGH-MOLECULAR-WEIGHT POLY[(*R*)-3-HYDROXYBUTYRATE]

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ABSTRACT

Ultra-high-molecular-weight poly[(*R*)-3-hydroxybutyrate](P(3HB)) was biosynthesized from glucose by a recombinant *Escherichia coli* XL-1 Blue (pSYL105) harboring *Alcaligenes eutrophus* PHB biosynthesis *phbCAB* genes. Six kinds of P(3HB) samples with different weight-average molecular weight (M_w) from 1.1×10^6 to 11×10^6 measured by multi-angle laser light scattering were respectively produced at pH values of 7.0 to 6.5 in culture media. Solvent-cast P(3HB) films of high-molecular-weights over M_w of 3.3×10^6 were stretched easily and reproducibly at 160°C to a draw ratio of 400–650%. Mechanical properties of the stretched P(3HB) films were markedly improved relative to those of solvent-cast film. The elongation to break, Young's modulus, and tensile strength of stretched film ($M_w = 11 \times 10^6$) were 58%, 1.1 GPa, and 62 MPa, respectively. X-ray diffraction patterns indicated that the stretched film was highly oriented and had a high crystallinity over 80%. When the stretched film was annealed at 160°C for 2 hours, the mechanical properties were further improved (elongation

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to break = 67%, Young's modulus = 1.8 GPa and tensile strength = 77 MPa). The mechanical properties of the stretched-annealed film remained almost unchanged for 6 months at room temperature, suggesting that a high crystallinity of the stretched-annealed film avoids a progress of secondary crystallization.

INTRODUCTION

Poly[(*R*)-3-hydroxybutyrate] (P(3HB)) is accumulated by a wide variety of microorganisms as intracellular carbon and energy storage material [1], and is extensively studied as a biodegradable and biocompatible thermoplastic [2-4]. However, it is well known that mechanical properties of P(3HB) homopolymer films markedly deteriorate to stiffness and brittleness by a process of secondary crystallization [5, 6]. The elongation to break, Young's modulus, and tensile strength of P(3HB) films were reported to be 7%, 3.5 GPa, and 40 MPa, respectively [4]. Accordingly, microbial P(3HB) has been regarded as a polymer required to copolymerize with other monomer components from the viewpoint of industrial applications because of its stiffness and brittleness [4, 7, 8].

Recently, some studies have been attempted to improve the mechanical properties of P(3HB) film. Holmes reported uniaxial and biaxial oriented films of P(3HB) to improve the mechanical properties [4]. However, the P(3HB) films were relatively difficult to draw uniaxially and biaxially. De Koning *et al.* reported that the stiffness and brittleness of P(3HB) film ($M_w = 5.4 \times 10^5$) was avoided by an annealing procedure at elevated temperatures (30-80°C below melting point), and that the annealed film had elongation to break values from 9 to 34% [9]. An alternative method of improving the ductility of brittle P(3HB) films is a cold-rolling procedure, which was reported by Barham and Keller [10]. When a cold-rolling ratio was applied over 1.3, the elongation to break and tensile strength of the P(3HB) films ($M_w = 2.1-2.9 \times 10^5$) were improved up to the values of 35-130% and 42-100 MPa, respectively. However, the authors did not report about the reproducibility of cold-rolling process.

Recently, we have succeeded in producing ultra-high-molecular-weight P(3HB) ($M_w = 5-20 \times 10^6$) under specific fermentation conditions [11] by using a recombinant *Escherichia coli* XL-I Blue (pSYL105) [12] harboring *Alcaligenes eutrophus* PHB biosynthesis *phaCAB* genes. The films of ultra-high-molecular-weight P(3HB) are expected to have improved mechanical properties by drawing procedure, as it has been demonstrated for polylactide [13] and polyethylene [14, 15].

In the present paper, we report the physical properties of films of ultra-high-molecular-weight P(3HB) produced from glucose by the recombinant *E. coli* XL-1 Blue (pSYL105).

