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Poly([*R*]-3-hydroxybutyrate-*co*-glycolate): A Novel PHB Derivative Chemically Synthesized by Copolymerization of a New Cyclic Diester Monomer [*R*]-4-Methyl-1,5-dioxepane-2,6-dione

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Introduction. Aliphatic polyesters such as poly(α-hydroxyalkanoates), poly(*β*-hydroxyalkanoates) (PHA), and poly(alkylene succinates) have been attracting much attention because of their excellent biomedical applicability, sustainability, and biodegradability in the natural environment.[1] The synthetic pathways for these polymers involve not only the ordinary chemical processes but also biochemical and biosynthetic methodologies. For example, poly(3-hydroxybutyrate) (PHB) and its copolymers are synthesized directly by bacterial fermentation,[2] while poly-L-lactide (PLLA) and polyglycolide (PGA) are chemically prepared by the ring-opening polymerization of cyclic monomers.[3] Although the biosynthesis may be preferable over the chemical synthesis in terms of their material sustainability, the latter is more highly reliable than the former in performing an elaborate molecular and material design of biodegradable polymers.

Bacterial polymer PHB can also be attained by chemical synthesis involving the ring-opening polymerization of [*R*]-*β*-butyrolactone ([*R*]-*β*BL).[4] The merit of this chemical synthesis is to obtain an extremely pure polymer that is free of contamination from a variety of cell substances that are often encountered in bacterial synthesis. Furthermore, the molecular weight and functionality of the products can easily be controlled by application of various chemical modification techniques such as copolymerization. Previously, we demonstrated a functionalization of PHB by using a novel cyclic diester monomer consisting of both glycolate (GA) and benzyl-L-malate (BM) units, i.e., [*S*]-3-[(benzyloxycarbonyl)-methyl]-1,4-dioxane-2,5-dione (BMD).[5] The ring-opening copolymerization of [*RS*]-*β*BL and BMD followed by deprotection of the benzyl groups of the BM units by catalytic hydrogenation gave a carboxyl-functionalized PHB for which various chemical modifications were possible. This technique using such diester monomers with mixed components is easy and thus attractive for the modification of various aliphatic polyesters synthesized by ring-opening polymerization.

This paper describes the synthesis of a novel cyclic diester monomer consisting of [*R*]-3-hydroxybutyrate (3HB) and GA, [*R*]-4-methyl-1,5-dioxepane-2,6-dione ([*R*]-MDP), and its copolymerization with [*R*]-*β*-butyrolactone ([*R*]-*β*BL) to prepare a GA-containing PHB, poly(3-hydroxybutyrate-*co*-glycolate) (P(3HB-GA), see Scheme 1b). This copolymerization using the new monomer [*R*]-MDP can afford a novel synthetic route to the PHB derivative comprising the C_2-hydroxy acid units that cannot be prepared by bacterial fermentation.

Experimental Section. [*R*]-3-Hydroxybutyric acid (97% ee) was supplied by NARD Chemicals, Ltd. (Amagasaki, Japan), and used without purification. [*R*]-*β*BL was supplied by Takasago International Corp. (Hiratsuka, Japan) and purified by repeated distillations over calcium hydride at reduced pressure. 1,3-Dichlorotetrabutyldistannoxane (DTD), which was used as the catalyst, was prepared using the method described in the literature (mp = 113−116 °C).[6]

The synthetic route to [*R*]-MDP is shown in Scheme 1a. In the first step, [*R*]-3-hydroxybutyric acid (20.0 g, 0.192 mmol) was mixed with bromoacetyl chloride (33.4 g, 212 mmol) in diethyl ether (300 mL) at 0 °C. To this mixture stirred vigorously in an ice/salt bath, triethylamine (24.8 g, 245 mmol) dissolved in diethyl ether (50 mL) was added dropwise below 3 °C over a period of 1

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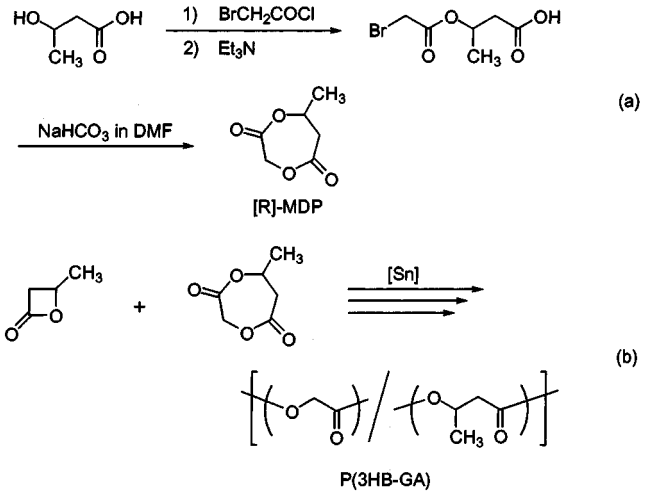
Scheme 1. Synthetic Routes to [*R*]-MDP and P(3HB-GA)



h. The resultant mixture was then stirred at room temperature for 6 h and filtered to remove triethylamine hydrochloride precipitates. The filtrate obtained was washed with a half volume of water several times and dried over sodium sulfate. Concentration of the ethereal solution in vacuo gave a pale yellow viscous liquid. This crude product was identified as [*R*]-3-(bromoacetoxy)-butyric acid (80% yield). ^{1}H NMR (400 MHz in $CDCl_3$), δ (ppm): 1.36 (d, J = 6.0 Hz, 3H, CCH_3), 2.59−2.64 (dd, J = 16.4, 5.2, 1H, CCH_aHCO), 2.71−2.77 (dd, J = 16.4, 7.6, 1H, CCH_bHCO), 3.83 (s, 2H, BrCH_2CO), 5.34 (m, 1H, CHCH$_3$).

In the second step, crude [*R*]-3-(bromoacetoxy)butyric acid (7.58 g, ca. 33.7 mmol) dissolved in *N*,*N*-dimethylformamide (DMF, 70 mL) was added dropwise to a dispersion of sodium bicarbonate (4.28 g, 50.9 mmol) in DMF (1.0 L) over a period of 8 h with vigorous stirring at room temperature. The resulting mixture was further stirred for 12 h and evaporated to dryness in vacuo to yield a brown residue. It was washed with 2-propanol several times, dissolved in acetone, and filtered to remove insoluble byproducts. The filtrate was evaporated in vacuo to recover a white crystalline product. It was sublimed in vacuo at 165 °C for purification. [*R*]-MDP (72% yield): $[\alpha]_D^{25} = -62.1°$ (c = 0.96 g/dL in acetone); mp = 160 °C. IR (KBr): 3005−2935, 1758 ($\nu_{C=O}$), 1733 ($\nu_{C=O}$), 1424−1364, 1306−1260, 1188, 1109, 1043, 960 cm^{-1}. ^{1}H NMR (500 MHz in $CDCl_3$), δ (ppm): 1.35 (d, J = 6.3 Hz, 3H, CCH_3), 2.57−2.60 (dd, J = 18.6, 2.3, 1H, CCH_aHCO), 2.71−2.77 (dd, J = 17.4, 11.0, 1H, CCH_bHCO), 4.26−4.87 (ABq, J = 289.8, 15.8 Hz, 2H, OCH_2CO), 5.33−5.39 (m, 1H, CHCH$_3$). ^{13}C NMR (50.3 MHz in d_6-acetone), δ (ppm): 19.9 (CCH_3), 40.0 (CCH_2-CO), 62.0 (OCH_2CO), 70.1 (OCHCH$_2$), 168.1 (*C*O of GA), 170.0 (*C*O of 3HB). Anal. Calcd for $C_6H_8O_4$: C, 50.00; H, 5.59. Found: C, 49.89; H, 5.55.

A typical procedure for the copolymerization of [*R*]-MDP and [*R*]-βBL was as follows. DTD (22.3 mg, 20.2 μmol) was first charged into a Schlenk-like glass reactor containing a stirrer bar and dried in vacuo at 90 °C for 10 h. Then, both [*R*]-MDP (146 mg, 1.01 mmol) and [*R*]-βBL (348 mg, 4.04 mmol) were added to the reactor, which was sealed and heated in an oil bath with stirring for 4 h. The polymeric product formed was dissolved in chloroform (3 mL) and precipitated into an excess methanol. The precipitate was thoroughly dried at 45 °C in vacuo. P(3HB-GA): IR (KBr) 2985−2930, 1739 ($\nu_{C=O}$), 1456−1383, 1305−1262, 1185−1137, 1102, 1059, 977 cm^{-1}. ^{1}H NMR (500 MHz in $CDCl_3$), δ (ppm): 1.28 (d, J = 6.0 Hz, 3H, CCH_3), 2.48−2.64 (m, 2H, CCH_2-CO), 4.60 (m, 2H, OCH_2CO), 5.27 (m, 1H, CHCH$_3$). ^{13}C NMR (125 MHz in $CDCl_3$), δ (ppm): 19.9 (CCH_3), 60.9 (OCH_2CO), 40.0−41.7 (CCH_2CO), 67.5−68.5 (OCHCH$_2$), 166.6 (CH$_2$*C*O of GA), 169.1 (CH$_2$*C*O of 3HB).

Results and Discussion. It has been known that the PHB copolymers consisting of 3-hydroxypropionate, 4-hydroxybutyrate, and 3-hydroxyalkanoates (3-hydroxyvalerate, 3-hydroxyhexanoate, etc.) can be synthesized by bacterial fermentation,[1] while those modified with 2-hydroxypropionate and hydroxyacetate units (i.e., GA units) can only be prepared by ring-opening copolymerization. Although the copolymerization of [*R*]-βBL and L-lactide can produce the corresponding PHB copolymer in a well-controlled manner,[7] the copolymerization of [*R*]-βBL and glycolide cannot be successfully accomplished because of the predominant formation of a homopolymer of PGA and the minor formation of the GA-containing PHB copolymer, as decribed in a former patent work.[8] To obtain a GA-modified PHB, therefore, development of a new process has been required. As a unique approach for such a new process, we designed a diester monomer [*R*]-MDP consisting of both GA and 3HB components. Its preparation was performed by the base-catalyzed cyclization of the coupling product of [*R*]-3-hydroxybutyric acid and bromoacetyl chloride (Scheme 1a). A high-dilution technique was used to obtain optically active [*R*]-MDP in high yield. Its structural identification was done as summarized in the Experimental Section. The characteristic AB proton signal at δ 4.26−4.87 ppm due to the GA methylene strongly supports the cyclic structure of the final product.

In our first attempt at polymerization, [*R*]-MDP did not homopolymerize by the action of any type of catalysts (cationic, anionic, or metal-coordinated). Despite the poor ring-opening polymerizability of [*R*]-MDP, we found that it underwent copolymerization with [*R*]-MDP using DTD as the initiator. Table 1 summarizes the results of the copolymerization of [*R*]-MDP and [*R*]-βBL examined at various monomer-to-initiator (M/I) and comonomer ratios (βBL/[*R*]-MDP) in feed and at different reaction temperatures. The isolated yield of the copolymers ranged from 55 to 87%, showing a decreasing tendency with an increasing [*R*]-MDP ratio in the monomer feed. The number-average molecular weight did not much change at the present reaction conditions. There was a tendency, however, that the M_n value significantly increased at high M/I ratio (runs 1 and 3) with the polydispersity being wider. In the ^{1}H NMR spectrum of the copolymers, the signals of the methyl, methylene, and methyne protons of the 3HB units can be detected, in addition to the methylene signal of the GA units at δ 4.62 ppm, which is quite different from the corresponding quartet AB methylene signal of [*R*]-MDP. The four large carbon signals at δ 19.9, 40.0−41.7, 67.5−68.5, and 169.1 ppm are reasonably assigned to the 3HB units, while the two small signals at δ 60.9 and 166.6 ppm are assigned to the GA units. The carbon signals of the methylene and methyne of 3HB exhibit a small splitting into two peaks where the larger and smaller peaks are assigned to the homodiad (3HB-3HB) and heterodiad (3HB-GA), respectively. These results indicate that the GA units have randomly been incorporated into the polymer backbone

Table 1. Results of Ring-Opening Copolymerization of [*R*]-*β*BL and [*R*]-MDP[a]

run	feed ratio *β*BL/MDP (3HB/GA)[b]	[M]/[I]	temp (°C)	yield[c] (%)	unit ratio 3HB/GA[d]	M_n[e] (10^{-4} Da)	M_w/M_n[e]	T_g[f] (°C)	T_m[f] (°C)
1	90/10 (91/9)	520	130	87	93/7	2.1	1.9	2.8	117, 137
2	90/10 (91/9)	280	130	82	95/5	1.6	1.5	1.0	106, 132
3	90/10 (91/9)	500	140	85	94/6	1.8	1.8		122, 142
4	80/20 (83/17)	250	120	64	93/7	1.2	1.5	-2.0	119, 137
5	80/20 (83/17)	250	130	72	88/12	1.4	1.4		102, 120
6	80/20 (83/17)	250	140	61	86/14	1.5	1.4	1.2	104, 114
7	80/20 (83/17)	250	150	61	86/14	1.0	1.4		100, 117
8	70/30 (77/23)	250	130	55	87/13	1.1	1.4		97, 115

[a] Polymerized with DTD as the initiator (I) for 4 h. [b] 3HB ratio (%) = 100 × {[*β*BL] + [MDP]}/{[*β*BL] + 2[MDP]} [c] After reprecipitation to methanol. [d] Determined by ^{1}H NMR spectra. [e] Determined by GPC (chloroform eluent) calibrated with polystyrene standards. [f] Determined by DSC in the second scan.

of [*R*]-PHB in the copolymerization of [*R*]-*β*BL and [*R*]-MDP, although further sequence analysis is needed for confirmation.

As summarized in Table 1, the GA content of the copolymers, calculated from the integral ratio of the methyne signals of 3HB and the methylene signals of GA, was slightly lower than that expected from the monomer ratio in feed. This may be attributed to the deposition of unreacted [*R*]-MDP from the reaction system with polymer formation as well as to the lower polymerizability of [*R*]-MDP. A relatively higher GA content was attained at higher reaction temperature (runs 6 and 7), because the deposition of [*R*]-MDP was more effectively inhibited. The GA content, however, did not increase even when the feed ratio of [*R*]-MDP was increased to 30% (run 8). The DSC curves of the copolymers revealed two melting endotherms at significantly lower temperatures than T_m (∼170 °C) of [*R*]-PHB. The endotherm shown at lower temperature can be attributed to the incomplete crystals of [*R*]-PHB that can recrystallize on melting.[9] It was confirmed that the T_m value became lower in the copolymers having a higher GA content. On the other hand, the T_g value of the copolymers was around 0 °C, being similar to that of [*R*]-PHB.

P(3HB-GA) thus prepared is a PHB derivative that is not obtainable by bacterial fermentation. While the ordinary PHB copolymers, consisting of 3- and 4-hydroxyalkanoates, are not susceptible to simple hydrolysis without the action of enzyme, P(3HB-GA) should receive both nonenzymatic and enzymatic hydrolyses. The increased nonenzymatic hydrolyzability of the latter is attributed to the GA units whose hydrolysis is naturally induced in an aqueous environment. The biodegradability of this unique copolymer is now under study.

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