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MINIREVIEW

Biosynthesis and Biodegradation of 3-Hydroxypropionate-Containing Polyesters[▽]

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3-Hydroxypropionate (3HP) is an important compound in the chemical industry, and the polymerized 3HP can be used as a bioplastic. In this review, we focus on polyesters consisting of 3HP monomers, including the homopolyester poly(3-hydroxypropionate) and copolyesters poly(3-hydroxybutyrate-*co*-3-hydroxypropionate), poly(3-hydroxypropionate-*co*-3-hydroxybutyrate-*co*-3-hydroxyhexanoate-*co*-3-hydroxyoctanoate), poly(4-hydroxybutyrate-*co*-3-hydroxypropionate-*co*-lactate), and poly(3-hydroxybutyrate-*co*-3-hydroxypropionate-*co*-4-hydroxybutyrate-*co*-lactate). Homopolyesters like poly(3-hydroxybutyrate) are often highly crystalline and brittle, which limits some of their applications. The incorporation of 3HP monomers reduces the glass transition temperature, the crystallinity, and also, at up to 60 to 70 mol% 3HP, the melting point of the copolymer. This review provides a survey of the synthesis and physical properties of different polyesters containing 3HP.

Bacterial polyhydroxyalkanoates (PHAs) are natural biodegradable thermoplastics produced by various microorganisms as intracellular energy and carbon storage compounds. PHAs have attracted increased attention as possible alternatives to petroleum-based polymers. They are biodegradable, insoluble in water, nontoxic, biocompatible, piezoelectric, thermoplastic, and/or elastomeric. These features make PHAs suitable for several applications in the packaging industry, medicine, pharmacy, agriculture, and food industry, as raw materials for the production of enantiomerically pure chemicals, and for the production of paints (2, 44). The best characterized PHA is poly(3-hydroxybutyrate) [poly(3HB)], which is synthesized by many bacteria (28, 31, 38, 41). Unfortunately, poly(3HB) is a highly crystalline and brittle polymer with a low elongation-to-break factor, which has prevented its use in a wide range of applications. To obtain bacterial PHAs with improved physical and mechanical properties, previous studies have demonstrated the biosynthesis of copolyesters consisting of 3-hydroxybutyrate (3HB) and a second constituent. Pathways for the biosynthesis of such PHA copolyesters, like poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [poly(3HB-*co*-3HV)], poly(3-hydroxybutyrate-*co*-4-hydroxyvalerate) [poly(3HB-*co*-4HV)], and poly(3-hydroxybutyrate-*co*-3-hydroxypropionate) [poly(3HB-*co*-3HP)], occur naturally in many bacteria or have been engineered (5, 40).

Some of these polyesters exhibit material characteristics comparable to those of petrochemical-derived polymers. However, in contrast to petrochemical-based polymers, PHAs are completely biodegradable to CO_2 and water. Another advantage is that they can be produced from renewable resources. Unfortunately, PHA production by bacterial fermentation is costly and, due to inefficient utilization of the resources, not necessarily environmentally convenient in all cases (13).

An example of a bacterium-synthesized copolymer which is competitive with polymers produced from petrochemicals in bulk is poly(3HB-*co*-3HV). *Cupriavidus necator* (32), formerly *Ralstonia eutropha* or *Alcaligenes eutrophus*, accumulates this copolyester when fed with glucose and propionic acid in a phosphate-depleted batch culture (30). The physical properties of poly(3HB-*co*-3HV) resemble those of polyethylene and polypropylene (18). Poly(3HB-*co*-3HV) has been commercially produced through fermentation using a glucose-utilizing mutant of *C. necator* that requires cofeeding of propionic acid for 3-hydroxyvalerate formation. This polymer was sold by ICI (in 1983) under the trade name Biopol.

In general, the morphology and several physical properties of copolymers strongly depend on their comonomer composition and sequence structure (20). A comonomer lowering the melting temperature, crystallinity, and fragility is 3-hydroxypropionate (3HP). 3HP is an industrially relevant product. The putative applications of 3HP are enormous. Among the possible uses as a monomer for (co)polymerization, it could be used as a precursor for the synthesis of other commercially valuable chemicals, like 1,3-propanediol, acrylic acid, or acrylamide (6).

3HP is only produced as a homopolymer from an unrelated carbon source by metabolic engineering in recombinant *Escherichia coli* (3); alternatively, it is chemically synthesized via ring-opening polymerization of β-propiolactone (4, 15, 49). In this paper, we review the synthesis and physical properties of 3HP-containing copolymers and the preparation of the 3HP units (Fig. 1).

