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Polyhydroxyalkanoates: Chemical Modifications Toward Biomedical Applications

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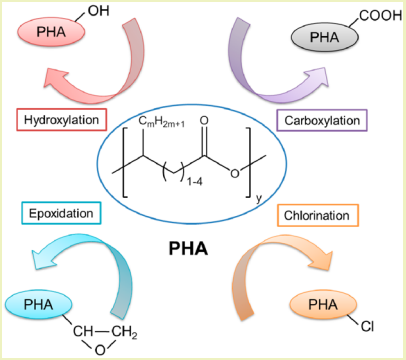
ABSTRACT: Polyhydroxyalkanoates (PHAs) have attracted great interest in recent years in research due to their potential wide applicability as biomaterials. However, PHAs are very hydrophobic, degrade slowly under physiological conditions, and lack chemical functionalities. These factors restrict the scope of their applications in the biomedical field. In order to expand on the use of these materials, many other properties such as mechanical strength, surface features, amphiphilicity, and degradation rate have to be modified to match the requirements of specific applications. Chemical modifications to introduce functional groups add valuable attributes to PHAs that cannot be easily achieved by bioconversion processes. These chemically modified PHAs, possessing improved properties, can be utilized as multifunctional materials. In this review, well-established chemical modification methods of PHAs are summarized and discussed. Furthermore, the biomedical significance of the functionalized PHAs in different applications is also discussed.



KEYWORDS: *Polyhydroxyalkanoates, Microbial polyesters, Chemical modification, Functionalization*

INTRODUCTION: AN OVERVIEW OF PHAS

Polyhydroxyalkanoates (PHAs) belong to a class of bioderived polyesters. These polyesters are produced by different microbes for energy storage purposes.[1] Through the use of different carbon substrates, culture conditions, and careful selection of bacterial species, a variety of biosynthetic PHA-based monomers have been identified, making them the largest group of natural polyesters.[2] The general formula of PHAs is presented in Figure 1.

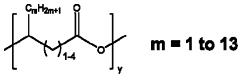


Figure 1. General chemical structure of PHAs.

Two groups of PHAs can be distinguished based on the carbon chain length of the polymers. The first consists of short-chain-length hydroxyalkanoic acids, (SCL-PHAs) such as poly(3-hydroxybutyrate) (PHB), poly(3-hydroxyvalerate) (PHV), and their copolymer poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV). The second consists of medium-chain-length hydroxyalkanoic acids, (MCL-PHAs), such as poly(3-hydroxyoctanoate) (PHO) and poly(3-hydroxynonanoate) (PHN), which typically contain 3-hydroxyhexanoate (HHx), 3-hydroxyheptanoate (HH), and/or 3-hydroxydecanoate (HD).[3−5] PHB was first isolated in 1926 from *Bacillus megaterium*.[6] The biosynthetic pathway to produce PHB consists of a three-step enzymatic reaction catalyzed by three different enzymes.[7] As shown in Figure 2, exemplified by Route 1, a phaA thiolase unit reacts with acetyl-CoA to form acetoacetyl-CoA in a 1:2 ratio. 3-Hydroxybutyrylis formed by reducing acetoacetyl-CoA with phaB reductase. The polymerization of 3-hydroxybutyryl-CoA is accomplished by P(3HB) polymerase to form PHB.[8] Most SCL-PHAs are rigid and brittle due to the high degree of crystallinity, and their poor mechanical properties are unsuitable for many biomedical and packaging applications.[9,10] In contrast, MCL-PHAs possess low melting and glass transition temperatures as well as a low degree of crystallinity. MCL-PHAs exist as semi-crystalline elastomers or amorphous liquids due to their low glass transition temperature (T_g).[11−14] As their T_g increases with increasing the average pendant chain length, MCL-PHAs form elastomeric biopolymers at certain side-chain lengths. Further increase in the side chain length leads to more viscous and tacky polymers.[15] MCL-PHAs function as elastomeric materials within a narrow temperature range because of their low melting temperature (T_m). At temperatures above or close to its T_m, the polymer loses its crystallinity and becomes tacky.[16] Hence, it is not easy to use MCL-PHAs as flexible biomaterials. Through the optimization of the structure of PHAs, PHAs with improved physical properties can be synthesized and customized for specific applications.

