**Co-crystallization phenomena in biosynthesized isotactic
poly[(*R*)-lactate-*co*-(*R*)-2-hydroxybutyrate]s with various lactate unit ratios**

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27 **Abstract**

28 Poly[(*R*)-lactate-*co*-(*R*)-2-hydroxybutyrate], which is biosynthesized as a random co-polyester by
29 microbial organisms, with various ratios of lactic acid (LA) to hydroxybutyrate monomers has been
30 isothermally crystallized to investigate its thermal properties and crystal structure changes.
31 Differential scanning calorimetry on the isothermally crystallized samples detected endothermic
32 peaks due to the melting of the crystals at all LA unit ratios. Melting temperatures of the
33 copolymers increased from ca. 107 °C to 146 °C as the LA unit ratio increased. Glass transition
34 temperatures also increased from 24 °C to 44 °C with increasing LA unit ratio. A melting
35 temperature was observed even at LA unit ratios around 50 %. Wide-angle X-ray measurements
36 also revealed that co-crystallization occurred at all samples, including at LA unit ratios of ca. 50%.
37 Despite of “jumping” phenomena many co-crystallizable random copolymers having, the lattice
38 constants changed “linearly” from resembling poly[(*R*)-lactate] to poly[(*R*)-2-hydroxybutyrate]
39 depending on the LA unit ratio. This unique *co*-crystallization behavior is discussed in detail.

40

41

42 **Keywords**

43 Microbial polyester; co-crystallization; Random copolymer

44

1. Introduction

Poly[(*R*)-3-hydroxybutyrate] P(3HB) is a microbial polyester that can be used as a thermoplastic material[1]. However, it is well known that P(3HB) is affected by deterioration at room temperature because of secondary crystallization[2, 3]. Accordingly, copolymerization has been studied with genetically modified microorganisms to prevent the deterioration of P(3HB). Many P(3HB) copolymers that include different secondary monomer units have been reported[4], poly[(*R*)-3HB-*co*-(*R*)-3-hydroxyvalerate] (P(3HB-*co*-3HV))[5, 6], poly[(*R*)-3HB-*co*-(*R*)-3-hydroxyhexanoate] (P(3HB-*co*-3HH))[7, 8], poly[(*R*)-3HB-*co*-4-hydroxybutyrate] (P(3HB-*co*-4HB))[9, 10], and poly[(*R*)-3HB-*co*-(*R*)-3-hydroxyproioate] (P(3HB-*co*-3HP))[11, 12]. Recently, the biosynthesis of some homopolymers has been reported, such as poly[(*R*)-3-hydroxyvalerate] (P(3HV))[13], poly[(*R*)-4-hydroxybutyrate] (P(4HB))[14], and the mid-length chain poly[(*R*)-3-hydroxyalkanoate] (mcl-P(3HA)) series[15, 16].

Poly[(*R*)-2-hydroxybutyrate] (P(2HB)) is a recently developed microbial polyester that shares the 2-hydroxy-based main-chain structure with poly[(*R*)-lactate] (PDLA) (Fig. 1). The polyester has an asymmetric carbon in the monomer unit, and this stereochemistry greatly affects the properties of the polymer. For example, a blend of the enantiomeric polymers, P[(*R*)-2HB] and P[(*S*)-2HB], forms a stereocomplex[17], which exhibits an elevated melting temperature, as has been seen in a blend of P[(*R*)-LA] (PDLA) and P[(*S*)-LA] (PLLA) as well. Interestingly, P(2HB) formed a hetero-stereocomplex when blended with PLA in a heterogeneous combination of enantiomeric polymers, P[(*R*)-2HB]/P[(*S*)-LA] and P[(*S*)-2HB]/P[(*R*)-LA][18-20]. However, despite the interesting phenomenon of P(2HB), a detailed analysis of the polymer properties, such as the mechanical properties and thermal properties, was not performed because it is difficult to synthesize in sufficient amounts.

Fig. 1. Chemical structures of poly[(*R*)-lactate] (PDLA), poly[(*R*)-2-hydroxybutyrate], and poly[(*R*)-lactate-*co*-(*R*)-2-hydroxybutyrate]. (In this paper, poly[(*R*)-lactate] = poly(*D*-lactate) = PDLA)

The chemical synthesis of P(2HB) using expensive enantiopure monomers has limited the amount of polymer that is available for detailed characterization. P(2HB)s have been synthesized via the polycondensation process of the corresponding free acids, and therefore, the isotacticity of the polymer seems to rely on the enantiopurity of the monomers[17], and this is also seen with poly(lactic acid)[21]. The preparation of chiral monomers continues to be a major contributor to the production cost. In a previous study, our group addressed this problem by using a microbial polyester synthetic system[22]. A key component of the system was a unique engineered polyhydroxyalkanoate (PHA) synthase (designated the lactate-polymerizing enzyme)[23], which has been modified to recognize 2-hydroxyacyl-CoAs as a monomer substrate as a standard PHA synthase would lactate. PHA synthases possess strict substrate specificity towards *R*-form monomers, and thus synthesize isotactic polymers even from racemic precursors.

Using this advantage, we succeeded in synthesizing isotactic P[(*R*)-2HB] from racemic 2HBs. Microbially synthesized P(2HB) film was as transparent as the film of chemically synthesized PDLA, but more flexible (elongation at break of 173%), in contrast to the normally rigid PDLA[22]. Furthermore, the copolymer P[14 mol%-(*R*)-LA-*co*-(*R*)-2HB], which was synthesized via a

modified biosynthetic pathway, was slightly harder compared with P(2HB) homopolymers, suggesting that LA units possess the potential to modulate this polymer property.

The aim of this study was to investigate the relationship between the thermal properties and the structure of P[(*R*)-LA-*co*-(*R*)-2HB] over a wide range of monomer composition. For this purpose, we biosynthesized P[(*R*)-LA-*co*-(*R*)-2HB)]s with various LA unit ratios and investigated their thermal properties. As a result, we have found that these copolymers exhibited melting peaks in the resulting thermogram. This phenomenon drew our attention to the co-crystallization of the copolymers. The crystal structure and crystallinity are important factors in determining the properties of a polymer. However, until now, our knowledge has been limited to conventional PHAs, which are composed of 3- and 4-hydroxyalkanoates, and there has been no report on a microbiologically synthesized 2-hydroxyalkanoate-based polymer. This paper describes the thermal and crystallization behaviors of P[(*R*)-LA-*co*-(*R*)-2HB)]s.

2. Materials and Methods

2.1 Biosynthesis of P[(*R*)-LA-*co*-(*R*)-2HB] with Various LA Monomer Ratios

P(2HB) and P(15 mol%-(*R*)-LA-*co*-(*R*)-2HB) copolymer were synthesized as described previously^[22]. P[20 mol%-(*R*)-LA-*co*-(*R*)-2HB] was produced using engineered *Escherichia coli* JW0885 harboring pTV118NpctC1(ST/FS/QK)[24]. The cells were grown on an LB medium containing 100 µg/L ampicillin in a 2-L jar fermenter, with aeration at 2.0 L/min, for 16 h to allow cell growth. The culture medium was modified to contain 20 g/L glucose, 10 mM calcium pantothenate, and 10 g/L sodium (*R,S*)-2HB and the cells were further cultivated for 32 h under a nitrogen flow of 0.3 L/min to promote LA synthesis. P[53 mol%-(*R*)-LA-*co*-(*R*)-2HB] was produced similarly, but 2.0 g/L sodium (*R,S*)-2HB was added. P[85 mol%-(*R*)-LA-*co*-(*R*)-2HB] and P[95 mol%-(*R*)-LA-*co*-(*R*)-2HB] were produced using engineered *Corynebacterium glutamicum* ATCC13803, which has been described previously[25, 26]. The culture medium was modified to

115 contain 3.0 and 2.0 g/L sodium (*R,S*)-2HB for the synthesis of P[85 mol%-(*R*)-LA-co-(*R*)-2HB] and
116 P[95 mol%-(*R*)-LA-co-(*R*)-2HB], respectively.

117 Chemically synthesized PDLA was purchased from Teijin Ltd. The weight-average molecular
118 weight (M_w), number-average molecular weight (M_n) and polydispersity were determined by using
119 gel permeation chromatography (10A series, Shimadzu Co., Japan) at 40 °C equipped with two
120 joined columns of Shodex K-802 and K-806M. Polystyrene standards were used to make a
121 molecular weight calibration curve.

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123 2.2 DSC Measurement

124 Glass transition temperature (T_g), crystallization temperature (T_c), and melting temperature (T_m)
125 of the samples were measured by differential scanning calorimetry (DSC) (DSC-8500, Perkin
126 Elmer Inc., USA). Temperature calibration of the instrument was carried out by referencing the
127 transition temperatures (T_t) of indium ($T_t=156.60$ °C) and tin ($T_t=231.88$ °C). The DSC properties of
128 all samples were measured under a nitrogen atmosphere. The first heating cycle involved heating at
129 a rate of 20 °C/min from 30 °C to 190 °C, maintaining at 190 °C for 1 min, and then quenching to
130 -50 °C at a rate of -200 °C/min. The second heating cycle was performed at heating rate of
131 20 °C/min from -50 °C to 190 °C. The T_g , T_c , T_m , enthalpy of crystallization, and melting (ΔH_c and
132 ΔH_m) were determined from the resulting thermogram.

133

134 2.3 Thermal Properties of the Isothermally Crystallized Samples

135 Isothermally crystallized samples were prepared to investigate the crystalline properties of
136 P[(*R*)-LA-co-(*R*)-2HB]. Each sample was melted at 30 °C above the melting temperature for 30 s,
137 and then placed in oven at 80 °C for 3 days. The melting temperature and enthalpy of melting of the
138 crystallized samples were determined by DSC measurement at the heating rate of 100 °C/min from
139 30 °C to 200 °C.

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141 2.4 Wide-angle X-ray Diffraction Measurement

142 Wide-angle X-ray diffraction (WAXD) measurements were carried out using synchrotron
143 radiation from BL-45XU of SPring-8, Harima, Japan. The wavelength was 0.1 nm, the sample to
144 CCD distance was 112 mm, and a CCD camera (C11440, Hamamatsu Photonics, Japan) was used
145 as the detector. Silicon powder was used as a standard sample. An image distortion correction was
146 performed using a grid mask pattern. Isothermally crystallized samples are measured as a film,
147 while P[20 mol%-(*R*)-LA-*co*-(*R*)-2HB] was measured in a capillary because of the small amount
148 available. The peak fitting of the 1-D WAXD profile was performed on Her-MesJ 1-D[27] software.
149 Based on the crystal lattice constant of PLLA[28], the crystal lattice constants of the orthorhombic
150 isothermal crystallized P[(*R*)-LA-*co*-(*R*)-2HB] at different LA unit ratios were calculated. Two very
151 strong diffraction peaks at low diffraction angles were assigned as the (110)/(200) and (113)/(203)
152 planes.

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154 3. Results and Discussion

155 3.1 Biosynthesis of P[(*R*)-LA-*co*-(*R*)-2HB] with Various LA Unit Ratios

156 The biosynthesized P[(*R*)-LA-*co*-(*R*)-2HB] with various LA unit ratios were processed using the
157 solvent-cast method. The polymers were prepared as transparent films (data not shown), which
158 were used for further analysis. The molecular weights of the P[(*R*)-LA-*co*-(*R*)-2HB]s are listed in
159 Table 1. The weight-average molecular weight (M_w) of the polymers was in the range of
160 10,000–40,000.

161

Table 1 Molecular weights of P(2HB), P[(R)-LA-co-(R)-2HB], and PDLA.

Sample ^a	M_w^b	M_n^b	M_w/M_n^b
P(2HB)	28,000	15,000	1.9
P[15mol%-(R)-LA-co-(R)-2HB]	39,000	28,000	1.4
P[20mol%-(R)-LA-co-(R)-2HB]	38,000	19,000	2.0
P[53mol%-(R)-LA-co-(R)-2HB]	18,000	10,000	1.8
P[85mol%-(R)-LA-co-(R)-2HB]	10,000	5,600	1.8
P[95mol%-(R)-LA-co-(R)-2HB]	16,000	8,800	1.8
PDLA ^c	144,000	103,000	1.4

^aSecond monomer unit ratio was measured by ¹H-NMR.^bWeight-average (M_w), Number-average molecular weight (M_n) and polydispersity (M_w/M_n) were determined from GPC measurements.^cIt was synthesized by chemical method.

3.2 DSC Measurements

Fig. 2 shows the DSC curves of the 1st and 2nd runs for P[(R)-LA-co-(R)-2HB] with different LA unit ratios, P(2HB), and PDLA. The thermal properties of each sample are listed in Table 2. The result 1st run DSC reflects the highly order structure, the lamellar thickness, and crystallinity of the cast films. On these 1st run DSC curves, melting temperatures (T_m s) of P(2HB) and PDLA were detected at ca. 100 °C and ca. 175 °C, respectively. The T_m of P[(R)-LA-co-(R)-2HB] decreases as the LA unit ratio increases to 50 %. When LA ratio exceeded 50 %, T_m increased again towards 175 °C, the value seen in PDLA. The T_m of P[(R)-LA-co-(R)-2HB] with a LA unit ratio of 53 mol% is of particular interest, because many copolymers fail to crystallize at monomer ratios of around 50/50.

The 2nd run of the DSC, after a melting and quenching process, reveals the glass-transition temperature (T_g). For the 2nd run DSC curves, T_g s were detected as a single shoulder peak in all samples. The T_g s of P[(R)-LA-co-(R)-2HB] increased from 25 °C to 60 °C as the LA unit ratio increased. It is well known that the introduction of a second monomer unit with a high glass temperature tends to increase the glass temperature of the copolymer. Melting peaks and cold-crystallization peaks of the P[(R)-LA-co-(R)-2HB]s were undetected in the 2nd run except for P[85mol%-(R)-LA-co-(R)-2HB]. This suggests that P[(R)-LA-co-(R)-2HB] cannot crystallize in limited time on the DSC heat profiles because of a slow crystallization rate.

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Table 2 Thermal properties of P(2HB), P[(*R*)-LA-*co*-(*R*)-2HB], and PDLA.

Sample	First run		Second run				
	T_m	ΔH_m^a	T_g	T_c	ΔH_c	T_m	ΔH_m
	(°C)	(J/g)	[°C]	[°C]	(J/g)	[°C]	(J/g)
P(2HB)	99, 106	27	23.9	— ^b	— ^b	— ^b	— ^b
P[15mol%-(<i>R</i>)-LA- <i>co</i> -(<i>R</i>)-2HB]	100	29	32.7	— ^b	— ^b	— ^b	— ^b
P[20mol%-(<i>R</i>)-LA- <i>co</i> -(<i>R</i>)-2HB]	89, 96	13	26.0	— ^b	— ^b	— ^b	— ^b
P[53mol%-(<i>R</i>)-LA- <i>co</i> -(<i>R</i>)-2HB]	48, 85	5, 12	33.0	— ^b	— ^b	— ^b	— ^b
P[85mol%-(<i>R</i>)-LA- <i>co</i> -2(<i>R</i>)-HB]	127, 135	34	34.0	109	4	130	8
P[95mol%-(<i>R</i>)-LA- <i>co</i> -(<i>R</i>)-2HB]	143, 150	48	43.5	106	40	140, 148	41
PDLA	175	62	58.5	101	48	174	69

^a ΔH_m are calculated from the area of bimodality peak.

^bThis value can not be detected.

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Fig. 2. DSC curves of P[(*R*)-LA-*co*-(*R*)-2HB]s, P(2HB), and PDLA. (A) is the first run and (B) is the second run.

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190 3.3 Thermal Properties of Isothermally Crystallized Samples

191 Melting temperatures and the fusion enthalpies of the isothermally crystallized samples were
192 measured by DSC in the 1st run. The relationship between melting temperature (T_m) and lamellar
193 thickness (l) is given by:

194

$$195 \quad T_m = T_m^\circ \left(1 - \frac{2\sigma_e}{l\Delta H_m}\right),$$

196

197 where T_m° , σ_e , and ΔH_m are the equilibrium melting temperature, surface free energy, and fusion
198 enthalpy, respectively. Therefore, a sample with a higher melting temperature means it has a thicker
199 lamellar crystal for the same macromolecular species.

200 DSC measurements at normal heating rates (ca. 5–20 °C/min) do not detect the characteristic
201 melting temperatures of isothermally crystallized films owing to the increased lamellar thickness by
202 recrystallization during the heating process. However, DSC at heating rate of 100 °C/min can
203 measure the melting temperature before recrystallization occurs because the heated time at
204 crystallizable temperature are very short in this heating rate. Thus, DSC at heating rate of 100 °C/min
205 measurements were carried out to reveal the highly order structure of the isothermally crystallized
206 films. Fig. 3 and Table 3 show the melting temperatures and heats of fusion (ΔH_m) of the
207 isothermally crystallized samples as a function of the LA unit ratio. ΔH_m is proportional to the
208 crystallinity. Despite the observation that the ΔH_m are constant until a LA unit ratio of 20 %, the
209 ΔH_m of P[53 mol%-(*R*)-LA-*co*-(*R*)-2HB] is drastically decreased. However, a T_m was still detected
210 at 108 °C for P[53 mol%-(*R*)-LA-*co*-(*R*)-2HB]. These results suggest that P[53
211 mol%-(*R*)-LA-*co*-(*R*)-2HB] is *co*-crystallized by the isothermal crystallization process.

Fig. 3. Melting temperature and heat of fusion (ΔH_m) of isothermally crystallized samples at an isothermal crystallization temperature of 80 °C.

Table 3 Melting temperature and ΔH_m of isothermally crystallized samples.

Sample	T_m	ΔH_m
	(°C)	(J/g)
P(2HB)	109	40
P[15mol%-(<i>R</i>)-LA-co-(<i>R</i>)-2HB]	108	42
P[20mol%-(<i>R</i>)-LA-co-(<i>R</i>)-2HB]	107	43
P[53mol%-(<i>R</i>)-LA-co-(<i>R</i>)-2HB]	108	21
P[85mol%-(<i>R</i>)-LA-co-(<i>R</i>)-2HB]	131	31
P[95mol%-(<i>R</i>)-LA-co-(<i>R</i>)-2HB]	146	58
PDLA	174	44

3.4 WAXD Analysis

WAXD patterns of the isothermally crystallized samples are shown in Fig. 4. Ring patterns, which indicate the presence of isotropic crystals, were detected in the WAXD patterns in all crystallized samples. Fig. 5 shows the 1-D profile from two-dimensional WAXD (Fig. 4). Two strong diffractions in the P(2HB) profile were detected at 2θ of 9.6 ° and 11.2 °. Additionally, two strong diffractions were detected at 2θ of 10.8° and 12.3° in the PDLA profile. For the P[(*R*)-LA-co-(*R*)-2HB] series with lower LA unit ratios, the diffraction patterns were the same as

227 P(2HB). When the LA unit ratio exceeded 85 %, the diffraction patterns are identical to that of
 228 PDLA. Interestingly, the two diffraction peaks from the P[53 mol%-(*R*)-LA-co-(*R*)-2HB] sample
 229 were between those of P(2HB) and PDLA. The diffraction angles of six peaks in each sample are
 230 listed in Table 4, and the calculated lattice constants from the two strongest peaks are listed in Table
 231 5. The crystallinity (X_c) was estimated by the following equation:

232

$$233 \quad X_c = \frac{\int_{2\theta_1}^{2\theta_2} I_c(2\theta) d(2\theta)}{\int_{2\theta_1}^{2\theta_2} I(2\theta) d(2\theta)} \times 100,$$

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235 where the $2\theta_1$ and $2\theta_2$ are varied from 8° to 25° . The X_c are plotted as a function of LA unit ratio in
 236 Fig. 6. It is interesting that P[53 mol%-(*R*)-LA-co-(*R*)-2HB], which was composed of nearly equal
 237 contents of 2HB and LA, was able to crystallize at an X_c of around 40 %.

238 The lattice constants of the crystallized samples were calculated from 1-D WAXD profiles. The
 239 crystal lattice parameters of P(2HB) have not been previously reported, to the best of our
 240 knowledge. Therefore, the lattice constants of P(2HB) were calculated based on the assumption it
 241 retained the orthorhombic crystal system of PLA crystal^[28]. Lattice constants and crystal volume,
 242 which are listed in Table 5, decreased with increasing LA unit ratios. These results are considered in
 243 detail in the following section.

Fig. 4. 2D-WAXD patterns of (a) P(2HB), (b) P[15 mol%-(*R*)-LA-*co*-(*R*)-2HB], (c) P[20 mol%-(*R*)-LA-*co*-(*R*)-2HB], (d) P[53 mol%-(*R*)-LA-*co*-(*R*)-2HB], (e) P[85 mol%-(*R*)-LA-*co*-(*R*)-2HB], (f) P[95 mol%-(*R*)-LA-*co*-(*R*)-2HB], and (g) PDLA. All samples were isothermally crystallized at 80 °C for 3 days.

Fig.5. 1D-WAXD patterns obtained from Fig. 4.

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Table 4. *d*-spacings of P(2HB), P[(*R*)-LA-*co*-(*R*)-2HB], and PDLA.

Specimen	<i>d</i> -spacing (nm)					
	Peak No.	1	2	3	4	5
P(2HB)		0.598	0.514	0.418	0.384	0.347
P[15 mol%-(<i>R</i>)-LA- <i>co</i> -(<i>R</i>)-2HB]		0.593	0.509	0.417	0.382	0.344
P[20 mol%-(<i>R</i>)-LA- <i>co</i> -(<i>R</i>)-2HB]		0.593	0.509	0.417	0.382	0.344
P[54 mol%-(<i>R</i>)-LA- <i>co</i> -(<i>R</i>)-2HB]		0.561	0.488	-	0.378	-
P[85 mol%-(<i>R</i>)-LA- <i>co</i> -(<i>R</i>)-2HB]		0.539	0.471	0.405	0.376	0.359
P[95 mol%-(<i>R</i>)-LA- <i>co</i> -(<i>R</i>)-2HB]		0.536	0.470	0.402	0.379	0.357

Specimen	<i>d</i> -spacing (nm)					
	Miller index	(110)/(200)	(203)	-	-	-
PDLA		0.538	0.471	-	0.308	-

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Table 5. Lattice constants and crystallinities of P(2HB), P[(*R*)-LA-*co*-(*R*)-2HB], and PDLA.

Specimen	Lattice constant (nm)			Volume (nm ³)	Crystallinity (%)
	<i>a</i>	<i>b</i>	<i>c</i>	<i>V</i>	<i>X_c</i>
P(2HB)	1.195	0.690	3.012	2.48	51
P[15 mol%-(<i>R</i>)-LA- <i>co</i> -(<i>R</i>)-2HB]	1.185	0.684	2.989	2.42	41
P[20 mol%-(<i>R</i>)-LA- <i>co</i> -(<i>R</i>)-2HB]	1.185	0.684	2.989	2.42	27
P[54 mol%-(<i>R</i>)-LA- <i>co</i> -(<i>R</i>)-2HB]	1.123	0.648	2.973	2.16	39
P[85 mol%-(<i>R</i>)-LA- <i>co</i> -(<i>R</i>)-2HB]	1.079	0.623	2.907	1.95	41
P[95 mol%-(<i>R</i>)-LA- <i>co</i> -(<i>R</i>)-2HB]	1.073	0.619	2.918	1.94	48
PDLA	1.075	0.621	2.939	1.96	43
PLA ²⁸	1.066	0.616	2.888	1.90	-

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Fig. 6. Crystallinities of the samples isothermally crystallized at 80 °C for 3 days as a function of the LA unit ratios.

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263 3.5 Co-crystallization Behavior

264 In the DSC curves (Fig. 2A), melting peaks are detected in all samples for all ratios of the
265 second monomer unit. In addition, X-ray diffraction peaks were observed in all isothermally
266 crystallized samples (Fig. 4). Furthermore, the lattice constants calculated from the *d*-spacings of
267 reflections are listed in Table 5 and the lattice constants are shown as a function of 2HB unit ratio in
268 Fig. 7. The lattice constant of P[(*R*)-LA-*co*-(*R*)-2HB] increased linearly with increasing 2HB unit
269 ratios. These results indicate that P[(*R*)-LA-*co*-(*R*)-2HB]s are co-crystallized at all 2HB unit ratio.

270 The *a*-axis lengths of P[(*R*)-LA-*co*-(*R*)-2HB]s, P(3HB-*co*-3HV)[29], and P(3HB-*co*-3HP)[30,
271 31] as a function of the P(3HB) copolymer are shown in Fig. 8A. In general, normal random
272 copolymers do not crystallize as the second monomer ratio approaches 50 %, such as in the P(3HB)
273 copolymers[32], that is, P(3HB-*co*-3HP)[30, 33], P(3HB-*co*-3HH)[34], and P(3HB-*co*-4HB)[35,
274 36], because the formation of polymer crystals requires the sequential arrangement of monomer
275 units in the polymer chain. However, co-crystallization is well known where the second monomer
276 units are similar in chemical structure to the major monomer units. For example, co-crystallization
277 has been reported in P(3HB-*co*-3HV)[29, 34, 37-40], which can be crystallized regardless of the
278 3HV monomer ratio[29, 41] because of the second monomer unit (3HV) fitting in the P(3HB) space
279 in the crystal[38].

280 The change of lattice constants depending on copolymer crystals can be classified as three types
281 as shown in Fig. 8B-8D. At second monomer unit ratios of 0–20% or 80–100%, as shown in Fig.
282 8B, two different types of homo-polymer crystals are generated. Generally, most of the random
283 copolymers cannot crystallize at second monomer unit ratios from 30 to 70 %. When
284 co-crystallization occurs, two different changes in the lattice constants can be envisioned, as shown
285 in Fig.s 8C and 8D. Until now, the ‘jumping’ phenomena in the lattice constants (8C type) have
286 been reported for P(3HB-*co*-3HV)[29, 35]. However, the lattice constant of P[(*R*)-LA-*co*-(*R*)-2HB]
287 changes “linearly” over a wide range of LA unit ratios, in the manor shown in Fig. 8D. This
288 continuous “linear change” of lattice constants is indicative of co-crystallization. This continuous

289 expansion of the lattice constant is caused by the continuous increase in the 2HB unit ratio.

290 It is seen that the “jump” changed parameter of co-crystallizable copolymer with changing
291 second monomer ratio occurs when the molecular chain packing model of second monomer
292 component become to more stable by second monomer ratio change. On the other hand, the
293 “continuous changing” of lattice parameter in P[(*R*)-LA-*co*-(*R*)-2HB] is very unique phenomenon.
294 This unique phenomenon seems to be due to the similarity of molecular chain conformation
295 between PDLA and P(3HB) in crystal. In the case of P(3HB-*co*-3HV), lattice constants of P(3HB)
296 crystal ($a=0.576$ nm, $b=1.320$ nm, $c=0.596$ nm) [42] and P(3HV) crystal ($a=0.932$ nm, $b=1.002$ nm,
297 $c=0.560$ nm) [43] are very different in despite of similar chemical structures between P(3HB) and
298 P(3HV), however that of P(2HB) crystal ($a=1.195$ nm, $b=0.690$ nm, $c=3.012$) and PDLA crystal
299 ($a=1.075$ nm, $b=0.620$ nm, $c=2.939$ nm in this paper) are similar. It seems that these similar
300 chemical structure and similar molecular chain conformation lead to “continuous changing”
301 phenomena with changing second monomer ratio in co-crystallization. The verification of this
302 hypothesis are needed the crystal structure analysis using P[(*R*)-LA-*co*-(*R*)-2HB] drawn film.

303

Fig. 7. Crystallographic parameters as a function of the 2HB unit ratio.

Fig. 8. (A) The changes in the a -axis and the lattice constant in P[(*R*)-LA-*co*-(*R*)-2HB]s and P(3HB) random copolymers as the second monomer unit ratio changes. (○) P[(*R*)-LA-*co*-(*R*)-2HB], (■) P(3HB-*co*-3HV)²⁹, (△) P(3HB-*co*-3HP)^{30,31}. Schematics of the changes in the lattice constant for; (B) crystallized co-polymers, (C) co-crystallizable co-polymers, and (D) P[(*R*)-LA-*co*-(*R*)-2HB].

312 4. Conclusion

313 In this article, we have investigated the thermal properties and changes in the crystal structure of
314 P[(*R*)-LA-*co*-(*R*)-2HB] as a random copolymer over a wide range of LA unit ratios. The melting
315 peaks of all isothermally crystallized P[(*R*)-LA-*co*-(*R*)-2HB]s were detected between 110 °C and
316 145 °C by DSC, which suggests that all of the P[(*R*)-LA-*co*-(*R*)-2HB]s crystallized at all LA unit
317 ratios. Wide-angle X-ray measurements also show the existence of crystalline material in the
318 P[(*R*)-LA-*co*-(*R*)-2HB]s at all LA unit ratios. This phenomenon is indicative of co-crystallization.
319 The lattice constants of P[(*R*)-LA-*co*-(*R*)-2HB] were calculated based on the assumption that the
320 P(2HB) crystal system retains the orthorhombic structure of PLA. The result shows a continuous
321 change from P(2HB) crystals to PDLA crystals depending on the LA unit ratio. The lattice constant
322 of P[(*R*)-LA-*co*-(*R*)-2HB]s changed linearly with the LA unit ratio, which is first reported here. This
323 unique co-crystallization phenomenon seems to be due to continuous expansion of the lattice
324 constant with a decreasing LA unit ratio. These co-crystallizing polymers, P[(*R*)-LA-*co*-(*R*)-2HB],
325 which can have a controlled T_g or mechanical properties without alteration of crystallinity, may be
326 developed as novel materials.

327

328

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338 Reference

- 339 [1] Lenz RW, Marchessault RH. Bacterial polyesters: biosynthesis, biodegradable plastics and
340 biotechnology. *Biomacromolecules*. 2005;6:1-8.
- 341 [2] Scandola M, Ceccorulli G, Pizzoli M. The Physical Aging of Bacterial Poly(D-Beta-Hydroxybutyrate).
342 *Makromolekulare Chemie-Rapid Communications*. 1989;10:47-50.
- 343 [3] Dekoning GJM, Lemstra PJ, Hill DJT, Carswell TG, Odonnell JH. Aging Phenomena in Bacterial
344 Poly[(R)-3-Hydroxybutyrate] .1. A Study on the Mobility in Poly[(R)-3-Hydroxybutyrate] Powders by
345 Monitoring the Radical Decay with Temperature after Gamma-Radiolysis at 77-K. *Polymer*.
346 1992;33:3295-7.
- 347 [4] Sudesh K, Abe H, Doi Y. Synthesis, structure and properties of polyhydroxyalkanoates: biological
348 polyesters. *Prog Polym Sci*. 2000;25:1503-55.
- 349 [5] Slater S, Gallaher T, Dennis D. Production of Poly-(3-Hydroxybutyrate-Co-3-Hydroxyvalerate) in a
350 Recombinant *Escherichia-Coli* Strain. *Appl Environ Microbiol*. 1992;58:1089-94.
- 351 [6] Akiyama M, Taima Y, Doi Y. Production of Poly(3-Hydroxyalkanoates) by a Bacterium of the Genus
352 *Alcaligenes* Utilizing Long-Chain Fatty-Acids. *Appl Microbiol Biotechnol*. 1992;37:698-701.
- 353 [7] Fukui T, Yokomizo S, Kobayashi G, Doi Y. Co-expression of polyhydroxyalkanoate synthase and
354 (R)-enoyl-CoA hydratase genes of *Aeromonas caviae* establishes copolyester biosynthesis pathway in
355 *Escherichia coli*. *FEMS Microbiol Lett*. 1999;170:69-75.
- 356 [8] Taguchi K, Aoyagi Y, Matsusaki H, Fukui T, Doi Y. Co-expression of 3-ketoacyl-ACP reductase and
357 polyhydroxyalkanoate synthase genes induces PHA production in *Escherichia coli* HB101 strain. *FEMS*
358 *Microbiol Lett*. 1999;176:183-90.
- 359 [9] Valentin HE, Dennis D. Production of poly(3-hydroxybutyrate-co-4-hydroxybutyrate) in recombinant
360 *Escherichia coli* grown on glucose. *J Biotechnol*. 1997;58:33-8.
- 361 [10] Hein S, Sohling B, Gottschalk G, Steinbuchel A. Biosynthesis of poly(4-hydroxybutyric acid) by
362 recombinant strains of *Escherichia coli*. *FEMS Microbiol Lett*. 1997;153:411-8.
- 363 [11] Nakamura S, Kunioka M, Doi Y. Biosynthesis and Characterization of Bacterial
364 Poly(3-Hydroxybutyrate-Co-3-Hydroxypropionate). *Journal of Macromolecular Science-Chemistry*.
365 1991;A28:15-24.
- 366 [12] Hiramitsu M, Doi Y. Microbial Synthesis and Characterization of
367 Poly(3-Hydroxybutyrate-Co-3-Hydroxypropionate). *Polymer*. 1993;34:4782-6.
- 368 [13] Wallen LL, Rohwedder WK. Poly-.beta.-hydroxyalkanoate from activated sludge. *Environ Sci*
369 *Technol*. 1974;8:576-9.
- 370 [14] Pazur RJ, Raymond S, Hocking PJ, Marchessault RH. Molecular modelling of helical and
371 extended-chain polyhydroxybutyrates and polytetramethylene succinate. *Polymer*. 1998;39:3065-72.
- 372 [15] Sato S, Ishii N, Hamada Y, Abe H, Tsuge T. Utilization of 2-alkenoic acids for biosynthesis of
373 medium-chain-length polyhydroxyalkanoates in metabolically engineered *Escherichia coli* to construct a
374 novel chemical recycling system. *Polym Degrad Stab*. 2012;97:329-36.
- 375 [16] Abe H, Ishii N, Sato S, Tsuge T. Thermal properties and crystallization behaviors of

medium-chain-length poly(3-hydroxyalkanoate)s. *Polymer*. 2012;53:3026-34.

[17] Tsuji H, Okumura A. Stereocomplex Formation between Enantiomeric Substituted Poly(lactide)s: Blends of Poly[(S)-2-hydroxybutyrate] and Poly[(R)-2-hydroxybutyrate]. *Macromolecules*. 2009;42:7263-6.

[18] Tsuji H, Yamamoto S, Okumura A, Sugiura Y. Heterostereocomplexation between Biodegradable and Optically Active Polyesters as a Versatile Preparation Method for Biodegradable Materials. *Biomacromolecules*. 2010;11:252-8.

[19] Tsuji H, Shimizu K, Sakamoto Y, Okumura A. Hetero-stereocomplex formation of stereoblock copolymer of substituted and non-substituted poly(lactide)s. *Polymer*. 2011;52:1318-25.

[20] Tsuji H, Yamamoto S, Okumura A. Homo- and Hetero-Stereocomplexes of Substituted Poly(lactide)s as Promising Biodegradable Crystallization-Accelerating Agents of Poly(L-lactide). *J Appl Polym Sci*. 2011;122:321-33.

[21] Mehta R, Kumar V, Bhunia H, Upadhyay SN. Synthesis of Poly(Lactic Acid): A Review. *Journal of Macromolecular Science, Part C*. 2005;45:325-49.

[22] Matsumoto K, Terai S, Ishiyama A, Sun J, Kabe T, Song Y, et al. One-pot microbial production, mechanical properties, and enzymatic degradation of isotactic P[(R)-2-hydroxybutyrate] and its copolymer with (R)-lactate. *Biomacromolecules*. 2013;14:1913-8.

[23] Taguchi S, Yamada M, Matsumoto Ki, Tajima K, Satoh Y, Munekata M, et al. A microbial factory for lactate-based polyesters using a lactate-polymerizing enzyme. *Proceedings of the National Academy of Sciences*. 2008;105:17323-7.

[24] Yamada M, Matsumoto Ki, Shimizu K, Uramoto S, Nakai T, Shozui F, et al. Adjustable Mutations in Lactate (LA)-Polymerizing Enzyme for the Microbial Production of LA-Based Polyesters with Tailor-Made Monomer Composition. *Biomacromolecules*. 2010;11:815-9.

[25] Song Y, Matsumoto Ki, Yamada M, Gohda A, Brigham C, Sinskey A, et al. Engineered *Corynebacterium glutamicum* as an endotoxin-free platform strain for lactate-based polyester production. *Appl Microbiol Biotechnol*. 2012;93:1917-25.

[26] Matsumoto Ki, Tobitani K, Aoki S, Song Y, Ooi T, Taguchi S. Improved production of poly(lactic acid)-like polyester based on metabolite analysis to address the rate-limiting step. *AMB Express*. 2014;4:83.

[27] Marubayashi H, Asai S, Sumita M. Complex Crystal Formation of Poly(L-lactide) with Solvent Molecules. *Macromolecules*. 2012;45:1384-97.

[28] Sasaki S, Asakura T. Helix Distortion and Crystal Structure of the α -Form of Poly(l-lactide). *Macromolecules*. 2003;36:8385-90.

[29] Wang Y, Yamada S, Asakawa N, Yamane T, Yoshie N, Inoue Y. Comonomer compositional distribution and thermal and morphological characteristics of bacterial poly(3-hydroxybutyrate-co-3-hydroxyvalerate)s with high 3-hydroxyvalerate content. *Biomacromolecules*. 2001;2:1315-23.

[30] Shimamura E, Scandola M, Doi Y. Microbial Synthesis and Characterization of

- 414 Poly(3-hydroxybutyrate-co-3-hydroxypropionate). *Macromolecules*. 1994;27:4429-35.
- 415 [31] Furuhashi Y, Iwata T, Sikorski P, Atkins E, Doi Y. Structure and Morphology of the Aliphatic
416 Polyester Poly- β -propiolactone in Solution-Grown Chain-Folded Lamellar Crystals. *Macromolecules*.
417 2000;33:9423-31.
- 418 [32] Yoshie N, Inoue Y. Chemical composition distribution of bacterial copolyesters. *Int J Biol Macromol*.
419 1999;25:193-200.
- 420 [33] Ichikawa M, Nakamura K, Yoshie N, Asakawa N, Inoue Y, Doi Y. Morphological study of bacterial
421 poly(3-hydroxybutyrate-co-3-hydroxypropionate). *Macromol Chem Phys*. 1996;197:2467-80.
- 422 [34] Doi Y, Kitamura S, Abe H. Microbial Synthesis and Characterization of
423 Poly(3-Hydroxybutyrate-Co-3-Hydroxyhexanoate). *Macromolecules*. 1995;28:4822-8.
- 424 [35] Kunioka M, Tamaki A, Doi Y. Crystalline and Thermal-Properties of Bacterial Copolyesters -
425 Poly(3-Hydroxybutyrate-Co-3-Hydroxyvalerate) and Poly(3-Hydroxybutyrate-Co-4-Hydroxybutyrate).
426 *Macromolecules*. 1989;22:694-7.
- 427 [36] Saito Y, Nakamura S, Hiramitsu M, Doi Y. Microbial synthesis and properties of
428 poly(3-hydroxybutyrate-co-4-hydroxybutyrate). *Polym Int*. 1996;39:169-74.
- 429 [37] Bluhm TL, Hamer GK, Marchessault RH, Fyfe CA, Veregin RP. Isodimorphism in Bacterial
430 Poly(Beta-Hydroxybutyrate-Co-Beta-Hydroxyvalerate). *Macromolecules*. 1986;19:2871-6.
- 431 [38] Kamiya N, Sakurai M, Inoue Y, Chujo R, Doi Y. Study of cocrystallization of
432 poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by solid-state high-resolution carbon-13 NMR
433 spectroscopy and differential scanning calorimetry. *Macromolecules*. 1991;24:2178-82.
- 434 [39] Yoshie N, Menju H, Sato H, Inoue Y. Complex Composition Distribution of
435 Poly(3-Hydroxybutyrate-Co-3-Hydroxyvalerate). *Macromolecules*. 1995;28:6516-21.
- 436 [40] Abe H, Doi Y, Aoki H, Akehata T. Solid-state structures and enzymatic degradabilities for
437 melt-crystallized films of copolymers of (R)-3-hydroxybutyric acid with different hydroxyalkanoic acids.
438 *Macromolecules*. 1998;31:1791-7.
- 439 [41] Kunioka M, Nakamura Y, Doi Y. New Bacterial Copolyesters Produced in *Alcaligenes-Eutrophus*
440 from Organic-Acids. *Polymer Communications*. 1988;29:174-6.
- 441 [42] Yokouchi M, Chatani Y, Tadokoro H, Teranish.K, Tani H. Structural Studies of Polyesters .5.
442 Molecular and Crystal-Structures of Optically-Active and Racemic Poly(Beta-Hydroxybutyrate). *Polymer*.
443 1973;14:267-72.
- 444 [43] Yokouchi M, Chatani Y, Tadokoro H, Tani H. Structural Studies of Polyesters. VII. Molecular and
445 Crystal Structures of Racemic Poly([beta]-ethyl-[beta]-propiolactone). *Polym J*. 1974;6:248-55.

447 Captions for Figures and Tables

449 Table 1 Molecular weights of P(2HB), P[(R)-LA-co-(R)-2HB], and PDLA.

450 Table 2 Thermal properties of P(2HB), P[(*R*)-LA-*co*-(*R*)-2HB], and PDLA.

451 Table 3 Melting temperature and ΔH_m of isothermally crystallized samples.

452 Table 4 *d*-spacings of P(2HB), P[(*R*)-LA-*co*-(*R*)-2HB], and PDLA.

453 Table 5 Lattice constants and crystallinities of P(2HB), P[(*R*)-LA-*co*-(*R*)-2HB], and PDLA.

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455

456 Fig. 1. Chemical structures of poly[(*R*)-lactate] (PDLA), poly[(*R*)-2-hydroxybutyrate], and
457 poly[(*R*)-lactate-*co*-(*R*)-2-hydroxybutyrate]. (In this paper, poly[(*R*)-lactate] =
458 poly(D-lactate) = PDLA)

459 Fig. 2. DSC curves of P[(*R*)-LA-*co*-(*R*)-2HB]s, P(2HB), and PDLA. (A) is the first run and (B) is
460 the second run.

461 Fig. 3. Melting temperature and heat of fusion (ΔH_m) of isothermally crystallized samples at an
462 isothermal crystallization temperature of 80 °C.

463 Fig. 4. 2D-WAXD patterns of (a) P(2HB), (b) P[15 mol%-(*R*)-LA-*co*-(*R*)-2HB], (c) P[20
464 mol%-(*R*)-LA-*co*-(*R*)-2HB], (d) P[53 mol%-(*R*)-LA-*co*-(*R*)-2HB], (e) P[85
465 mol%-(*R*)-LA-*co*-(*R*)-2HB], (f) P[95 mol%-(*R*)-LA-*co*-(*R*)-2HB], and (g) PDLA. All
466 samples were isothermally crystallized at 80 °C for 3 days.

467 Fig. 5. 1D-WAXD patterns obtained from Fig. 4.

468 Fig. 6. Crystallinities of the samples isothermally crystallized at 80 °C for 3 days as a function of
469 the LA unit ratios.

470 Fig. 7. Crystallographic parameters as a function of the 2HB unit ratio.

471 Fig. 8. (A) The changes in the *a*-axis and the lattice constant in P[(*R*)-LA-*co*-(*R*)-2HB]s and
472 P(3HB) random copolymers as the second monomer unit ratio changes. (○)
473 P[(*R*)-LA-*co*-(*R*)-2HB], (■) P(3HB-*co*-3HV)²⁹, (△) P(3HB-*co*-3HP)^{30,31}. Schematics of

474 the changes in the lattice constant for; (B) crystallized co-polymers, (C) co-crystallizable
475 co-polymers, and (D) P[(*R*)-LA-*co*-(*R*)-2HB].

476

477

Table
Table 1
Molecular weights of P(2HB), P[(R)-LA-co-(R)-2HB], and PDLA.

Sample ^a	M_w^b	M_n^b	M_w/M_n^b
P(2HB)	28,000	15,000	1.9
P[15mol%-(R)-LA-co-(R)-2HB]	39,000	28,000	1.4
P[20mol%-(R)-LA-co-(R)-2HB]	38,000	19,000	2.0
P[53mol%-(R)-LA-co-(R)-2HB]	18,000	10,000	1.8
P[85mol%-(R)-LA-co-(R)-2HB]	10,000	5,600	1.8
P[95mol%-(R)-LA-co-(R)-2HB]	16,000	8,800	1.8
PDLA ^c	144,000	103,000	1.4

^aSecond monomer unit ratio was measured by ¹H-NMR.
^bWeight-average (M_w), Number-average molecular weight (M_n) and polydispersity (M_w/M_n) were determined from GPC measurements.
^cIt was synthesized by chemical method.

Table 2

Thermal properties of P(2HB), P[(R)-LA-co-(R)-2HB], and PDLA.

Sample	First run		Second run				
	T_m	ΔH_m^a	T_g	T_c	ΔH_c	T_m	ΔH_m
	(°C)	(J/g)	[°C]	[°C]	(J/g)	[°C]	(J/g)
P(2HB)	99, 106	27	23.9	_ ^b	_ ^b	_ ^b	_ ^b
P[15mol%-(R)-LA-co-(R)-2HB]	100	29	32.7	_ ^b	_ ^b	_ ^b	_ ^b
P[20mol%-(R)-LA-co-(R)-2HB]	89, 96	13	26.0	_ ^b	_ ^b	_ ^b	_ ^b
P[53mol%-(R)-LA-co-(R)-2HB]	48, 85	5, 12	33.0	_ ^b	_ ^b	_ ^b	_ ^b
P[85mol%-(R)-LA-co-2(R)-HB]	127, 135	34	34.0	109	4	130	8
P[95mol%-(R)-LA-co-(R)-2HB]	143, 150	48	43.5	106	40	140, 148	41
PDLA	175	62	58.5	101	48	174	69

^a ΔH_m are calculated from the area of bimodality peak.^b This value can not be detected.

Table 3

Melting temperature and ΔH_m of isothermally crystallized samples.

Sample	T_m	ΔH_m
	(°C)	(J/g)
P(2HB)	109	40
P[15mol%-(<i>R</i>)-LA-co-(<i>R</i>)-2HB]	108	42
P[20mol%-(<i>R</i>)-LA-co-(<i>R</i>)-2HB]	107	43
P[53mol%-(<i>R</i>)-LA-co-(<i>R</i>)-2HB]	108	21
P[85mol%-(<i>R</i>)-LA-co-(<i>R</i>)-2HB]	131	31
P[95mol%-(<i>R</i>)-LA-co-(<i>R</i>)-2HB]	146	58
PDLA	174	44

Table 4
 d -spacings of P(2HB), P[(R)-LA-co-(R)-2HB], and PDLA.

Specimen	<i>d</i> -spacing (nm)						
	Peak No.	1	2	3	4	5	6
P(2HB)		0.598	0.514	0.418	0.384	0.347	0.281
P[15 mol%-(<i>R</i>)-LA- <i>co</i> -(<i>R</i>)-2HB]		0.593	0.509	0.417	0.382	0.344	0.281
P[20 mol%-(<i>R</i>)-LA- <i>co</i> -(<i>R</i>)-2HB]		0.593	0.509	0.417	0.382	0.344	0.281
P[54 mol%-(<i>R</i>)-LA- <i>co</i> -(<i>R</i>)-2HB]		0.561	0.488	-	0.378	-	0.278
P[85 mol%-(<i>R</i>)-LA- <i>co</i> -(<i>R</i>)-2HB]		0.539	0.471	0.405	0.376	0.359	0.310
P[95 mol%-(<i>R</i>)-LA- <i>co</i> -(<i>R</i>)-2HB]		0.536	0.470	0.402	0.379	0.357	0.310
Specimen	<i>d</i> -spacing (nm)						
	Miller index	(110)/(200)	(203)	-	-	-	-
PDLA		0.538	0.471	-	0.308	-	0.308

Table 5

Lattice constants and crystallinities of P(2HB), P[(R)-LA-co-(R)-2HB], and PDLA.

Specimen	Lattice constant (nm)			Volume (nm ³)	Crystallinity (%)
	<i>a</i>	<i>b</i>	<i>c</i>	<i>V</i>	<i>X_c</i>
P(2HB)	1.195	0.690	3.012	2.48	51
P[15 mol%-(<i>R</i>)-LA-co-(<i>R</i>)-2HB]	1.185	0.684	2.989	2.42	41
P[20 mol%-(<i>R</i>)-LA-co-(<i>R</i>)-2HB]	1.185	0.684	2.989	2.42	27
P[54 mol%-(<i>R</i>)-LA-co-(<i>R</i>)-2HB]	1.123	0.648	2.973	2.16	39
P[85 mol%-(<i>R</i>)-LA-co-(<i>R</i>)-2HB]	1.079	0.623	2.907	1.95	41
P[95 mol%-(<i>R</i>)-LA-co-(<i>R</i>)-2HB]	1.073	0.619	2.918	1.94	48
PDLA	1.075	0.621	2.939	1.96	43
PLA ²⁸	1.066	0.616	2.888	1.90	-