CHEMICAL SYNTHESIS OF POLY(3-HYDROXYPROPIONATE)

For the synthesis of 3HP-containing polyesters, a 3HP supply is required. There are several chemical approaches to provide 3HP for copolymer synthesis (45).

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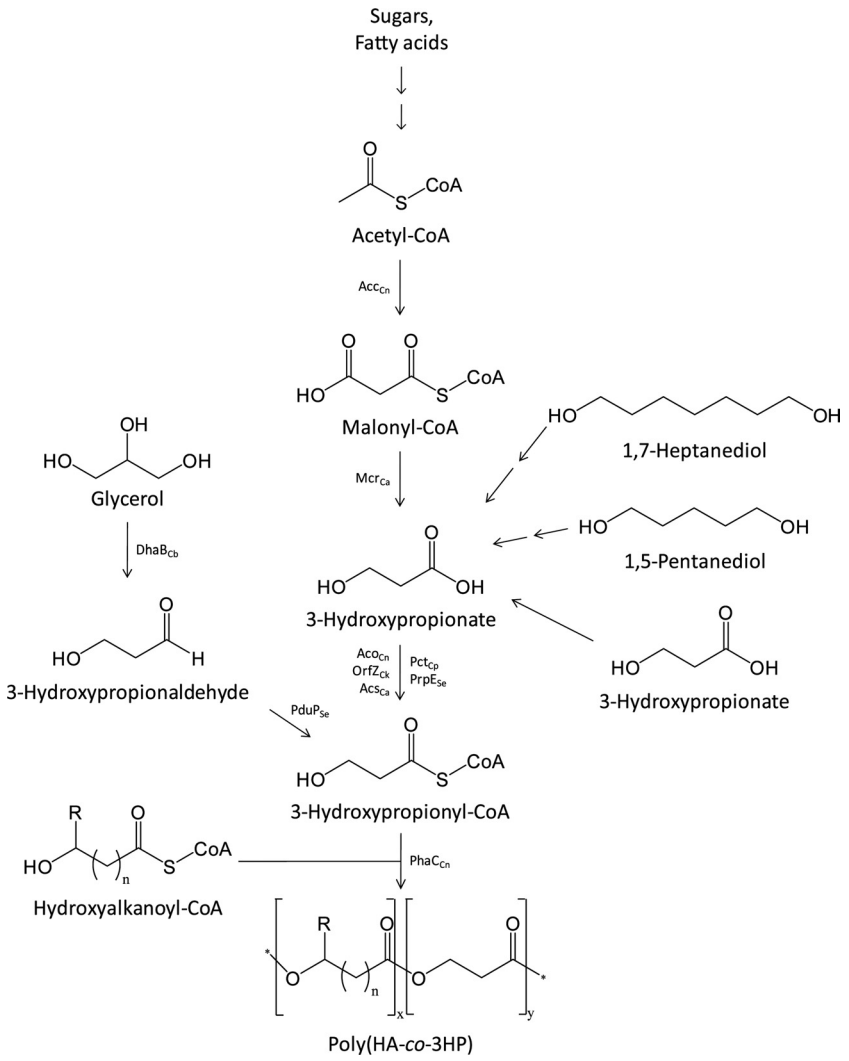

FIG. 1. Artificial pathways for poly(HA-*co*-3HP) accumulation from different carbon sources. Acc$_{Cn}$, acetyl-CoA carboxylase (*C. necator*); Aco$_{Cn}$, acetyl-CoA synthase (*C. necator*); Acs$_{Ca}$, 3HP-CoA synthase domain of propionyl-CoA synthase (*C. aurantiacus*); DhaB$_{Cb}$, glycerol dehydratase (*C. butyricum*); Mcr$_{Ca}$, malonyl-CoA reductase (*C. aurantiacus*); OrfZ$_{Ck}$, acetyl-CoA:4-hydroxybutyrate-CoA transferase (*C. kluyveri*); Pct$_{Cp}$, propionyl-CoA transferase (*C. propionicum*); PduP$_{Se}$, propionaldehyde dehydrogenase (*Salmonella enterica* serovar Typhimurium LT2); PhaC$_{Cn}$, PHA synthase (*C. necator*); PrpE$_{Se}$, propionyl-CoA synthetase (*S. enterica*). The indices (n) at the hydroxyalkanoate moieties indicate the presence of lactate ($n = 0$), 3-hydroxyalkanoates ($n = 1$), and 4-hydroxybutyrate ($n = 4$).

