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Biosynthesis of Novel Poly(3-hydroxyalkanoates) Containing Benzoyl Groups

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We studied novel poly(3-hydroxyalkanoates) (PHAs) containing benzoyl groups. Production of PHAs by *Pseudomonas cichorii* strain YN2 cultured with 4-benzoylbutyrate, 5-benzoylvalerate, 6-benzoylhexanoate, 7-benzoylheptanoate, or 8-benzoyloctanoate was investigated. NMR and GC-MS analysis indicated that the polymer consisted of 3-hydroxybenzoylalkanoates as monomeric units. The polymer, which was biosynthesized from 5-benzoylvalerate, contained 3-hydroxy-5-benzoylvalerate units. The average molecular number (M_n), the average molecular weight (M_w), the glass transition temperature (T_g), and the melting point (T_m) of this polymer were higher (330,000, 1,300,000, 36°C, and 150°C, respectively) than for other unusual-PHAs (PHAs having substituents other than alkyl groups introduced in the side chains) previously reported. This polymer has potential for use to some purpose, to which existing biosynthetic PHAs cannot be used because of their thermal properties.

Key words: poly(3-hydroxyalkanoate), benzoyl group, *Pseudomonas*, thermal property

1. Introduction

Many microorganisms produce and accumulate poly(3-hydroxybutyrate) (PHB) or other poly(3-hydroxyalkanoates) (PHAs) in cells[4,20]. These polymers can be used to produce various kinds of products by using melting processes, etc., like with other conventional plastics. PHAs have the advantage of being completely decomposed by microorganisms in nature because of their biodegradability, and so they would not remain in the natural environment to cause pollution unlike many conventional synthetic polymers. They are also excellent in biological compatibility and are expected to be used in medicine, etc.

Such microbial PHAs may have a variety of compositions and structures depending on the types of microorganisms, compositions of culture media, culture conditions, etc., used for their production. Studies of PHAs have been done so far on controlling such compositions and structures. For example, *Ralstonia eutropha* can synthesize PHAs containing short-chain-length 3-hydroxyalkanoate units which have three to five carbon atoms (scl-PHA), i.e., 3-hydroxypropionate unit, 3-hydroxybutyrate unit, 3-hydroxyvalerate unit and 4-hydroxybutyrate unit[4,19,20]. PHAs containing medium-chain-length 3-hydroxyalkanoate units, which have 6 to 14 carbon atoms (mcl-PHAs), have been produced by *Pseudomonas oleovorans*[4,19], etc.

However, if a wider range of applications using microbial PHA, such as a functional polymer, is considered, PHAs having substituents other than alkyl groups introduced in the side chains (unusual-PHAs) are expected to be useful. Examples of these substituents include those containing an aromatic ring (such as a phenyl group[9,12], a phenoxy group[9,13], a thiophenoxy group[21]), a cyclohexyl group[1,9], an unsaturated hydrocarbon[6], a cyano group[16], a halogenated hydrocarbon[16], or an epoxide[2,11].

In this study, we investigated PHA produced by *Pseudomonas cichorii* strain YN2[9,11] cultured with a benzoylalkanoate. The PHAs biosynthesized from benzoylalkanoates contained monomer units having benzoyl groups. The average molecular number (M_n), the average molecular weight (M_w), the glass transition temperature (T_g) and the melting point (T_m) of the PHA biosynthesized from 5-benzoylvalerate (5-BzVA) were higher than for other unusual-PHAs previously reported.

2. Materials and Methods

2.1. Materials

4-Benzoylbutyrate (4-BzBA), 5-BzVA, 6-benzoylhexanoate (6-BzHxA), 7-benzoylheptanoate (7-BzHpA), and 8-benzoyloctanoate (8-BzOA) were used as "unusual carbon sources" in this study. These chemicals were of the highest purity grade available from Lancaster Synthesis Ltd. (Manchester, UK), and were used without further purifica-

tion.