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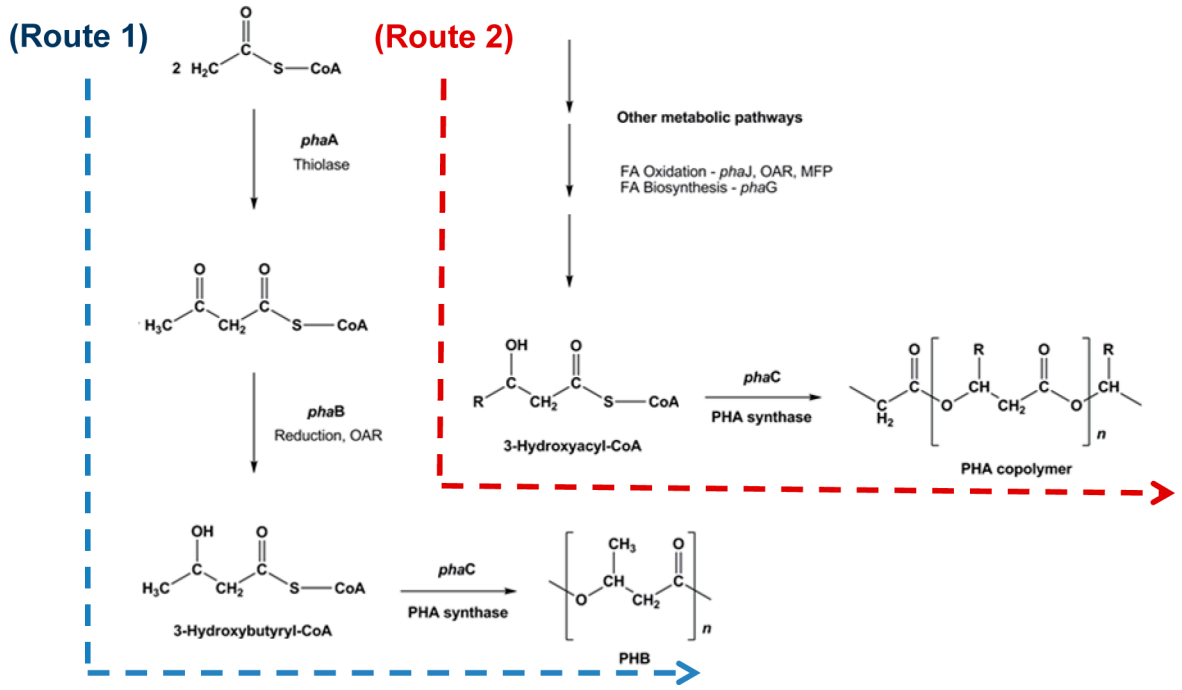

Figure 2. Biosynthetic pathway of producing PHB and PHA copolymers.

PHA copolymers containing SCL-PHA monomeric units and MCL-PHA monomeric units can be prepared by metabolic engineering by using recombinant strains in a new metabolic pathway.[17] This approach allows for the preparation of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) containing polymers, PHBHHx, through biosynthesis by recombinant *Escherichia coli*.[18] PHBHHx copolymers with a low 3HHx fraction have superior mechanical properties compared with PHB and polylactic acid (PLA).[19] It possesses similar mechanical properties to low density polyethylene, and the rigidity of the PHBHHx biopolymer can be finely adjusted through the 3HHx monomer content control, which affects the T_g value and crystallinity of the polymer.[19,20] The typical biosynthesis pathway of the PHBHHx copolymer is shown in Figure 2 (Route 2). Another route for the biosynthesis of PHBHHx involves fatty acid biosynthesis and subsequent oxidation. This will lead to the synthesis of other long-chain 3-hydroxyacyl-CoA units. PHA copolymers are formed by copolymerizing 3HB-CoA and 3HA-CoA with phaC PHA synthase.[21] PHAs' crystallinity and mechanical properties can be tailored in various copolymers to make these biopolymers more suitable for biomedical applications.[22,23]