Polymerization of β-propiolactone is a well-known method for the preparation of poly(3HP). Gresham et al. (15) observed the polymerization of β-propiolactone by heating (150°C) in the presence of different acids, bases, and salts. The most effective catalysts were ferric chloride, stannic chloride, sulfuric acid, and sodium hydroxide. Ring-opening polymerization with lanthanide alkoxides such as decamethylsamaroceneethylate diethyl ether [SmOEt(C_5Me_5)$_2$(Et_2O)], di-decamethylyttrocenemethylate ([YOMe(C_5H_5)$_2$]$_2$), and decamethylyttrocenemethylate tetrahydrofuran [YOMe-(C_5Me_5)$_2$(THF)] at 0°C is very effective to obtain high-molecular-weight poly(3HP)s. They have average molecular numbers of up to 63,000 and were formed with conversion rates from 86 to 91% (49).

As β-propiolactone is a human carcinogen, other methods of synthesis were attempted. Based on the commercially available and noncarcinogenic 3HP, poly(3HP) was synthesized by Nanba et al. (35). The polymer was prepared by condensation of the corresponding hydroxycarboxylic acid in the presence of a transesterification catalyst at 70°C. In comparison to the ring-opening reaction, the condensation process has a lower degree of control over molecular weight, comonomer incorporation, and end-group definition. To combine the benefits of the ring-opening reaction with the advantages of 3HP condensation, poly(3HP) was polymerized from macrocyclic esters constructed from 3HP (51). The macrocyclic 3HP polyesters were obtained by acid-catalyzed self-condensation with concomitant removal of water.

To the best of our knowledge, chemical synthesis of 3HP-containing copolyesters has not yet been described.

BACTERIAL SYNTHESIS OF POLY(3HB-*co*-3HP) COPOLYMERS

The best characterized copolymer containing 3HP constituents is poly(3HB-*co*-3HP). The homopolymer of 3-hydroxybu-

TABLE 1. Physical properties of 3-hydroxypropionate-containing polyesters and their natural or artificial synthesis from different carbon sources

Polymer	Carbon substrate(s)[a]	Polyester content (% CDW)	Monomer composition (mol% [range])	M_n (10^{-5})	M_w/M_n	Organism	Gene(s) involved	Reference(s)
Poly(3HP)	Glycerol	5–12	3HP (100)			*E. coli*	*dhaB1B2*$_{Cp}$, *pduP*$_{Se}$, *phaC1*$_{Cn}$	3
Poly(3HB-*co*-3HP)	1,5-Pentanediol	1–42	3HP (1–3), 3HB (97–99)	0.4–2.1	2–6.5	*C. necator*	*phaC1AB*$_{Cn}$, *acoE*$_{Cn}$	34
	1,7-Heptanediol	10–13	3HP (2–4), 3HB (96–98)	1.4–1.8	2.4–2.6	*C. necator*	*phaC1AB*$_{Cn}$, *acoE*$_{Cn}$	34
	3-Hydroxypropionate	11–50	3HP (13–22), 3HB (12–87)	1.1–4.0	2.1–4.5	*A. lata*	*phaC1AB*$_{Cn}$	19, 39
	3-Hydroxypropionate	1–23	3HP (4–7), 3HB (93–96)	5.9–14	1.6–2.5	*C. necator*	*phaC1AB*$_{Cn}$, *acoE*$_{Cn}$	34
	3-Hydroxypropionate	12–16	3HP (13–27), 3HB (73–97)			*E. coli*	*phaC1AB*$_{Cn}$, *prpE*$_{Se}$	43
	3-Hydroxypropionate	10–14	3HP (17–21), 3HB (79–83)			*E. coli*	*phaC1AB*$_{Cn}$, *orfZ*$_{Ck}$	43
	3-Hydroxypropionate	3.9–11.8	3HP (4.3–10.5), 3HB (89.5–95.7)			*Methylobacterium* sp. strain KCTC 0048	*phaC1AB*$_{Cn}$	23
	Sugars, fatty acids	31–53	3HP (0.4–2.1), 3HB (97.9–99.6)			*C. necator*	*phaC1AB*$_{Cn}$, *mcr*$_{Ca}$, *acs*$_{Ca}$	16
Poly(4HB-*co*-3HP-*co*-2HP)	3-Hydroxypropionate					*E. coli*	*pct*$_{Cp}$, *ptb*$_{Ca}$, *buk*$_{Ca}$	37
Poly(3HB-*co*-3HP-*co*-4HB-*co*-2HP)	3-Hydroxypropionate					*E. coli*	*pct*$_{Cp}$, *ptb*$_{Ca}$, *buk*$_{Ca}$	37
Poly(3HP-*co*-3HB-*co*-3HH-*co*-3HO)	Octanoate, acrylic acid	11.8	3HP (1.4–6.5), 3HB (81.7–95.9), 3HH (2.1–10.2), 3HO (0.6–1.6)	6.3–8.0	2.3–2.5	*C. necator*	*phaC1AB*$_{Cn}$, *phaC1*$_{Ps}$	14

[a] Carbon substrate converted into the 3HP monomer.

tyrate (3HB) is synthesized by various bacteria, including *C. necator*, *Bacillus megaterium*, and *Azotobacter vinelandii* (28).

The 3HB-based copolyesters containing an additional monomer unit without a side chain, poly(3HB-*co*-3HP), could be accumulated by several (natural or artificial) pathways (Table 1). For example, it was synthesized via the *phaC1AB* operon of *C. necator* (*phaC1AB*$_{Cn}$) with 3-hydroxypropionic acid, 1,5-pentanediol, or 1,7-heptanediol as the sole carbon source (Fig. 1) and accumulated only up to 7 mol% of the copolymer. In the case of the diols, the authors postulated the formation of 3-hydroxypropionyl-coenzyme A (CoA) via an oxidative cycle which was not defined in detail (34).

Another attempt to assemble poly(3HB-*co*-3HP) was described by Kang et al. (23). The authors cultivated *Methylobacterium* sp. strain KCTC 0048 with methanol and 3HP and obtained poly(3HB-*co*-3HP) yields which were comparable to those achieved by Nakamura et al. (34). The copolymer contents ranged between 3.9 and 11.8% (wt/wt) of the cell dry weight (CDW), with 3HP fractions between 4.3 and 10.5 mol%.

Higher copolymer contents in wild-type strains were obtained by using *Azohydromonas lata* (formerly *Alcaligenes latus* [47]). Poly(3HB-*co*-3HP) copolyesters with a wide range of compositions, varying from 0 to 88 mol% 3HP, were produced by *A. lata* from mixed carbon substrates consisting of 3-hydroxypropionic acid and sucrose or 3-hydroxybutyric acid (19, 39, 46). *A. lata* did not grow on 3-hydroxypropionic acid as sole carbon source. The cell densities and polyester contents of the cells decreased as the 3-hydroxypropionic acid fraction in the carbon source mix (3-hydroxypropionic acid/sucrose) increased. In contrast, the 3HP fraction in the copolymer increased to 29 mol%. With 3-hydroxybutyric acid and 3-hydroxypropionic acid, the 3HP content rose from 25 to 76 mol%. The chirality of the carbon source has a direct influence on the 3HP molecules incorporated and the copolymer content. When (*R*)-(−)-3-hydroxybutyric acid was used instead of (*S*)-(+)-3-hydroxybutyric acid, the 3HP fraction increased up to 88 mol%, but the cells' polyester content decreased to 6 weight percent (wt%).

The first production of poly(3HB-*co*-3HP) in recombinant *E. coli* was reported by Valentin et al. (43). When *E. coli* cells harboring the *phaC1AB*$_{Cn}$ operon of *C. necator* and expressing the propionyl-CoA synthase PrpE$_{Se}$ from *Salmonella enterica* serovar Typhimurium LT2 or the acetyl-CoA:4-hydroxybutyrate CoA transferase OrfZ$_{Ck}$ from *Clostridium kluyveri* were grown in LB medium containing 1% 3HP, polyesters containing approximately 90 mol% 3HP were accumulated. Cultivation in the presence of 1% (wt/vol) mannitol plus 1% (wt/vol) 3HP resulted in a similar accumulation of 3HP. Without mannitol, the 3HP fraction decreased to only 20 to 25 mol%. The expression of the acetyl-CoA synthase AcoE$_{Cn}$ from *C. necator* under such growth conditions resulted in significantly lower levels of 3HP in the polyester. The total amount of polyesters accumulated in these strains did not vary significantly (12 to 14% CDW).

Fukui et al. (12) showed the microbial synthesis of poly(3HB-*co*-3HP) from an unrelated carbon source, such as fructose, by a metabolically engineered *C. necator* strain. For this, the authors established an artificial 3HP biosynthesis pathway. The genes for malonyl-CoA reductase (*mcr*$_{Ca}$) and the N-terminal 3HP-CoA synthase domain of propionyl-CoA synthase (*acs*$_{Ca}$)

from *Chloroflexus aurantiacus* were tandemly organized and inserted downstream of the p_{BAD} promoter of the pBBad broad-host-range vector (16). The expression of the respective genes in *C. necator* was induced with L-arabinose. When mcr_{Ca} and acs_{Ca} were coexpressed in the recombinant *C. necator* H16, 3HP units were detected by gas chromatography analysis after methanolysis and subsequent *O*-trimethylsilylation of the dried cells. While a small fraction (0.2 mol%) of 3HP was observed even in the absence of an inducer, the 3HP content in the copolymer increased from 0.2 to 1.0 mol% concomitantly with the rise from 0.001 to 0.1% (vol/vol) L-arabinose. The cell densities and PHA contents of the induced cultures (1.8 to 1.9 g/liter and 57 to 59 wt%, respectively) were comparable to those of the strain harboring only the vector. Supplementation with gluconate instead of fructose also resulted in copolyester biosynthesis despite a lower productivity. With octanoate as the sole carbon source, poly(3HB-*co*-3-HP) was accumulated up to 76 wt%, with a 3HP content of 0.6 mol%. With longer alkyl chains, dodecanoate, or soybean oil, no or only trace amounts of 3HP units were incorporated into the polymer.

BACTERIAL SYNTHESIS OF POLY(3HP) HOMOPOLYMERS

The first biotechnological approach accumulating the poly (3HP) homopolymer and producing a 3HP-containing polyester independently from 3HP as a precursor was recently established in our laboratory by engineering a nonnatural pathway (3). For construction of the novel pathway, we expressed the genes for the glycerol dehydratase ($dhaB1_{Cb}$) of *Clostridium butyricum*, the propionaldehyde dehydrogenase ($pduP_{Se}$) of *Salmonella enterica* serovar Typhimurium LT2, and the PHA synthase ($phaC1_{Cn}$) of *C. necator* in a recombinant *E. coli* strain. Poly(3HP) was accumulated up to 12% of the CDW in two-step fed-batch fermentations.

Glycerol dehydratases are mainly present in a few *Clostridia* and enteric bacteria. They were also used for the conversion of glycerol to 1,3-propanediol, which can be chemically polymerized with terephthalic acid or dimethyl terephthalate (26). The resulting polymer is distributed by DuPont as Sorona.

IN VITRO SYNTHESIS OF POLY(3HB-*co*-3HP)

The copolymer of 3HB and 3HP was not only synthesized *in vivo*. Han et al. (17) used an improved two-phase reaction system (TPRS) for chemoenzymatic poly(3HB-*co*-3HP) synthesis. For this, they constructed a fusion protein of the PHA synthase ($PhaC1_{Cn}$) from *C. necator* and the propionyl-CoA transferase (Pct_{Cp}) from *Clostridium propionicum* JCM1430. The TPRS contained a sodium phosphate-buffered water phase with 3HB and 3HP as substrates, the purified fusion protein, and coenzyme A. Hexane and acetyl thioester of ethyl thioglycolate (AcETG) constituted the organic solvent phase. An ester exchange reaction between AcETG in the organic solvent phase and coenzyme A in the water phase was performed on the interface to synthesize acetyl-CoA. 3HB-CoA and 3HP-CoA were generated by transfer reactions between acetyl-CoA and a free molecule of 3HB or 3HP, respectively, catalyzed by Pct_{Cp}. The hydroxyacyl moieties of 3HB-CoA and 3HP-CoA were then polymerized sequentially by $PhaC_{Cn}$. The molar ratio of 3HP in the copolyester increased with increased 3HP concentrations in the reaction mixture. It was possible to control the monomer composition of the polymer thereby. Due to the higher affinity toward 3HP than for 3HB, as indicated by a lower Michaelis-Menten constant of Pct_{Cp} for 3HP, the molar ratios of 3HP units in the polymers were significantly higher than those in the corresponding reaction mixture (17).

BACTERIAL SYNTHESIS OF OTHER COPOLYESTERS CONTAINING 3HP

Green et al. (14) showed that *C. necator* synthesizes poly(3-hydroxypropionate-*co*-3-hydroxybutyrate-*co*-3-hydroxyhexanoate-*co*-3-hydroxyoctanoate) [poly(3HP-*co*-3HB-*co*-3HH-*co*-3HO)] copolyesters in the presence of acrylic acid and sodium octanoate as the sole carbon source. It is known that acrylic acid inhibits fatty acid β-oxidation and that the incubation of *C. necator* in the presence of this inhibitor leads to the incorporation of medium-chain-length 3-hydroxyalkanoates (3HAs) into the PHA. Since sodium acrylate is toxic to bacteria, no growth occurred if its concentration exceeded 5 mM in the mineral salt medium. To test the effects of higher levels of acrylate, Green et al. (14) established a two-stage cultivation and PHA production protocol. The bacteria were first grown to high cell densities in LB medium and then harvested and suspended in mineral salt medium containing different concentrations of sodium acrylate. The ratios of 3HP, 3HH, and 3HO in the copolymer increased as the concentration of acrylate increased, while the 3HB content decreased. When *C. necator* was incubated with 10.6 mM acrylate, the copolyesters contained 1.4 mol% 3HP, 95.9 mol% 3HB, 2.1 mol% 3HH, and 0.6 mol% 3HO. If the acrylate concentration was increased to 29.3 mM, the 3HB fraction decreased to 81.7 mol% and the fractions of 3HP, 3HH, and 3HO in the copolyesters increased to 6.5, 10.2, and 1.6 mol%, respectively (Table 1). Unlike for poly(3HB-*co*-3HP), the melting point decreased concomitantly with the decline of the 3HB content. In contrast to this, the glass transition temperature of poly(3HB-*co*-3HP) was only marginally influenced.

Park et al. (37) described a method to synthesize different polymers containing 3HP, namely, poly(4-HB-*co*-3-HP-*co*-lactate), poly(4HB-*co*-3HP-*co*-2HP), poly(3-HB-*co*-3-HP-*co*-4-HB-*co*-lactate), and poly(3HB-*co*-3HP-*co*-4HB-*co*-2HP). They incubated a recombinant *E. coli* TOP10 strain harboring the propionyl-CoA transferase (Pct_{Cp}) from *Clostridium propionicum*, the phosphate butyryltransferase (Ptb_{Ca}) and the butyrate kinase (Buk_{Ca}) from *Clostridium acetobutyricum*, and the PHA synthase from *Pseudomonas* sp. strain 6-19 in the presence of lactate, 4HB, and 3HP or lactate, 4HB, 3HP, and 3HB, respectively, as substrates (Table 1). This process was related to a process previously described by Liu and Steinbüchel (27) and Lütke-Eversloh et al. (29).

THERMAL AND PHYSICAL PROPERTIES OF POLY(3HB-*co*-3HP)

It is well known that the physical properties of the given bacterial copolyesters will, in principle, be regulated by factors such as comonomer structure, average comonomer composition, comonomer compositional distribution, etc.

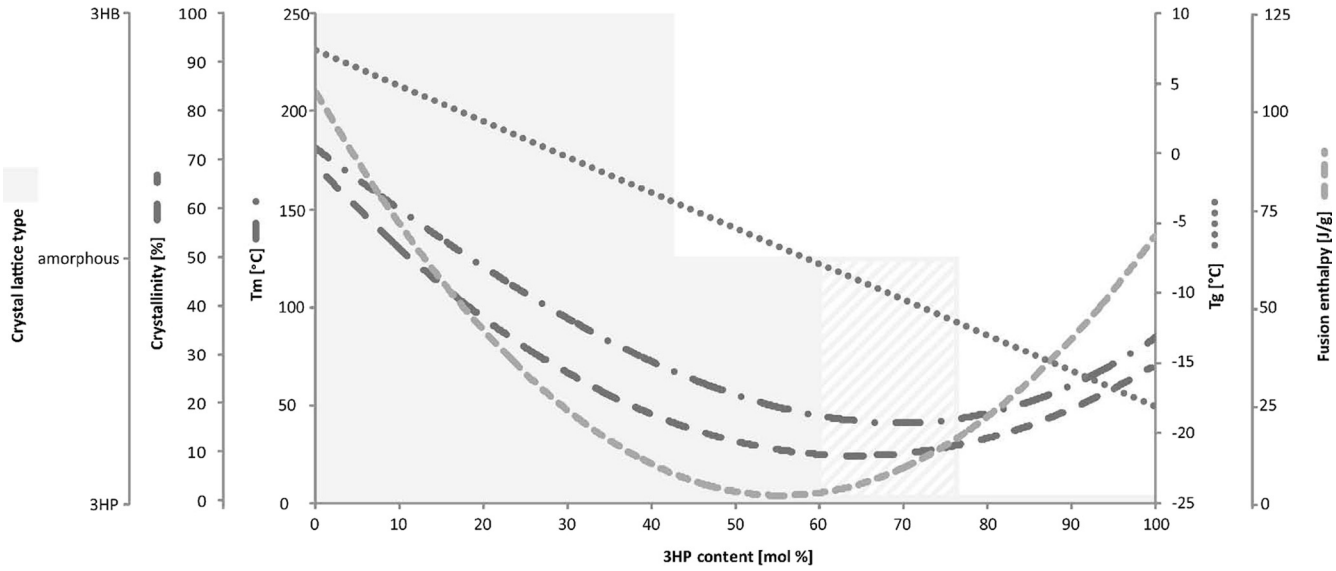

FIG. 2. Changes in the physical properties of the copolymer poly(3HB-*co*-3HP) by varying the 3HP content. The glass transition temperatures (Tg) and melting temperatures (Tm), crystallinity, crystal lattice type, and fusion enthalpy are plotted against the 3HP content. The figure compiles the data of Shimamura et al. (39), Ischikawa et al. (19), Cao et al. (7, 8, 9), Na et al. (33), Wang et al. (46), and Feng et al. (11). For the hatched area of the crystal lattice type, the data are contrary. Both the amorphous and the 3HP crystal lattice type have been reported.

The influence of varying the 3HP content in poly(3HB-*co*-3HP) on the physical and thermal properties of the polyester has been investigated in detail. The glass transition temperature decreased from +4 to −19°C as the 3HP fraction increased from 0 to 100 mol%. This effect also occurred in other copolyesters in which 3HB was partially replaced by a different hydroxyalkanoic acid constituent. Poly(3HB-*co*-3HH) and poly(3HB-*co*-4HB) behave in a similar manner (27, 36). The alteration of the melting temperature shows a parabolic behavior. Poly(3HB) has a melting temperature of 177°C. The incorporation of 3HP units up to 67 mol% led to a decrease to 44°C, whereas further increasing the 3HP content raised it again, to 77°C [100 mol% 3HP]. The melting temperatures of other copolyesters similar to poly(3HB-*co*-3HH) and poly(3HB-*co*-4HB) showed the same tendency. This obviously implies that the reduction of the melting temperature value is not solely influenced by the chemical structure, due to the side chains, but is also an effect of the secondary structure and the crystallinity (11). The enthalpy of fusion showed a similar behavior. First, it decreased from 97 J/g (0 mol% 3HP) to 2 J/g (43 mol% 3HP) and then increased up to 74 J/g (100 mol% 3HP). Thermal degradation of poly(3HB-*co*-3HP) increased with the fraction of 3HP (19, 39). It was shown by Wang et al. (46) that poly(3HB-*co*-3HP) with 3HP contents below 42 mol% formed only the poly(3HB)-type crystalline structure, while samples with 3HP contents higher than 56 mol% formed only the poly(3HP)-type crystalline structure. Both types of crystals occurred with a 3HP content of 48.8 mol%.

The secondary structures of poly(3HB-*co*-3HP) also differ with the 3HP fraction. Cao et al. (9) have reported, based on wide-angle X-ray diffraction of poly(3HB-*co*-3HP)s, that 3HB-rich (0 to 38.3 mol% 3HP) and 3HP-rich (77.9 to 100 mol% 3HP) copolyesters form distinct helix structures, while copolyesters with 3HP contents ranging from about 45 to 75 mol% appear in the amorphous state. The results of ^{13}C nuclear magnetic resonance (NMR) experiments by Cao et al. (7) confirmed that 3HP units are essential to comprise the poly(3HP) lattice-type crystallites for the 3HP-rich semicrystalline copolyesters. Isomorphism, a phenomenon of cocrystallization observed in the bacterial poly(3HB-*co*-3HV) system, does not occur in the poly(3HB-*co*-3HP) system. The 3HB or 3HP units were entirely excluded from the respective poly(3HB)- or poly(3HP)-type crystallites and constituted the amorphous regions with another structural unit. In addition, the 3HB units showed more significantly suppressing effects on the crystallization in the 3HP-rich semicrystalline copolyesters. A possible reason for that could be the large difference in molecular mobility between the 3HB and 3HP units.

Different from poly(3HB-*co*-3HV) copolymers, the degree of crystallinity of poly(3HB-*co*-3HP) copolymers decreased from about 68% to less than 10% when the 3HP fraction of the copolymer increased from 0 to 48.8 mol%. It remained nearly constant at 3HP contents ranging from 48.8 to 74.2 mol% and increased with the increase of the 3HP fraction from 74.2 to 100 mol% (46). The main influences of the 3HP fraction in poly(3HB-*co*-3HP) copolyesters are summarized in Fig. 2.

ENZYMATIC DEGRADATION OF 3HP-CONTAINING POLYESTERS

The ability to utilize PHAs as a sole carbon source for growth is widely distributed among bacteria, as well as eukaryotic microorganisms. For this, they have evolved several carboxyesterases, also referred to as PHA depolymerases, which are secreted into the medium. All PHA depolymerase proteins (PhaZ) have composite domain structures and consist of a signal peptide segment, a large catalytic domain at the N terminus, a C-terminal substrate-binding domain, and a linking domain between the catalytic and binding domains. Three strictly conserved amino acids, serine, aspartate, and histidine,

constitute the active center of the catalytic domain. The extracellular PHA depolymerases are classified into four types by differences in the linker domain structure or in the position of the lipase box in the catalytic domain (21, 22). The enzymatic hydrolysis of polymers has been studied on solution cast films (10) and melt-crystallized films (25, 42), which demonstrated that the enzymatic hydrolysis occurred first at the amorphous region and subsequently at the crystal region. For poly(3HB) films with similar degrees of crystallinity, the rate of enzymatic hydrolysis is influenced by spherulite and crystal sizes.

Kasuya et al. (24) described the degradation of poly(3HP), which was synthesized by ring-opening polymerization of β-propiolactone, by the extracellular PHB depolymerases from *Ralstonia pickettii* (previously referred to as *Alcaligenes faecalis* [48]) ($PhaZ_{Rp}$), *Pseudomonas stutzeri* ($PhaZ_{Ps}$), and *Comamonas acidovorans* ($PhaZ_{Ca}$). The rates of erosion occurring in solid specimens of the material ranged between 0.1 ($PhaZ_{Rp}$) and nearly 0.3 mg/h/cm^2 ($PhaZ_{Ps}$). Surprisingly, the corresponding values for the actual substrate poly(3HB) of all three depolymerases were lower (24).

The best characterized degradation of a 3HP-containing copolymer is the hydrolysis of poly(3HP-*co*-3HB). Shimamura et al. (39) degraded poly(3HB-*co*-3HP) films with different 3HP contents with $PhaZ_{Rp}$. These results, in addition to those of Cao et al. (8), suggested that the rates of enzymatic degradation were controlled not only by the crystallinity of the polymer but also by the chemical structure of the building blocks and the substrate specificity of PHA depolymerases. The crystalline structure and the degree of crystallinity of poly(3HB-*co*-3HP) copolymers were shown to change with alterations in the 3HP content. The poly(3HP) homopolymer-type crystalline structure of the copolymer could be degraded at a higher rate than poly(3HB) homopolymer crystallites. Although the erosion rates for the poly(3HP)-type crystalline structures were higher than those of the poly(3HB) type, the maximum degradation rate has been observed for the poly(3HB-*co*-3HP) copolyester with a 3HP content of about 20 to 30 mol% (8). A similar effect was observed during the degradation of poly(3HB-*co*-3HP) samples by PhaZ from *Acidovorax* sp. strain TP4 ($PhaZ_{Ac}$). The rate increased in parallel with the increase of the 3HP content from 0 to 56.2 mol%. Samples with 3HP contents from 56.2 to 83.9 mol% showed almost similar degradation rates. When the 3HP content was higher than 84 mol%, the enzymatic hydrolysis decreased with the increase of the copolymer crystallinity. ^{1}H NMR spectra of the degradation products of unfractionated poly(3HB-*co*–21.0 mol% 3HP) indicated that $PhaZ_{Ac}$ degrades poly(3HB-*co*-3HP) films first into trimers or dimers and then into the monomers. In contrast to $PhaZ_{Rp}$, $PhaZ_{Ac}$ depolymerizes poly(3HB-*co*-3HP) films with lower degrees of crystallinity at higher rates (46). The water-soluble degradation products obtained from poly(3HB-*co*-3HP) by $PhaZ_{Ac}$ were monomers (3HB and 3HP), dimers (3HB-3HB, 3HB-3HP, 3HP-3HB, and 3HP-3HP), and trimers (3HB-3HP-3HP, 3HB-3HB-3HP, and 3HP-3HP-3HP) (1).

FUTURE DIRECTIONS AND PERSPECTIVES

This review has focused on our current knowledge of the synthesis of the poly(3HP) homopolymer and 3HP-containing copolymers. Some enzymes and artificial pathways have been identified until now, but via metabolic engineering, new approaches may still be found. One significant challenge is to synthesize poly(3HP) and its copolymers from structurally unrelated carbon sources. For competitiveness with petrochemically synthesized polyesters, there is an urgent need for independence from 3HP as a precursor. Glycerol could be a good candidate for a cheap carbon source due to its occurrence as a by-product of biodiesel production (50).

The studies of Fukui et al. (12) and Andreeßen et al. (3) represent first demonstrations of methods to solve this problem. To achieve higher 3HP fractions in the copolymer, more investigations concerning the accumulation of 3HP are needed. Further research should also aim at synthesizing new copolyesters containing 3HP together with other, hitherto unidentified hydroxyalkanoic acids in the polymer backbone. The structure and physical properties of 3HP exhibit several advantages: (i) the homopolymer exhibits high rigidity, ductility, and exceptional tensile strength in drawn films and, (ii) in copolyesters, it reduces the glass transition temperature and crystallinity but increases the enzymatic degradability, due to the missing methyl groups in the polymer backbone (Figs. 1 and 2).

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