5-Phenylvalerate (5-PVA), 5-phenoxyvalerate (5-PxVA) and 4-cyclohexylbutyrate (4-CHBA) were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan), and *n*-nonanoate was purchased from Kishida Chemical Co., Ltd. (Osaka, Japan).

2.2. Biosynthesis of polymers

The composition of M9 medium used in this study was (per liter): Na_2HPO_4, 6.2 g; KH_2PO_4, 3.0 g; NaCl, 0.5 g; NH_4Cl, 1.0 g; and 3 ml of a trace element solution. The trace element solution contained (per liter): nitrilotriacetic acid, 1.5 g; $MgSO_4$, 3.0 g; $MnSO_4$, 0.5 g; NaCl, 1.0 g; $FeSO_4$, 0.1 g; $CaCl_2$, 0.1 g; $CoCl_2$, 0.1 g; $ZnSO_4$, 0.1 g; $CuSO_4$, 0.1 g; $AlK(SO_4)_2$, 0.1 g; H_3BO_3, 0.1 g; Na_2MoO_4, 0.1 g; $NiCl_2$, 0.1 g. The medium was sterilized by autoclaving at 121°C for 10 min.

Two-stage shake flask cultures of *P. cichorii* strain YN2 were done aerobically in 500 ml shaking flasks (Sakaguchi flasks) containing 200 ml of the medium. *P. cichorii* strain YN2 was inoculated into M9 medium containing 0.5% (wt./vol.) D-glucose and 0.1% (wt./vol.) of an unusual carbon source and then was cultured with shaking at 125 rpm and 30°C on a reciprocating shaker. After the culture for 48 hours, the cells were harvested by centrifugation (9,000× *g*, 4°C, 10 min.), were resuspended in M9 medium containing 0.5% (wt./vol.) D-glucose and 0.1% (wt./vol.) of an unusual carbon source but not containing a nitrogen source (NH_4Cl), and were further cultured with shaking at 125 rpm and 30°C on a reciprocating shaker. After the culture for 48 hours, the cells were harvested by centrifugation (9,000× *g*, 4°C, 10 min.), were washed once with cold methanol and were lyophilized.

The lyophilized cells were suspended in 20 ml of chloroform and was stirred at 60°C for 20 hours to extract the polymer. The extract was filtered through a membrane filter of pore size 0.45 μm. The filtrate was concentrated by using a rotary evaporator. The concentrate was reprecipitated in cold methanol, and then only the precipitate was recovered and was dried *in vacuo* to obtain the polymer.

Poly(3-hydroxy-5-phenylvalerate) (PHPV), poly(3-hydroxy-5-phenoxyvalerate) (PHPxV), poly(3-hydroxy-4-cyclohexylbutyrate) (PHCHB), mcl-PHA and PHB were biosynthesized for comparative experiments in this study. PHPV and PHPxV were biosynthesized from 5-PVA and 5-PxVA, respectively, by using the method reported previously by us[10]. PHCHB was biosynthesized by culturing *P. cichorii* strain YN2 in M9 medium containing 4-CHBA and yeast extract (Bacto® Yeast Extract, Difco Laboratories, Detroit, USA) for 24 hours. Mcl-PHA was biosynthesized by using the same method as described in this paper, excepts for using *n*-nonanoate as a solo carbon source instead of benzoylalkanoate and D-glucose. PHB was obtained by culturing *Alcaligenes* sp. strain TL2 with sodium lactate as a solo carbon source[8].

2.3. Analytical methods

The molecular weight of the polymer was measured by using gel permeation chromatography (GPC) at 30°C with a TOSOH HLC-8020 GPC system and a refractive index detector with a PL gel MIXED-C column (5 μM, Polymer Laboratory). Chloroform (HPLC grade) was used as an eluent. Polystyrene standards with low polydispersity (Polymer Laboratory) were used to generate calibration curves.

To find the polymer composition, 5 mg of polymer sample was mixed with 2 ml of 3.0% (vol./vol.) sulfuric acid in methanol and 2 ml of chloroform, and the mixture was refluxed for 3.5 hours at 100°C to convert monomer units to their corresponding methyl ester. The mixture was cooled and washed with 10 ml of distilled water. The organic phase was collected, was dried over $MgSO_4$, and was analyzed by using a gas chromatography-mass spectrometer (GC-MS) (Shimadzu QP-5050A) with a J&W DB-WAXETR capillary column (30 m×0.32 mm×0.5 μm; carrier gas, He, 2.2 ml/min.; temperature program, 80°C for 5 min., then the temperature was increased at 5°C/min. to 200°C and remained at this temperature).

Proton (^1H) and carbon (^{13}C) NMR spectra were recorded by using a Brucker FT-NMR DPX400 spectrometer at 400 MHz and 100 MHz, respectively, at the following experimental conditions: 0.5% (wt./vol.) polymer sample in chloroform-*d*, 298 K, tetramethylsilane was used as an internal reference.

Differential scanning calorimetry (DSC) was done by using Perkin Elmer Pyris 1. A polymer sample was tested from –50 to 200°C in a nitrogen atmosphere at a heating rate of 10°C/min. The T_g and the T_m were taken from the inflection point.

Thermogravimetry-differential thermalanalysis (TG-DTA) was done by using MAC Science TG-DTA 2000S. A polymer sample was tested from room temperature to 300°C in an air flow (100 ml/min.) at a heating rate of 10°C/min.

3. Results and Discussion

3.1. Biosynthesis of polymers

Table 1 lists the cell dry weights and polymer yields of the cultures of *P. cichorii* strain YN2 grown with different unusual carbon sources. Cell growth and PHA production depend on the used unusual carbon source. Table 1 shows that the produced cell and polymer using 5-BzVA and 7-BzHpA were greater than those obtained by using 4-BzBA, 6-BzHxA and 8-BzOA.

The cell dry weight results of the cultures of *P. cichorii* strain YN2 grown with 5-BzVA, 5-PVA and 5-PxVA were 0.89 g/L, 1.30 g/L and 0.84 g/L, respectively. These results show that benzoylalkanoate has not marked growth inhibitory effect compared with phenoxyalkanoate. The polymer yields of the cultures of *P. cichorii* strain YN2 grown with 5-BzVA, 5-PVA and 5-PxVA were 0.30 g/L, 0.94 g/L and 0.40 g/L, respectively, and indicate that benzoylalkanoate

Table 1. Production of PHAs by *Pseudomonas cichorii* strain YN2 in a two-stage cultivation in the media containing benzoylalkanoate.

polymer	Unusual carbon source	Dry cell weight (g/l)	Polymer yield (g/l)	Polymer content[a] (%wt.)
1	4-BzBA	0.31	0.01	3
2	5-BzVA	0.89	0.30	34
3	6-BzHxA	0.71	0.18	25
4	7-BzHpA	1.44	0.59	41
5	8-BzOA	0.71	0.17	24
PHPV	5-PVA	1.30	0.94	72
PHPxV	5-PxVA	0.84	0.40	48
PHCHB	4-CHBA	1.10	0.23	21
mcl-PHA	—	1.19	0.51	43
PHB	—	0.71	0.56	79

[a] Percentage of polymer yield per dry cell weight

and phenoxyalkanoate are comparatively hard to incorporate into polymer compared with phenylalkanoate.

3.2. Characterization of polymers

The polymers obtained from *P. cichorii* strain YN2 were methanolized to the methyl ester of the repeating units, and the products were analyzed by using a GC-MS.

Some total ion chromatogram (TIC) peaks were detected in the gas chromatogram of the polymer biosynthesized from 5-BzVA (data not shown). TIC minor peaks were identified as the methyl ester from medium-chain-length alkyl 3-hydroxyalkanoate units (3-hydroxyoctanoate methyl ester, 3-hydroxydecanoate methyl ester, 3-hydroxydodecanoate methyl ester and 3-hydroxydodecenoate methyl ester). But the main peak (retention time, R.T.=54.8 min.), which was 80% of the total peak area, was previously unknown. Unknown peaks were also detected in the chromatograms of the analysis of the other polymer samples biosynthesized from 4-BzBA (R.T.=52.6 min.), 6-BzHxA (R.T.=69.8 min.), 7-BzHpA (R.T.=54.8 and 192.5 min.) and 8-BzOA (R.T.=70.5 and approx. 550 min.; the latter was a broad peak).

These unknown GC peaks were identified by mass spectroscopy measurements (Table 2). Characteristic *m/z* peaks derived from the benzoyl structure (*m/z*=77, 105) were detected in both the unknown peaks. The *m/z* peaks of the fragment of the benzoyl structure and proximal methylene group (*m/z*=120, 121) were also detected in the unknown peaks except for the R.T. 52.6 min. peak. Therefore these unknown GC peaks were derived from methyl esters of monomeric units of PHAs that have benzoyl groups. Judging from the retention times and the maximum *m/z* peaks in each mass pattern, the unknown GC peaks were estimated to derive from the structures shown in Table 2.

The polymers were characterized by NMR spectroscopy. Fig. 1 and 2 show ^{1}H- and ^{13}C-NMR spectra of the polymer biosynthesized from 5-BzVA. Chemical shifts and patterns of peaks corresponded to those expected from a PHA containing 3-hydroxy-5-benzoylvalerate units. Therefore the polymer was found to be a PHA containing 3-hydroxy-5-benzoylvalerate units. Similarly, the polymers biosynthesized from 4-BzBA, 6-BzHxA, 7-BzHpA or 8-BzOA were judged from their ^{1}H-NMR spectra to be PHAs containing 3-hydroxybenzoylalkanoate units (data not shown). We believe that PHAs containing benzoyl groups have not been reported, and therefore this study may be the first report.

Table 3 shows monomer unit compositions (GC-MS, TIC peak area proportion) of the polymers. The polymer biosynthesized from 5-BzVA contained 3-hydroxy-5-benzoylvalerate units and usual (alkyl) 3-hydroxyalkanoate

Table 2. Mass spectroscopy measurement of PHAs produced by *Pseudomonas cichorii* strain YN2 cultivated in the media containing benzoylalkanoate.

polymer	Retention time of GC peak (min.)	Main *m/z* peaks	Structure	Molecular weight
1, 3, 5	52.6	51, 58, 63, 77, 95, 105, 115, 144, 172	3-hydroxy-4-benzoylbutyrate methyl ester	222
2, 4	54.8	41, 51, 59, 71, 75, 77, 85, 89, 105, 117, 120, 131, 144, 159,177, 187, 218	3-hydroxy-5-benzoylvalerate methyl ester	236
3, 5	68.8, 70.5	41, 51, 59, 71, 75, 77, 85, 105, 117, 120, 133, 147, 159, 173, 191, 200, 232	3-hydroxy-6-benzoylhexanoate methyl ester	250
4	192.5	43, 51, 55, 71, 77, 91, 105, 120, 133, 145, 159, 173, 197, 215, 228, 246	3-hydroxy-7-benzoylheptanoate methyl ester	264
5	applox. 550	41, 44, 57, 63, 69, 77, 91, 105, 121, 256	3-hydroxy-8-benzoyloctanoate methyl ester	278

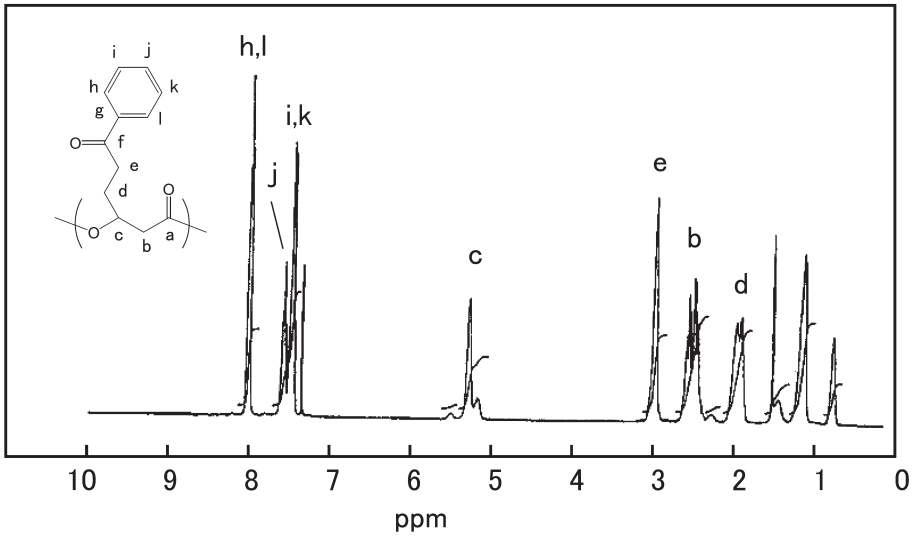

Fig. 1. ^{1}H-NMR spectrum of the polymer synthesized from 5-BzVA by *Pseudomonas cichorii* strain YN2.

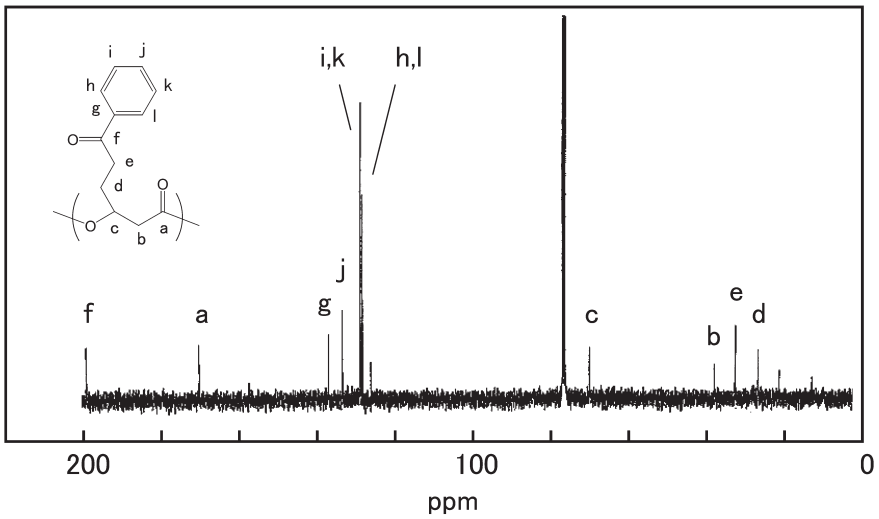

Fig. 2. ^{13}C-NMR spectrum of the polymer synthesized from 5-BzVA by *Pseudomonas cichorii* strain YN2.

Table 3. Relative amount of repeating units in PHA produced by *Pseudomonas cichorii* strain YN2 with two-stage cultivation in the media containing benzoylalkanoate.

polymer	Unusual carbon source	Relative amount of monomeric units in polymer (%)[a]					
		3HBzB[b]	3HBzV[c]	3HBzHx[d]	3HBzHp[e]	3HBzO[f]	usual-3HA[g]
1	4-BzBA	8.3	—	—	—	—	91.7
2	5-BzVA	—	79.8	—	—	—	20.2
3	6-BzHxA	—	—	69.8	—	—	30.2
4	7-BzHpA	—	30.8	—	34.2	—	35.0
5	8-BzOA	—	0.2	37.8	—	49.0	13.0

[a] GC-MS, peak area %, [b] 3-hydroxy-4-benzoylbutyrate, [c] 3-hydroxy-5-benzoylvalerate, [d] 3-hydroxy-6-benzoylhexanoate, [e] 3-hydroxy-7-benzoylheptanoate, [f] 3-hydroxy-8-benzoyloctanoate, [g] alkyl 3-hydroxyalkanoate (C_6–C_{14}).

units at 79.8% and 20.2%, respectively. Similarly, the polymer biosynthesized from 6-BzHxA contained 3-hydroxy-6-benzoylhexanoate units at 69.8%. However, the polymer biosynthesized from 4-BzBA contained only 8.3% of 3-hydroxy-4-benzoylbutyrate units and the major part (91.7%) consisted of usual 3-hydroxyalkanoate units. Because the yield of the polymer was also smaller than from other benzoylalkanoates, 4-BzBA may be hard to incorporate

into PHA because of the shortage of its alkyl chain length.

The polymer biosynthesized from 7-BzHpA contained 3-hydroxy-7-benzoylheptanoate units and 3-hydroxy-5-benzoylvalerate units (the chain length decreased by two $-CH_2-$). The polymer biosynthesized from 8-BzOA contained 3-hydroxy-8-benzoyloctanoate units and 3-hydroxy-6-benzoylhexanoate units (the chain length decreased by two $-CH_2-$). These short length units may be produced by β-oxidation reported by previous studies[13,21].

In GC-MS experiments of PHPV, PHPxV, PHCHB, mcl-PHA and PHB, PHPV, PHPxV, PHCHB and PHB were homopolymers consisting of 3-hydroxy-5-phenylvalerate units, 3-hydroxy-5-phenoxyvalerate units, 3-hydroxy-4-cyclohexylbutyrate units and 3-hydroxybutyrate units, respectively. The relative amount of monomeric units in mcl-PHA was (GC-MS, TIC peak area %): 3-hydroxyvalerate unit, 1%; 3-hydroxyheptanoate unit, 29%; 3-hydroxyoctanoate unit, 3%; 3-hydroxynonanoate unit, 67%.

Table 4 show the molecular weights of the polymers measured by GPC experiments. The molecular weight of the polymer biosynthesized from 5-BzVA (M_n=330,000, M_w=1,300,000) was markedly higher than for other polymer samples (M_n=18,000 to 41,000, M_w=42,000 to 93,000).

Incorporation of a 3-hydroxy-5-benzoylvalerate unit may influence the molecular weight of a polymer, but this mechanism is unknown. 5-BzVA or 3-hydroxy-5-benzoylvalerate units may inhibit the end of the biosynthesis of the polymer chain, of which cause is unknown. But the comparatively high M_w/M_n value of the polymer (M_w/M_n=3.9) suggests the intermolecular binding is caused in some way by the benzoyl groups.

The biodegradation property of the polymer biosynthesized in this study is of high interest to us, but it remains unknown.

One of the expected characteristics of biosynthesized PHAs would be their biodegradability. Some scl- and mcl-PHAs are degraded by microorganisms in the natural environment with relative ease, and the details have been studied[18,20], but information on biodegradation of unusual-PHAs is limited.

We have not tested the biodegradability of the PHA containing benzoyl groups, but the unusual-PHA containing similar aromatic side chains were degraded rapidly in compost (data not shown). PHAs containing 3-hydroxy-5-phenylvalerate or 3-hydroxy-6-(4-cyanophenoxy)hexanoate as unusual monomeric units were degraded by some *Pseudomonas* strains[3,5,7]. These results indicate a potential of the PHA containing benzoyl groups to be degraded by natural microbes; further study is needed to show the biodegradation property of the PHA containing benzoyl groups.

3.3. Thermal analysis of polymers

The texture of the polymer biosynthesized from 5-BzVA is relatively hard at room temperature, which indicates that this polymer has characteristic thermal properties.

To find if incorporation of benzoyl groups influences thermal properties of the polymer, DSC and TG-DTA analyses were done. As references, PHPV, PHPxV, PHCHB, mcl-PHA, and PHB were also tested.

Table 5 shows the thermal properties of these PHAs, and Fig. 3 shows the DSC thermogram of the polymer biosynthesized from 5-BzVA. The T_g value estimated by the result of the second heating scan was 36°C, and the T_m value estimated by the result of the first heating scan was 150°C (Fig. 3). T_g and T_m values of the other unusual- and mcl-PHAs tested were lower than these values. The thermal decomposition temperatures of the polymers were about the same, and a crystallization of the polymer was not clearly observed, except for PHB.

We believe such high T_g and T_m values of biosynthesized unusual-PHAs have not been reported. So the thermal properties of the polymer in this study stand out from those of other biosynthesized unusual-PHAs.

A high molecular weight can cause high T_g or T_m values or both, but mcl-PHA (M_n=170,000, M_w=350,000) and PHB (M_n=1,800,000, M_w=2,600,000), which have relatively

Table 4. Molecular weight of PHA produced by *Pseudomonas cichorii* strain YN2 in a two-stage cultivation in the media containing benzoylalkanoate.

polymer	Unusual carbon source	Molecular weight of polymer		
		M_n[a]	M_w[b]	M_w/M_n
1	4-BzBA	18,000	42,000	2.3
2	5-BzVA	330,000	1,300,000	3.9
3	6-BzHxA	24,000	62,000	2.6
4	7-BzHpA	23,000	53,000	2.3
5	8-BzOA	41,000	93,000	2.3
PHPV	5-PVA	69,000	120,000	1.7
PHPxV	5-PxVA	74,000	150,000	2.0
PHCHB	4-CHBA	49,000	100,000	2.0
mcl-PHA	—	170,000	350,000	2.1
PHB	—	1,800,000	2,600,000	1.4

[a] Average molecular number estimated by GPC, [b] Average molecular weight estimated by GPC

Table 5. Thermal properties of PHA.

polymer	Unusual carbon source	T_g[a] (°C)	T_m[b] (°C)	Thermal decomposition[c] (°C)
2	5-BzVA	36	150	278
PHPV	5-PVA	19	58	281
PHPxV	5-PxVA	23	88	284
PHCHB	5-CHBA	23	n.d.[d]	n.t.[e]
mcl-PHA	—	–35	50	280
PHB	—	5	182	274

[a] Glass transition temperature detected by DSC, [b] melting point detected by DSC, [c] detected by TG-DTA, [d] not detected, and [e] not tested.

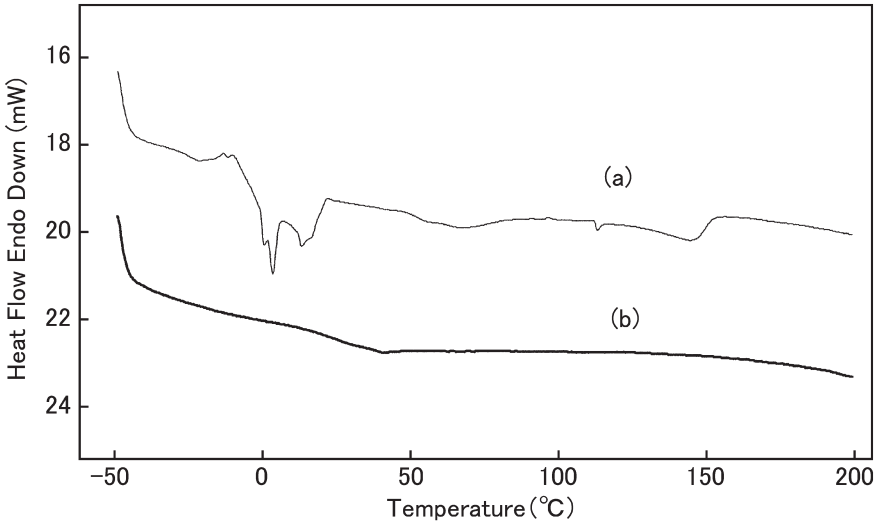

Fig. 3. DSC thermogram of the polymer synthesized from 5-BzVA by *Pseudomonas cichorii* strain YN2.
(a) First heating scan of the polymer, (b) Second heating scan of the polymer.

high molecular weights, had no such high T_g or T_m values. Therefore, incorporation of benzoyl groups may influence the thermal properties of the polymer.

However, the appearances and the thermal properties of the polymers biosynthesized from 4-BzBA, 6-BzHxA, 7-BzHpA, and 8-BzOA were similar to those of the other unusual-PHAs (data not shown). Therefore, in addition to molecular weight and incorporation of benzoyl groups, the length of the side chain containing a benzoyl group and the number of monomeric units can also influence thermal properties of a polymer.

Incorporation of 3-hydroxy-5-benzoylvalerate units into a PHA at a certain monomeric-unit-proportion may affect the molecular weight and the thermal properties of the PHA. The mechanism of this is unknown, but one hypothesis may be that the steric hindrance and the intermolecular binding derived by the benzoyl group cause the molecular weight and the thermal properties of the polymer. If the steric hindrance derived by the benzoyl group limits the molecular motion and the stereochemical configuration of the polymer intra- or inter-molecularly or both, the T_g and T_m values of the polymer will increase.

Usual-PHAs, consisting of scl- or mcl-3-hydroxyalkanoate units (3-hydroxybutyrate unit, 3-hydroxyvalerate unit, 3-hydroxyhexanoate unit etc.) or both, have been studied in great detail, for example, by Doi et al.[4,17,20]. But this study differs from other studies in the chemical structure of PHAs, which causes important differences in properties and functions of the polymer.

PHA containing 3-hydroxy-5-benzoylvalerate units shows characteristic thermal properties. Especially, the T_g value of the polymer is much higher than for PHB, a representative scl-PHA, and typical mcl-PHA. Unusual-PHAs containing aromatic groups, such as benzoyl substituents, are estimated to differ in the solubility in organic solvent from usual-PHAs. These differences, which may be caused by the chemical structure, are important when considering industrial process and practical use.

The polymers in this study are classified as unusual-PHAs, not usual-PHAs. Usual-PHAs are generally expected to be 'earth-friendly plastics' or 'biomaterials' that are biodegradable, reproducible and biocompatible. Unusual-PHAs are expected to be 'functional polymers' that have useful functions derived from their functional groups, in addition to environmental suitability and biocompatibility.

This study is basic research on novel PHAs containing

benzoyl groups, and its main purposes were: (1) to test if monomeric units that have a benzoyl group can be incorporated into biosynthetic PHA, (2) to check if the unit can be easily incorporated compared with other monomeric units, (3) to show some basic properties of PHAs containing benzoyl groups. Also examining the cost of using PHA in the future is necessary.

A wide range of applications of PHAs have been hampered by high production cost of PHAs compared with conventional petrochemical-based plastics. Much effort has been devoted to reduce the cost of PHA by increasing the cell density and polymer yield in the fermentation process, and positive results have been achieved in producing scl-PHAs (PHB, etc.) and mcl-PHAs by microorganisms[14,15]. For example, a mcl-PHA is produced by *P. putida* at a high cell density and high polymer yield (141 g/L and 72.6 g/L, respectively) when using the high-cell-density fed-batch fermentation technique with phosphorus limitation[15]. Cell dry weights (0.31 to 1.44 g/L) and polymer yields (0.01 to 0.59 g/L) in this study may not be sufficient for a wide use, so using such a fermentation technique will be necessary to increase cell dry weight and polymer yield.

We believe this is the first report on PHAs containing benzoyl groups. Our results suggest that PHAs containing benzoyl groups have potential for use, to which existing unusual-PHAs cannot be used because of their thermal properties. Especially, polymers that have a hard texture at room temperature are useful. Our novel polymers widen the range of use of biodegradable polymers.

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