Need for Functionalization of PHAs. PHAs containing hydroxylated, methylated, brominated, and nitro phenyl derivatives were produced by some organisms.[24] The functional groups containing polyesters expanded the applications of PHAs in biomedical fields by allowing further chemical modification of these polyesters.[15] However, such biosynthetic approaches remain inaccessible to most chemists and material scientists working on the optimization of the properties of PHAs. As opposed to biological synthesis via fermentation, the ability to chemically modify the polyesters allows for the precise modulation of the polymer structure with predictable variations in molecular weight and functionality. Moreover, chemical reactions allow for batch-to-batch uniformity, and some functional groups can be further tailored to obtain more useful polymers (e.g., PHA-based graft/block copolymer) with special properties for specific applications. In the following sections, various functional groups introduced to PHAs by chemical reaction methods will be summarized. (Figure 3)

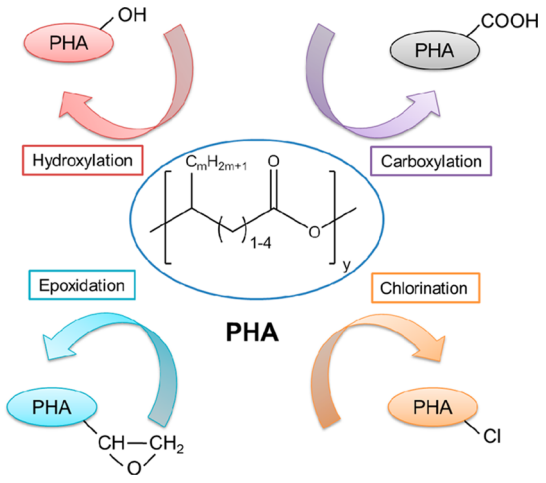

Figure 3. Overview of the chemical modification of PHA copolymers to afford different functionalities.

INTRODUCTION OF HYDROXYL GROUP

Monohydroxylated PHA Macromonomer. Chemical methods such as base- or acid-catalyzed reactions with *para*-toluene sulfonic acid monohydrate (APTS) and methanolysis have produced PHA oligomers containing a terminal hydroxyl group.[25−28] According to Timbart's report, the ester bonds of poly(3-hydroxyoctanoate) (PHO) were stable at pH 10 and 12, while hydrolysis occurred immediately when pH was 14, resulting in the formation of a hydroxyl group at one end of the chain. Nevertheless, NMR results obtained on the PHO oligomer prepared under basic conditions showed an unsaturated end group for the products, which may result

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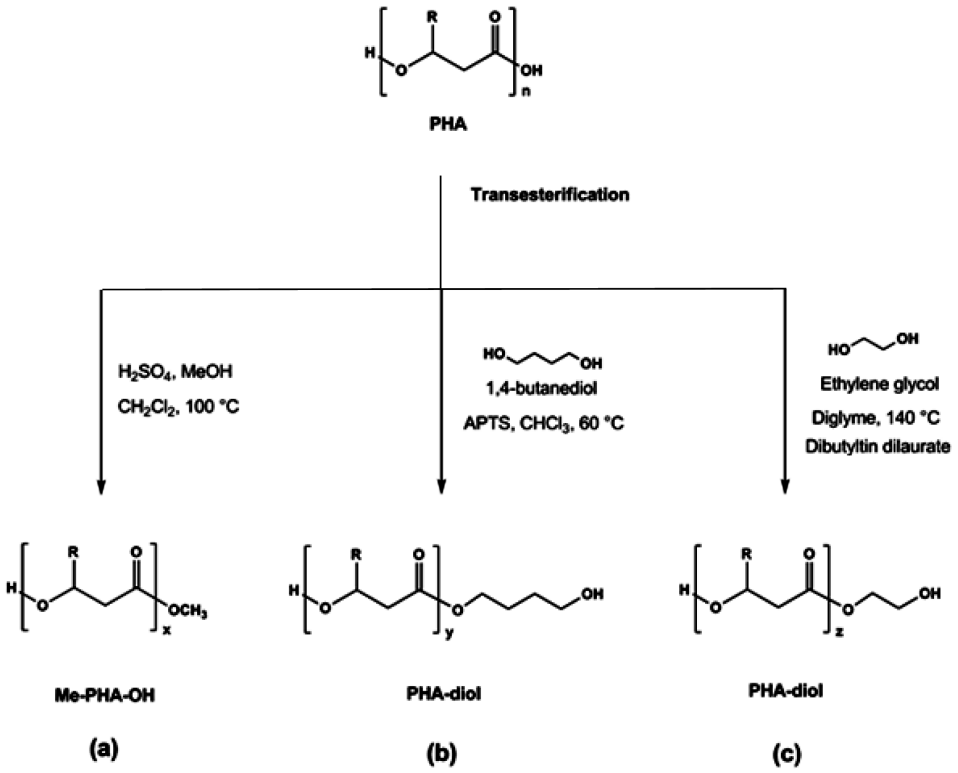

Figure 4. Preparation of hydroxylated PHA macromonomers by different methods.

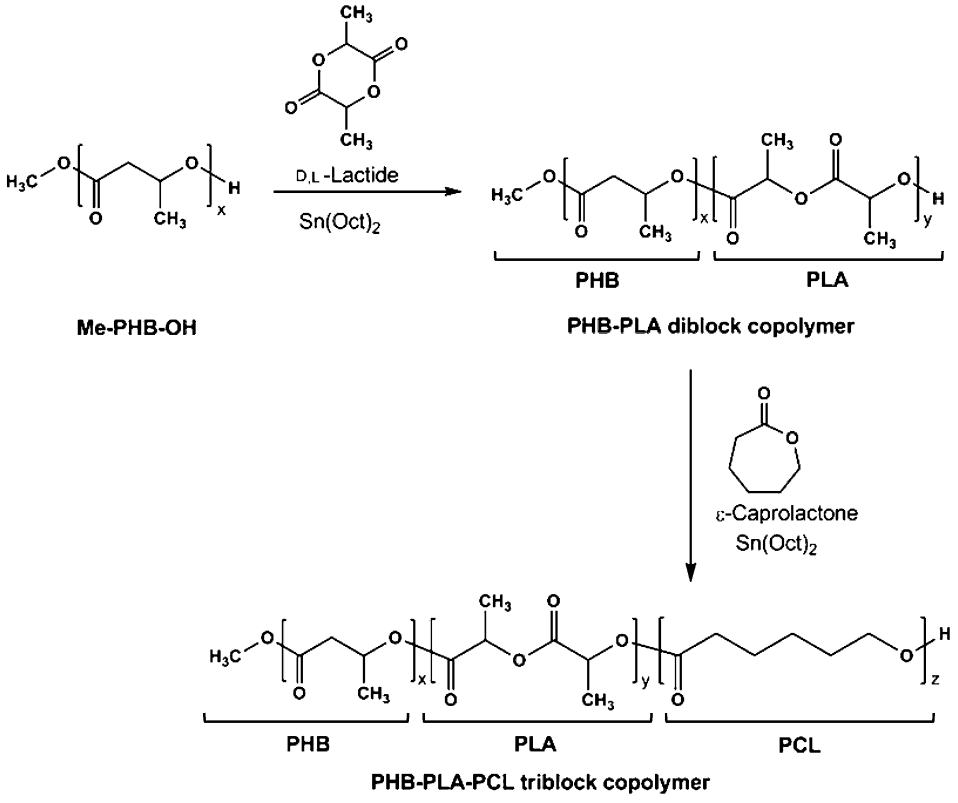

Figure 5. Synthesis of the PHB−PLA−PCL triblock copolymer by consecutive ring-opening polymerization.

from the *cis*-elimination reaction accounted for with PHB.[25,29] On the other hand, the acid-catalyzed reaction for monohydroxylated PHO oligomer preparation was more efficient. The molar mass decrease was dependent on the solvent nature. At the same conditions, the PHO chain cleavage occurred more rapidly in toluene than in dichloroethane. This was attributed to the enhanced solubility of PHO in toluene. As revealed from MALDI-TOF spectra, the acid-catalyzed condition with APTS

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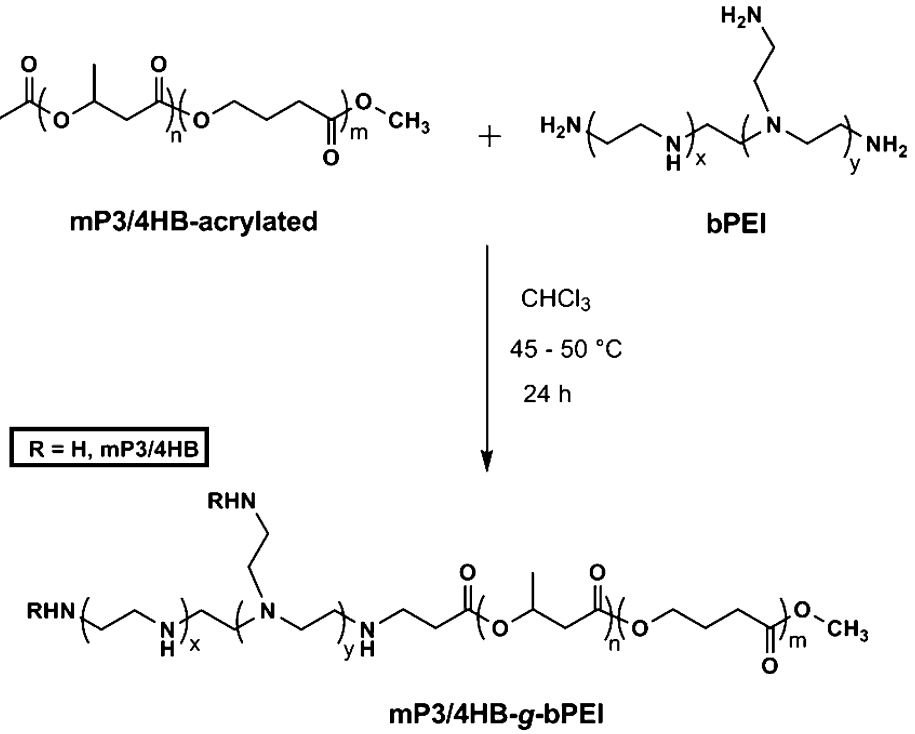

Figure 6. Synthesis route of mP3/4HB-g-bPEI copolymer by Michael addition.

resulted in linear PHOs with a hydroxyl group at one end and a carboxylic group at the other end. Cyclic structures were observed in very low quantities.[25] When high molecular weight PHAs were exposed to methanolysis using sulfuric acid as the catalyst, monohydroxylated PHA macromonomers with a protected carboxylic acid functionality as an ester were formed (Figure 4a).[25,26,28,30] With precise control in reaction time and temperature, monohydroxylated PHAs of different molecular weights can be prepared.[26,30] The monohydroxyl-terminated PHAs prepared by this transesterification reaction can be further used to initiate the ring-opening polymerization (ROP) of lactones and lactides.

The segment effect in modification of PHAs block copolymer properties is obvious even in the simplest A−B diblock. Monohydroxyl group end-functionalized PHBs (average DP = 26) were reacted in the presence of $AlEt_3$ for the ROP of ε-caprolactone and lactide monomers to prepare PHB−PCL (PHB DP = 26, PCL DP = 12, 38, 51), PHB−P(D,L)LA (PHB DP = 26, D,L-PLA DP = 13), and PHB−PLLA (PHB DP = 26, L-PLA DP = 13, 23) diblock copolymers. Different segment lengths in the obtained diblock copolymer could be tuned by reaction time and temperature.[31] The crystallization of PHB−PCL and PHB−PLLA varied with different chain lengths of the PCL or PLLA chain segments.[31] In a similar fashion, the PHO−PCL diblock copolymer (average $M_{n,\text{block PHO}}$ = 800−20000, average $M_{n,\text{ block PCL}}$ = 13500−18500) can be prepared using PHO oligomers as the soft segment and PCL as the hard segment. The PHO−PCL diblock copolyesters were semi-crystalline, and two T_gs were observed when the PHO molecular weight was 20 kDa.[32] Timbart and co-workers reported that the crystallinity of the PHOU−PCL copolymers (average $M_{n,\text{block PHOU}}$ = 4800−20500, average $M_{n,\text{ block PCL}}$ = 5000−25000) could be modified by tuning the two different block lengths.[25]

Consecutive ROP of D,L-lactide followed by ε-caprolactone yields PHB−PLA−PCL triblock copolymers (Figure 5). These triblock copolymers show microphase separation induced by the PHB−PLA hard segment and the PCL soft segment where the hard segment domains serve as physical cross-linkers for the matrix.[27] Recently, L-lactide was successfully copolymerized with PHBHHx using tin(II) 2-ethylhexanoate as a catalyst to generate a copolyester. Random transesterification of high molecular weight PHA along with chain scission reactions of PHA with lactide gives a random multiblock copolymer with molecular weights of between 25 and 50 kDa. The final products exhibited improved mechanical properties compared to pure PLA.[33]

Zhou et al. reported a PHA-grafted-branched poly(ethyleneimine) (PHA-*g*-bPEI) that was synthesized by Michael addition between acrylated monomethoxy-PHA (mPHA-acrylated) and branched PEI (Figure 6). As a potential carrier for siRNA delivery, PHA-*g*-bPEI copolymers were nontoxic, had good cellular uptake, and high transfection efficiency similar to the commercial Lipofectamine 2000.[28]

Dihydroxylated PHA Macromonomer. One way to prepare dihydroxylated PHAs (PHA-diol) is from the transesterification with diol, such as 1,4-butanediol, in the presence of acidic catalyst *p*-toluenesulfonic acid.[34−37] Figure 4b illustrates the typical reaction equation by using 1,4-butanediol as a demonstration. During the reaction, the hydroxyl group in the small molecule cleaves the ester bond forming a telechelic dihydroxyl-terminated polymer. Because random cleavage was considered as the main mechanism during the reaction, the molecular weight of the degraded products decreases exponentially initially. With further reaction, oil-like products would be obtained.[37−41] Alternatively, PHBV-diol prepared in semi-preparative scale were reported through the transesterification procedure with excess ethylene glycol in diglyme at 140 °C using dibutyltin dilaurate as the catalyst.[42] This

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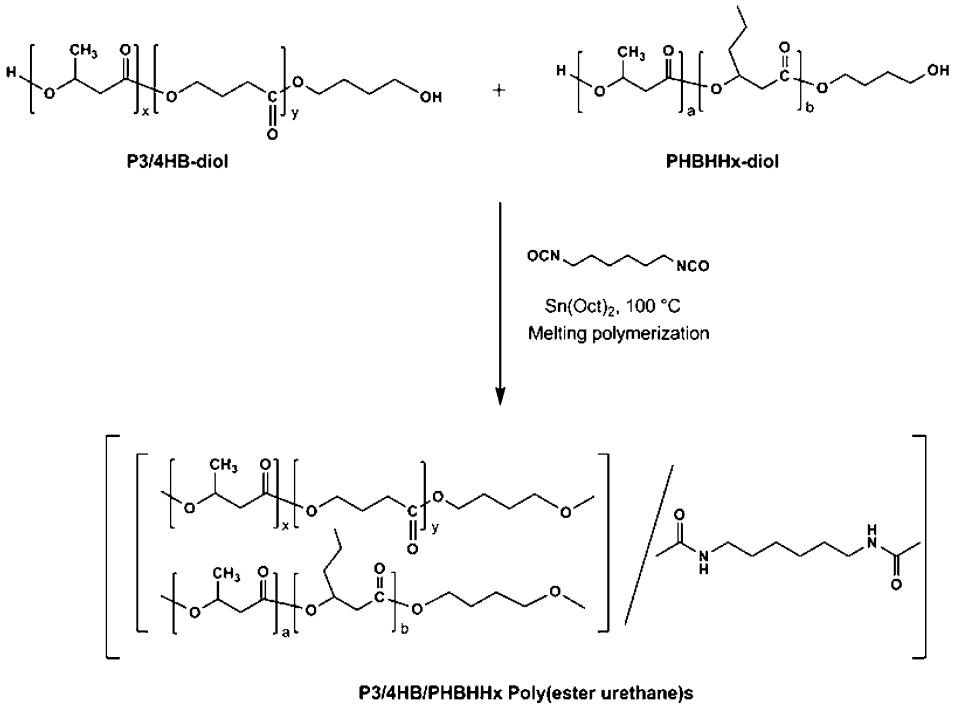

Figure 7. Synthesis of PHA-based poly(ester urethane)s containing different PHAs repeating units as building block by melting polymerization.

approach could be done in high PHA concentration, and the product could be precipitated in cold water. After the purification steps of filtration through Celite and silicate and precipitation in petroleum ether, the purified products at yield of 74% could be obtained. A typical reaction illustration is presented in Figure 4c. Since Hirt's report, many other types of PHAs-diol containing different SCL−PHA and MCL−PHA units have been reported by this facial method. A large group of new functional block copolymers have been diversified by applying PHAs-diols as building blocks due to the predominantly reactive telechelic hydroxyl groups in PHAs-diol.[9,43−48] Similar to the PHAs-diol prepared under acidic catalyst, a small portion of crotonic acid ester (∼ 2.4%) was also produced as byproduct. Crotonic acid ester end groups may form from the water elimination after the transesterification.[42]

Various dihydroxy telechelic PHA oligomers have been coupled form PHA block copolymers. Andrade et al. reported the copolymerization of PHO-diol and PHB-diol by polycondensation with terephthaloyl chloride (TeCl).[9] Unlike the soft and tacky starting PHO block, the copolymer showed good thermoplastic properties with a melting temperature of about 130 °C and glass transition of about −40 °C. On the other hand, the reaction between the diisocyanate and dihydroxyl groups in both PHA oligomers have been used to develop PHAs-containing polyurethane block copolymers. Because there is no small molecule (-e.g. H_2O, HCl) generated from the urethane reactions, this reaction presents a versatile option for the industrial scale-up of block copolymer synthesis.[49] A large group of new PHAs materials with adjustable mechanical properties, surface features, and degradation rate have been diversified through this technique, which would expand the potential applications of PHAs as wound-healing and hemostatic materials. For example, changing the 3HB and 4HB content in P3/4HB polyesters affords polymers with properties ranging from soft elastomers to high crystallinity plastics.[50] Recently, Xu and co-workers reported a coupling reaction of different P3/4HB-diols by HDI in molten state of 4HB with the content ranging from 9.3 to 32.3% in the prepolymers.[40] The physical and surface properties of the polymer was altered by changing the compositions, segment block lengths and linking unit content in P3/4HB poly(ester urethane)s.[40] