

SHORT COMMUNICATIONS

## Comparative Study of Effects of Thio/Oxo Ester Linkages on Thermal Properties of Bacterial Poly[3-hydroxybutyrate-co-3-(mercapto/hydroxy)propionate]s

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Bacterially synthesized poly(3-hydroxybutyrate) [P(3HB)], a naturally occurring thermoplastic, has attracted much attention of industrialists and academia for its biodegradability and biocompatibility. Wide range of properties of P(3HB), such as microstructure, thermal behavior, mechanical properties, enzymatical degradation process, and so forth, have been intensively studied.<sup>1–3</sup> However, there exists several deficiencies of P(3HB) as an engineering material, such as its inherent brittleness and thermal instability above its melting point.

To modify the properties of P(3HB), a variety of poly(hydroxyalkanoate)s [PHAs] containing 3HB unit have been reported.<sup>3–5</sup> For example, a series of poly(3-hydroxybutyrate-co-3-hydroxypropionate)s [P(3HB-co-3HP)s]<sup>6–8</sup> and poly(3-hydroxybutyrate-co-3-hydroxyvalerate)s [P(3HB-co-3HV)s].<sup>9–11</sup>

It has been known that the bacterially as-produced copolymers, such as P(3HB-co-3HP)<sup>8,12,13</sup> and P(3HB-co-3HV),<sup>9–11,14</sup> have a broad comonomer unit compositional distribution, and they are practically mixtures (blends) of random copolymers having different comonomer unit composition. It has been found that solid-state structures and properties of bacterial copoly(hydroxyalkanoate)s depend not only on their chemical structures but also on their comonomer unit compositional distribution.<sup>6–15</sup>

Recently, poly(3-hydroxybutyrate-co-3-mercaptopropionate) [P(3HB-co-3MP)] has been bacterially synthesized.<sup>16–18</sup> It was the first example on a bacterial PHA containing sulfur atoms in the backbone. The properties of polymers containing sulfur atoms in the backbone linkage are expected to be different from those of polymers containing oxygen atoms in the corresponding linkage. For example, the melting point of poly(ethylene sulfide) ( $T_m = 216^\circ\text{C}$ )<sup>19</sup> is significantly

higher than that of its counterpart polymer with oxygen atoms at the corresponding positions, that is, poly(ethylene oxide) ( $T_m = 66–69^\circ\text{C}$ ).<sup>20,21</sup> It is reasonable to expect that physical properties of P(3HB-co-3MP) differ more or less from those of its counterpart, that is, P(3HB-co-3HP).

In this communication, thermal properties of bacterial P(3HB-co-3MP)s with different 3MP content are characterized. As mentioned above, bacterial copoly(hydroxyalkanoate)s have more or less comonomer unit compositional distribution, at first we investigate the compositional distribution for P(3HB-co-3MP) samples. Then, the effects of comonomer unit composition and its distribution on the thermal properties of P(3HB-co-3MP)s will be investigated by using compositionally well-fractionated samples. The results will be compared with those of compositionally well-fractionated P(3HB-co-3HP)s.

### EXPERIMENTAL

#### *Materials and Methods*

P(3HB-co-3MP) was synthesized by *Ralstonia eutropha* H16 using sodium gluconate and 3,3'-thiodipropionic acid as the carbon sources.<sup>16–18</sup> The biosynthetic procedures in details were described elsewhere.<sup>16–18</sup>

#### *Fractionation of as-produced P(3HB-co-3MP)*

Bacterial P(3HB-co-3MP) denoted as “original” sample was fractionated with a chloroform/*n*-hexane mixed solvent at ambient temperature according to the procedures, previously applied for P(3HB-co-3HV) and P(3HB-co-3HP) as described elsewhere.<sup>10–15</sup>

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### <sup>1</sup>H NMR Measurement

To determine the 3MP unit composition of as-produced as well as fractionated samples of P(3HB-co-3MP), 270 MHz <sup>1</sup>H NMR spectra were recorded on a JEOL GSX-270 spectrometer at 30 °C in CDCl<sub>3</sub> solutions.

### Characterization by Gel Permeation Chromatography (GPC)

Number averaged molecular weight ( $M_n$ ), weight averaged molecular weight ( $M_w$ ) and polydispersity ( $M_w/M_n$ ) of as-produced as well as fractionated samples were characterized by a Tosoh HLC-8020 GPC system with TSK GEL G2000Hxl and GMHxl columns. Chloroform was used as the eluant at a flow rate of 1.0 mL/min. Polystyrenes with narrow molecular distribution were used as standards to calibrate the GPC elution curve.

### Measurement of Differential Scanning Calorimetry (DSC)

Calorimetric measurement of the copolyester (3–5 mg), which was encapsulated in an aluminum pan, was carried out on a Seiko DSC-220. Before the measurement, the film samples cast from chloroform solution were kept for more than 1 month at ambient temperature. The DSC thermogram was recorded by heating sample from –10 °C to 200 °C (first heating run) in order to measure the melting point  $T_m$ . After the first heating run, the sample was quenched to –70 °C with liquid N<sub>2</sub>. Then the sample was heated again from –70 °C to 200 °C. (second heating run) to measure glass transition temperature  $T_g$ .

## RESULTS AND DISCUSSION

Several P(3HB-co-3MP) samples with different 3MP unit contents were synthesized by fermentation of *Ralstonia eutropha* H16 with 3,3'-thiodipropionic acid and sodium gluconate as the carbon sources. In this communication, the results of only one typical example of P(3HB-co-3MP) samples with the original 3MP unit content of 18 mol % are reported. The as-biosynthesized P(3HB-co-3MP), which was denoted "original" sample in order to distinguish from their fractions, was fractionated with a chloroform/*n*-hexane mixed solvent. The molecular characteristics of the original and its fractions were described in Table I, indicating that the 3MP content of the fractionated P(3HB-co-3MP) gradually decreases with increasing the concentration of *n*-hexane in mixed solvent, at least up to the fraction f-5. The molecular weight of the following fractions gradually decreased with an increasing concentration of *n*-hexane, and it seemed that the copolymer was also fractionated based on the molecu-

**Table I.** Fractionated results for P(3HB-co-18 mol %3MP) sample

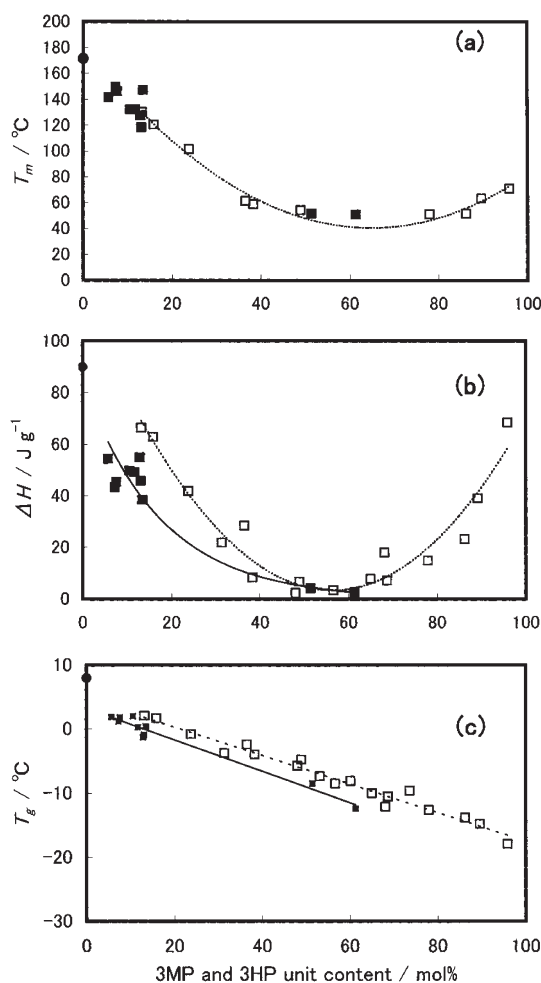
Fraction no.	Conc. of <i>n</i> -hexane	wt %	3MP content <sup>b</sup>	10 <sup>-5</sup> $M_n$	$M_w/M_n$
	%		mol %		
f-0 <sup>a</sup>	—	100	18	3.3	2.4
f-1	46.4	9.6	62	1.9	1.8
f-2 <sup>c</sup>	50.8	16.5	31	1.7	3.9
f-3	53.1	14.7	12	6.1	1.7
f-4	53.8	10.7	8	7.1	1.6
f-5	54.5	14.7	7	7.2	1.5
f-6	55.2	3.1	13	5.3	2.0
f-7	56.5	17.7	9	4.7	1.9
f-8	58.0	2.8	12	2.9	1.6
f-9	60.0	2.5	13	1.1	1.5
f-10 <sup>d</sup>	—	5.2	23	0.2	2.3

<sup>a</sup>Original sample. <sup>b</sup>Average 3MP content calculated from the 3MP unit contents of fractions was 18.6 mol %. <sup>c</sup>f-2 was re-fractionated into two-fractions, soluble and insoluble in 1/2 (v/v) chloroform/*n*-hexane mixed solvent at 70 °C, with 3MP unit content 14 and 51 mol %, respectively. <sup>d</sup>The residue recovered from the supernatant solution of the former fractionation step.

lar weight as well as the 3MP content. As mentioned below the f-2 was re-fractionated.

To investigate the effects of compositional distribution on the physical properties of P(3HB-co-3MP), the thermal properties were studied by DSC measurements. The DSC thermogram of the original P(3HB-co-3MP) showed the multiple melting peaks in the first heating run, indicating the original sample is the mixture of P(3HB-co-3MP)s with different 3MP unit content. The f-2 showed two melting peaks (data are not shown here), indicating the first comonomer unit compositional fractionation was not effective for the f-2. Thus, this fraction was re-fractionated into two fractions, that is, the soluble and insoluble fractions with 3MP unit content 14 mol % and 51 mol %, respectively, in the mixed solvent 1/2 (v/v) chloroform/*n*-hexane at 70 °C. Each of all fractions, including two fractions obtained by re-fractionation of the f-2, showed well defined DSC single melting peak, indicating that the comonomer unit compositional distribution became narrower after fractionation than that of original sample.

In the previous papers, the thermal properties have been reported for fractionated samples of P(3HB-co-3HP).<sup>8</sup> The effects of monomeric unit content on the thermal properties of bacterial P(3HB-co-3MP)s were investigated and the results are compared with those of bacterial P(3HB-co-3HP)s. The values of  $T_m$ ,  $\Delta H$ , and  $T_g$  are plotted against the content of the second monomer units (3HP, 3MP) for these copolymers as shown in Figure 1. P(3HB-co-3MP)s exhibit marked thermal properties similarity to P(3HB-co-3HP)s.



**Figure 1.** Plots of (a) melting temperature ( $T_m$ ), (b) heat of fusion ( $\Delta H$ ) and (c) glass transition temperature ( $T_g$ ) vs. 3MP and 3HP unit content (mol%) for fractionated copolymers. ■: P(3HB-*co*-3MP) fractions, □: P(3HB-*co*-3HP) fractions, ●: P(3HB) homopolymer.

As shown in Figure 1, the values of  $T_m$  of P(3HB-*co*-3MP)s as well as P(3HB-*co*-3HP)s decrease significantly with the increase of 3MP/3HP unit content in a similar fashion, suggesting both copolymers form almost the same crystalline morphology. It has been reported that P(3HB-*co*-3HP)s with higher 3HB unit content form the P(3HB) homopolymer type crystalline lattice and only the 3HB units are included in the crystalline phase.<sup>8,12,13</sup> Thus, it is reasonable to consider that P(3HB-*co*-3MP)s with higher 3HB unit content also form similar P(3HB) homopolymer-type crystalline lattice, and the 3MP units are excluded from the crystalline phase.

The values of  $\Delta H$  of both copolymers also decrease markedly with the increase of 3MP/3HP unit content, and reach minimum values close to  $\Delta H \approx 0$  J/g at 3MP/3HP content of about 50–60 mol%. The  $\Delta H$  value reflects the degree of crystallinity. Thus, in the range of 3MP/3HP unit content 0–60 mol%, the de-

gree of crystallinity of P(3HB-*co*-3MP)s as well as P(3HB-*co*-3HP)s decreases with the increase of 3MP/3HP unit content. The  $\Delta H$  values of copolymers with 3MP/3HP unit content about 50–60 mol% are almost zero, and so they are almost amorphous in this compositional range. In the 3MP/3HP unit content ranged from 7 to 15 mol%, the  $\Delta H$  values of P(3HB-*co*-3MP) are clearly lower than those of P(3HB-*co*-3HP)s when both copolymers have the same 3HB unit content, indicating the destructive effect of the 3MP unit on the P(3HB) homopolymer-type crystalline lattice is larger than that of the 3HP unit.

In Figure 1 are also shown the correlations between the glass transition temperature ( $T_g$ ) and the 3MP/3HP unit content of copolyesters. It is seen that the  $T_g$  value of P(3HB-*co*-3MP) decreases linearly with increasing 3MP unit content within the comonomer composition range investigated here. This result is similar to that of P(3HB-*co*-3HP).

In general, higher the segmental mobility of polymer chains in the amorphous phase, lower the value of  $T_g$ . For P(3HB-*co*-3MP) copolymers, the  $T_g$  value decreases with the increase of content of the 3MP unit. Furthermore, the  $T_g$  values of P(3HB-*co*-3MP)s are systematically lower a little than those of P(3HB-*co*-3HP)s at the same 3HB unit content, which may be attributable to the lower crystallinity of the P(3HB-*co*-3MP) as mentioned above.

In conclusion, as-produced P(3HB-*co*-3MP) biosynthesized by *Ralostnia eutropha* from sodium gluconate and 3,3'-thiodipropionic acid was found to be the blend of copolymers with a wide range of comonomer unit content. Although the thermal properties of both P(3HB-*co*-3MP)s and P(3HB-*co*-3HP)s show quite similar dependences on the comonomer unit content, the destructive effect of the 3MP unit on the P(3HB) homopolymer-type crystalline structure was found to be larger than that of the 3HP unit.

It has been reported that the melting point of bacterial poly(3-mercaptopropionate) [P(3MP)] synthesized by recombinant *E. coli* strains was 170 °C,<sup>22</sup> which is much higher than that of chemosynthesized poly(3-hydroxypropionate)[P(3HP);  $T_m \sim 73$  °C<sup>23</sup>]. Thus, it is reasonable to expect that 3MP-unit rich P(3HB-*co*-3MP)s, including P(3MP), may form the crystal with much higher crystallinity or even form the crystalline lattice different from that of 3HP-unit rich P(3HB-*co*-3HP)s with similar 3HB unit content. It will be interesting to investigate the 3MP-unit content dependence of crystalline morphology of 3MP-unit rich P(3HB-*co*-3MP)s, although such polymers except for P(3MP) have so far not been biosynthesized.

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