EXPERIMENTAL

Biosynthesis of High-Molecular-Weight P(3HB)

High-molecular-weight P(3HB) samples ($M_w = 1.1-11 \times 10^6$) were produced by *E. coli* XL-1 Blue (pSYL105) [12] containing a stable plasmid harboring the *Alcaligenes eutrophus* H16 (ATCC 17699) PHB biosynthesis gene operon *phaCAB*. Two-step cultivation of the recombinant *E. coli* was applied for the production of high-molecular-weight P(3HB) [11]. Luria-Bertani (LB) medium containing 10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl was used for growth of recombinant cells in the first step cultivation. The cells were grown for 20 hours at 37°C under aerobic conditions in a 500 mL Sakaguchi flask with 75 mL LB medium. These grown cells (about 100 mg) were transferred to LB medium of 1.4 L containing 20 g/L glucose, and the cells were aerobically cultivated at 37°C and pH 6.5-7.0 for 10-20 hours in a 2.6 L jar fermenter equipped with six conventional turbine impellers and three baffles. Solutions of 2 N NaOH and 2 N H₂SO₄ were automatically added for controlling the pH of culture medium. The cells were collected by centrifugation, washed with distilled water, and lyophilized. P(3HB) was extracted from the lyophilized cells by stirring with chloroform over 24 hours at room temperature, and then cells were removed by filtration. P(3HB) was purified by reprecipitation in *n*-hexane and dried *in vacuo*.

Film Preparation

High-molecular-weight P(3HB) (0.5 g) was swelled in 50 mL chloroform for 24 hours, and then dissolved in a pressure-resistant glass tube at 100°C for 30 minutes. Then, the films of high-molecular-weight P(3HB) were prepared by a conventional solvent-casting technique from chloroform solutions using glass petri dishes as casting surface. Solution-cast films were oriented by stretching almost 650% of their initial length by loosely hung weights in a silicone oil bath at 160°C. The stretched films were further annealed at 160°C for 2 hours to increase their crystallinity. All samples were used after aging for at least 7 days at room temperature. The contents of silicone in the stretched films were measured by elemental analysis and found to be as low as 0.19 wt%.

Analytical Procedures

Molecular weight distribution data of P(3HB) samples were obtained by gel-permeation chromatography (GPC) at 40°C, using a Shimadzu 10A GPC system and 6A refractive-index detector with joint columns of Shodex K-802 and K-806M. Chloroform was used as an eluent at a flow rate of 0.8 mL/min, and a sample concentration of 0.25 mg/mL was applied. Eight polystyrene standard samples with low polydispersity ($M_w = 1.3 \times 10^3$ - 3.1×10^6) were used to construct a calibration curve.

Weight-average molecular weights (M_w) were measured statically with a Wyatt Technology DAWN DSP multiangle laser light scattering (MALLS) photometer at 25°C. This instrument, equipped with a He-Ne laser, operates at a wavelength of 633 nm. 2,2,2-trifluoroethanol filtered through a 0.02 μ membrane filter was used as the solvent. Samples of P(3HB) solutions were filtered through a 0.5 μ membrane filter. Refractive index increment with respect to the change of high-molecular-weight P(3HB) concentration (dn/dc) at 633 nm was determined to be 0.134 by means of a Wyatt Technology Optilab DSP interferometric refractometer. Molecular weight analysis from light scattering data, plots of (Kc/R_θ) versus $\sin^2(\theta/2) + kc$ (Zimm plot), were performed with Wyatt Technology DAWN software, where K is an optical constant, c is the polymer concentration, R_θ is the excess Rayleigh ratio of the solvent and k is a stretch factor which scales the contributions from $\sin^2(\theta/2)$.

X-ray diffraction patterns were recorded in a flat-plate camera on Fuji New RX films, using Ni-filtered Cu-K α radiation with a Rigaku D-9C type X-ray generator operated at 40 kV and 30 mA. Crystallinity of films was calculated from X-ray diffracted intensity data in the 2θ range 6-40° recorded on a Rigaku RAV-1VB system according to Vonk's method [16].

Stress-strain tests of P(3HB) films (35 mm x 5 mm) were performed at room temperature with a strain rate of 20 mm/min on an Imada tensile machine (model SV-50). Mechanical tensile data were calculated from such curves on an average of three specimens.

Differential scanning calorimetry data of P(3HB) films were recorded at a heating rate of 10°C/min on a Shimadzu DSC-50 under a nitrogen flow of 30 mL/min.

Dynamic mechanical spectra of P(3HB) films (30 mm x 2.5 mm) were recorded at a heating rate of 2°C/min and a frequency of 1 Hz in the temperature range -100°C to 120°C, using a Seiko Instruments DMS210 dynamic mechanical thermal analyzer.

RESULTS AND DISCUSSION

Biosynthesis and Film Preparation of High-Molecular-Weight P(3HB)

High-molecular-weight P(3HB) samples 1 to 6 listed in Table 1 were produced by a recombinant *E. coli* XL1-Blue (pSYL105) grown in LB medium with glucose as a sole carbon source. The formation of P(3HB) homopolymer was confirmed by analysis of ^1H - and ^{13}C -NMR spectra. In a previous paper [11], we showed that molecular weights of P(3HB) produced within cells were strongly dependent of the pH value of culture medium containing glucose as a carbon source. Accordingly, in this study three pH values of 6.5, 6.7 and 7.0 were selected to produce P(3HB) samples of different molecular weights from glucose for at 37°C . In the cultivation conditions, the growth of recombinant *E. coli* was quite ideal and P(3HB) productions reached up to 4.0-5.0 g/L.

The molecular weight data of P(3HB) samples produced from glucose by a recombinant *E. coli* XL1-Blue (pSYL105) are summarized in Table 1. The molecular weights were determined by both methods of gel-permeation chromatography (GPC) and of multiangle laser light scattering (MALLS). GPC was used to determine the molecular weight distribution of P(3HB), although the M_w and M_n values are relative to polystyrene standard. Figure 1 shows the GPC traces of three P(3HB) samples (1, 3 and 6). The GPC traces of samples 3 and 6 are unimodal, while that of ultra-high-molecular-weight P(3HB) sample 1 is bimodal. The limitation of the GPC joint columns (Shodex K-802 and K-806M) to resolve the high-molecular-weight product seems to be around 5×10^7 . The polydispersities (M_w/M_n) of P(3HB) samples ranged from 1.4 to 3.4.

The absolute M_w values of P(3HB) samples were determined by MALLS analysis. Figure 2 shows the relation between $M_w(\text{MALLS})$ and $M_w(\text{GPC})$ values of six kinds of P(3HB) samples with different $M_w(\text{MALLS})$ values of 1.1×10^6 to 11×10^6 prepared by the recombinant *E. coli*. Furthermore, P(3HB) sample 7 with $M_w(\text{MALLS})$ of 6×10^5 prepared by *A. eutrophus* from butyric acid [7] is also plotted in Figure 2. On the basis of the plotting in Figure 2, the following equation is concluded

$$M_w(\text{MALLS}) = 0.70 \times M_w(\text{GPC}) \quad (1)$$

The ultra-high-molecular-weight P(3HB) accumulated within cells of *E. coli* was apparently amorphous and quantitatively extracted with chloroform over 24 hours at room temperature. However, after the P(3HB) sample was crystallized,

TABLE 1. Molecular Weights of Poly[(*R*)-3-hydroxybutyrate] Produced from Glucose by a Recombinant *E. coli* XL1-Blue (pSYL105) at 37 °C and the Reproducibility of Hot-Drawings for P(3HB) Films

Sample no	pH of medium	Molecular weights			Reproducibility of hot-drawings for P(3HB) films ^c
		$M_n(\text{GPC})^a$ $\times 10^6$	$M_w(\text{GPC})^a$ $\times 10^6$	$M_w(\text{MALLS})^b$ $\times 10^6$	
1	6.5	6.0	16	11	○
2	6.5	9.6	13	9.6	○
3	6.7	3.5	7.0	4.9	○
4	6.7	1.9	6.5	4.2	○
5	7.0	1.7	4.6	3.3	○
6	7.0	0.78	1.3	1.1	×
7 ^d	-	0.35	0.76	0.60	×

^aDetermined by gel-permeation chromatography (GPC) relative to polystyrene standard in chloroform at 40°C.

^bAbsolute weight-average molecular weight determined by multiangle laser light scattering (MALLS) in 2,2,2-trifluoroethanol at 25 °C.

^cWhen all of three P(3HB) films were stretched over 400% against its initial length, reproducibility is represented by "○".

^dProduced by *Alcaligenes eutrophus* H16 from butyric acid [7].

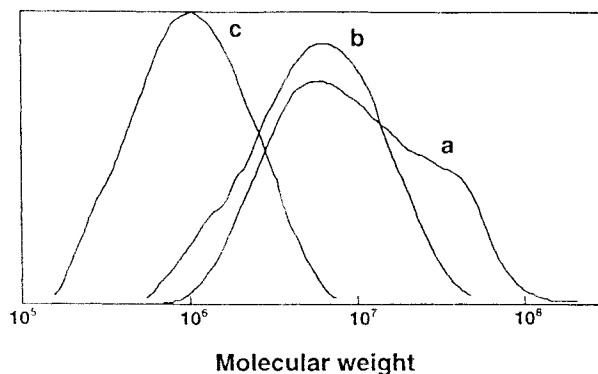


Figure 1. GPC traces of three P(3HB) samples: (a) sample 1, (b) sample 3 and (c) sample 6.

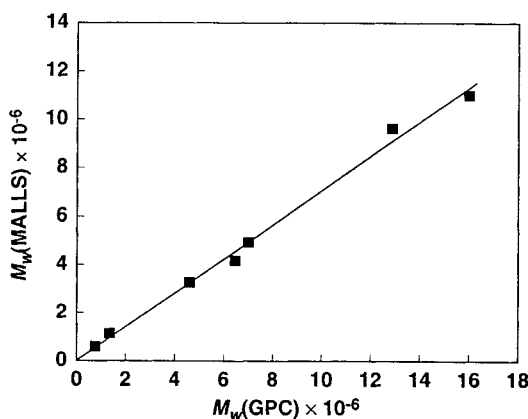


Figure 2. Correlation between $M_w(\text{MALLS})$ and $M_w(\text{GPC})$ values.

dissolution of the sample in chloroform required pre-swelling and high temperature, as described in the experimental section.

The solvent-cast films of seven P(3HB) samples 1-7 were prepared, and the thicknesses of these films were around 40 μm . The solvent-cast films of ultra-high-molecular-weight P(3HB) (sample 1) were stretched easily and reproducibly by a hot-drawing at 160°C, and the thicknesses were almost 15 μm . The preparation conditions for draw ratio, annealing temperature and annealing time of P(3HB) films (sample 1) are given in Table 2.

It is of important to note that the stability and reproducibility of hot-drawing process is controlled by molecular weight of P(3HB). When the hot-drawings were performed against P(3HB) films of various $M_w(\text{MALLS})$ over 0.6×10^6 , it was found that the M_w of 3×10^6 was a critical value whether hot-drawings over 400% against its initial length were reproducible or not (see Table 1).

X-ray Diffraction Diagrams of P(3HB) Films

Typical X-ray diffraction patterns for solvent-cast, stretched, and stretched-annealed films of the ultra-high-molecular-weight P(3HB) (sample 1) are respectively shown in Figures 3a-c. Well-defined Debye-Scherrer rings are shown in Figure 3a, which indicates that solvent-cast P(3HB) film has a high crystallinity in spite of its high molecular weight. Figures 3b and c indicate that the stretched film (b) and the stretched-annealed film (c) are well oriented by hot-drawing of solvent-cast films. All reflections of X-ray fiber diagrams of the stretched film and the stretched-annealed film were indexed with orthorhombic unit cell parameters: $a = 0.576 \text{ nm}$, $b = 1.320 \text{ nm}$, and c (fiber axis) = 0.596 nm as reported previously [17, 18].

TABLE 2. Physical Properties of Various Films of Ultra-High-Molecular-Weight P(3HB) Sample 1

Sample no.	Draw ratio (%)	Annealing time at 160°C	Aging time at r.t.	X-ray crystallinity (%)	Mechanical properties		
					Elongation to break (%)	Young's modulus (GPa)	Tensile strength (MPa)
1-a	0	0	7days	65±5	7±3	2.3±0.5	41±4
1-b	0	1sec	7days	65±5	6±3	2.4±0.3	41±3
1-c	650	1sec	7days	80±5	58±1	1.1±0.1	62±5
1-d	650	1sec	190days	75±5	30±1	2.5±0.2	88±8
1-e	650	2hrs	7days	>85	67±1	1.8±0.3	77±10
1-f	650	2hrs	180days	>85	67±2	2.5±0.2	100±10

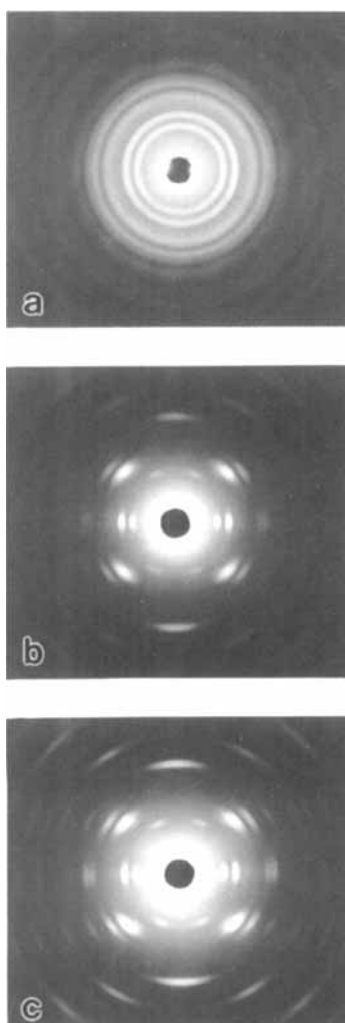


Figure 3. X-ray diffraction patterns of ultra-high-molecular-weight P(3HB) films: (a) solvent-cast film (sample 1-a) (b) stretched film (sample 1-c) and (c) stretched-annealed film (sample 1-e).

It has been reported that the conformational transformation of poly(L-lactide) [19, 20], poly(α -methyl- α -*n*-propyl- β -lactone) [21], and poly[(*R*)-3-hydroxybutyrate- ω -21%-(*R*)-3-hydroxyvalerate] (PHB/V) [22] takes place by hot- or cold-drawing. For example, Orts *et al.* [22] reported the X-ray fiber diagram of stretched PHB/V film that P(3HB) chains in PHB/V film transformed from helix

conformation to planar zigzag one by cold-drawing. In our X-ray fiber diagrams of stretched P(3HB) films, however, any reflections indicative of the conformational transformation from the 2_1 helix to the planar zigzag conformation could not be detected.

Mechanical and Thermal Properties of P(3HB) Films

Mechanical properties and X-ray crystallinities of ultra-high-molecular-weight P(3HB) films (sample 1) are summarized in Table 2, together with preparation conditions of films. All mechanical properties of the solvent-cast film (1-a) are almost identical with those of conventional P(3HB) film [4], in spite of large difference in the M_w (MALLS) values from 0.6×10^6 to 11×10^6 . These data indicate that the solvent-cast film is stiff and brittle as same as low-molecular-weight P(3HB) solvent-cast film measured by Abe *et al.* [23]. Accordingly, it seems that molecular weight is not an important factor in determining the mechanical properties of P(3HB) films with the M_w (MALLS) values over 0.6×10^6 . However, the stability and reproducibility of hot-drawing process is controlled by molecular weight, as indicated in Table 1.

The ultra-high-molecular-weight P(3HB) film was drawn readily and reproducibly to draw ratio 650% at 160°C, and the stretched film (1-c) showed the improved mechanical properties as listed in Table 2. Elongation to break and tensile strength increased from 7 to 58% and from 41 to 62 MPa, respectively, when solvent-cast film was stretched. In contrast, Young's modulus decreased from 2.3 to 1.1 GPa. This result demonstrates that the stiff and brittle solvent-cast P(3HB) film is improved to ductile and flexible material by a hot-drawing process. Furthermore, when an annealing procedure was applied to the stretched film (1-c), the mechanical properties of stretched-annealed film (1-e) were even more improved. Elongation to break and tensile strength of the stretched-annealed film (1-e) increased approximately 20% in comparison with those of stretched film (1-c). The change in the molecular weights of P(3HB) during the hot-drawing procedure was measured on solvent-cast film (1-a) and stretched film (1-c). The M_w (GPC) values were almost unchanged in the range $16 \pm 2 \times 10^6$ during the hot-drawing procedure. However, we could not measure the molecular weight of stretched-annealed film (1-e), because the film did not dissolve in chloroform.

The improvement of mechanical properties seems to arise from an increase in the crystallinity and from a decrease in the amount of amorphous polymer chains constrained between lamellar crystals depending on hot-drawing and annealing processes. De Koning and Lemstra [5] reported that the constriction of polymer

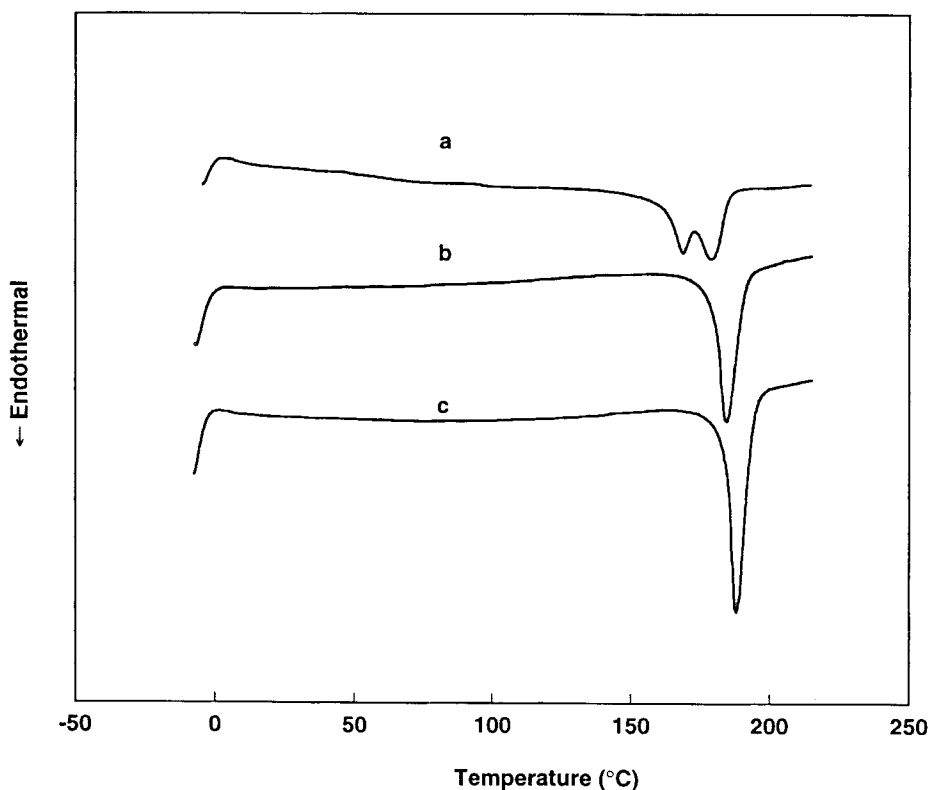


Figure 4. Differential scanning calorimetry curves of ultra-high-molecular-weight P(3HB) films: (a) solvent-cast film (sample 1-a) (b) stretched film (sample 1-c) and (c) stretched-annealed film (sample 1-e).

chains in amorphous phase between lamellar crystals due to the secondary crystallization made P(3HB) films stiff and brittle. The X-ray crystallinities of three P(3HB) films of ultra-high-molecular-weight were respectively 65, 80, and >85% for solvent-cast, stretched, and stretched-annealed films (1-a, 1-c, and 1-e). High crystallinity and high orientation of polymer chains in the stretched and stretched-annealed films are likely to avoid the secondary crystallization. The procedures of hot-drawing and annealing against the ultra-high-molecular-weight P(3HB) films are methods which drastically reduce the secondary crystallization.

Figure 4 shows the differential scanning calorimetry (DSC) curves of solvent-cast film (1-a), stretched film (1-c), and stretched-annealed film (1-e). The solvent-cast film has two melting peaks at 170°C and 180°C. The first peak at

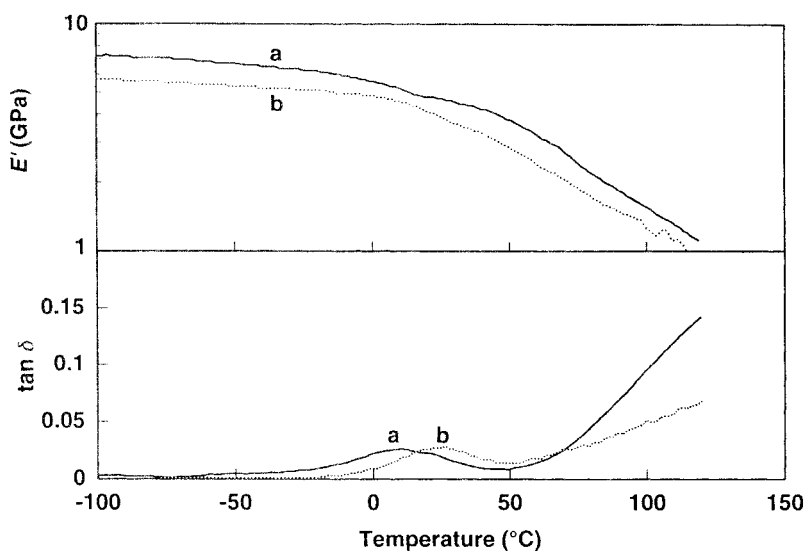


Figure 5. Dynamic mechanical spectra of ultra-high-molecular-weight P(3HB) films: (a; solid line) solvent-cast film (sample 1-a) and (b; dotted line) stretched film (sample 1-c).

170°C corresponds to a melting peak of original crystals in the solvent-cast film, while the second peak at 180°C is due to reorganized crystals formed during sample heating [24]. Only one melting peaks appeared on the DSC curves of the stretched film and the stretched-annealed film at 185°C and 188°C, respectively. The shift of melting peaks toward higher temperatures over 185°C reflects an increase in the thickness of lamellar crystals due to drawing and annealing procedures. Mitomo *et al.* reported that the lamellar crystal thickness of P(3HB) was drastically increased when annealed up to 145°C [25]. This may be caused by the reorganization of lamellar crystals and the crystallization of amorphous regions.

The dynamic mechanical spectra of the solvent-cast and stretched films (1-a and 1-c) of ultra-high-molecular-weight P(3HB) are shown in Figure 5. Storage modulus (E') of stretched film (curve b) was lower than that of solvent-cast film (curve a). This result is consistent with Young's modulus data in Table 2. Loss tangent ($\tan \delta$) peak of β transition, which attributes to glass transition of the amorphous regions [26, 27], shifted from 10°C to 26°C by hot-drawing. This transition is due to the reduction of micro-Brownian motion in the main chains and the local mode relaxation in the amorphous region [28]. Shift of the β relaxation

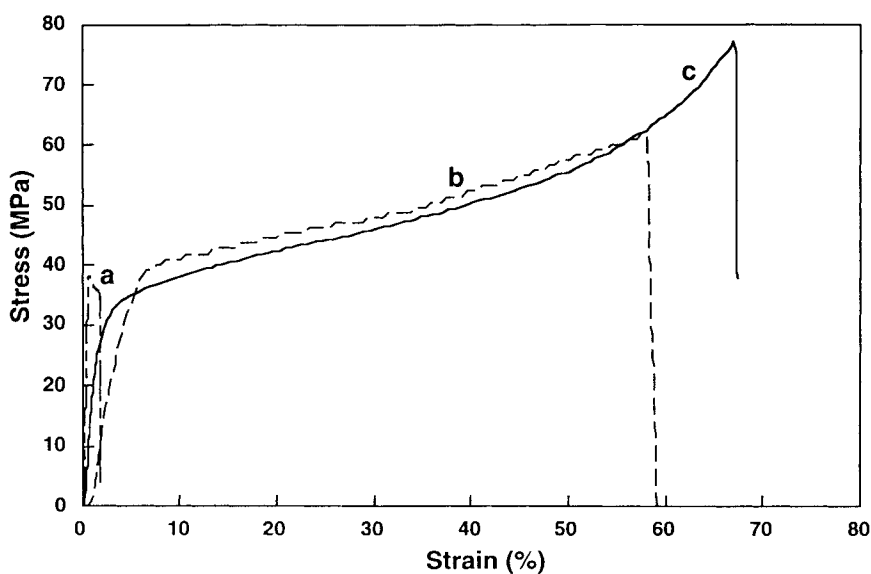


Figure 6. Stress-strain curves of ultra-high-molecular-weight P(3HB) films: (a) solvent-cast film (sample 1-a), (b) stretched film (sample 1-c) and (c) stretched-annealed film (sample 1-e).

peak of the $\tan \delta$ to higher temperature would arise from a low mobility of the polymer chains in the amorphous region between crystal regions. A decrease in the mobility of polymer chains in the stretched film may be explained as follows. The polymer chains in the amorphous region were melted at 160°C . Subsequently, polymer chains were stretched and organized with orientation of the crystalline phase by hot-drawing, and the mobility of polymer chains was decreased.

Figure 6 shows the typical stress-strain curves of the stretched film (1-c) and stretched-annealed film (1-e) of ultra-high-molecular-weight P(3HB), together with that of solvent-cast P(3HB) film (1-a). There were no yield points in the stress-strain curves (b and c) of stretched and stretched-annealed films, suggesting that the elongation of films are not plastic but elastic. Embrittlements of P(3HB) films due to the secondary crystallization have been reported to occur at room temperature above the glass transition temperature (approximately 4°C) [5, 10]. In our stretched-annealed films, however, any embrittlement and deterioration did not take place because of small amounts of amorphous region and high crystallinity, which will be described later. Elongation to break as an elastic type, much increased as the

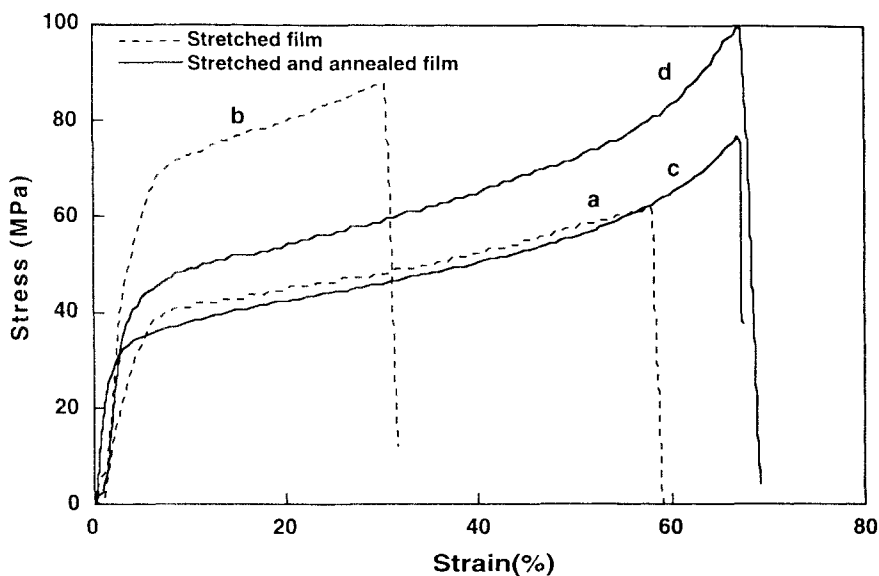


Figure 7. Stress-strain curves of ultra-high-molecular-weight P(3HB) films after different storage times at room temperature: (a) stretched film stored for 7 days (sample 1-c), (b) stretched film stored for 190 days (sample 1-d), (c) stretched-annealed film stored for 7 days (sample 1-e), and (d) stretched-annealed film stored for 180 days (sample 1-f).

crystallinity was increased by hot-drawing and annealing processes. This elastic elongation may arise from the three-dimensional linkage of crystal regions by the small amount of amorphous phase located perpendicularly and horizontally to the drawing direction.

Effect of Thermal History by Hot-Drawing Against Mechanical Properties

De Koning and Lemstra reported that the mechanical properties of brittle P(3HB) samples, which had progressed a secondary crystallization, were rejuvenated by a simple annealing treatment at 110°C for 10 hours [5]. In this study, sample films were heated at 160°C for about 1 second in hot-drawing process. Therefore, it was necessary to define whether this heating process for 1 second affected directly the mechanical properties or not. The solvent-cast P(3HB) films (sample 1) were annealed in the silicone oil bath for 1 second at 160°C, stored at room temperature for 1 week, and their stress-strain tests were performed. The mechanical properties of the annealed film (1-b) was consistent with those of

original solvent-cast film (1-a), as given in Table 2. This result indicates that annealing at 160°C for 1 second did not affect the mechanical properties.

Effect of Storage Time on Mechanical Properties

The stretched film and stretched-annealed film of P(3HB) (sample 1) were stored for about 6 months at room temperature to study the time-dependent change of the mechanical properties, and the stress-strain tests were performed. The mechanical properties data (1-d and 1-f) are listed in Table 2, and the stress-strain curves are shown in Figure 7. The tensile strength of the stretched film increased from 62 to 88 MPa for 190 days, while the elongation to break decreased from 58 to 30%. On the other hand, tensile strength of the stretched-annealed film increased from 77 to 100 MPa for 180 days, and the elongation to break was constant at 67%. It is of importance to note that the mechanical properties of the stretched-annealed film did not deteriorate during 6 months. It is concluded that a highly oriented and crystallized P(3HB) film keeps superior mechanical properties for long periods.

CONCLUSION

In this paper, we reported the biosynthesis and physical properties of ultra-high-molecular-weight P(3HB). Biosynthesis of ultra-high-molecular-weight P(3HB) from glucose using a recombinant *E. coli* is a novel method to produce a new polymer because of its high growth rate and high polymer content. The P(3HB) accumulated within cells was easily extracted with chloroform and was purified by reprecipitation as a conventional method. Solvent-cast film of ultra-high-molecular-weight P(3HB) was stretched easily and reproducibly by hot-drawing in a silicone oil bath at 160°C for 1 second. The stretched film had a high crystallinity and acceptable mechanical properties. The mechanical properties were even more improved by an annealing treatment. In addition, the properties did not deteriorate during the aging for 6 months. It has been suggested that the embrittlement of P(3HB) film due to secondary crystallization is avoided by its high crystallinity and high degree of orientation. After the best drawing conditions are selected, the mechanical properties of P(3HB) film may be even more improved.

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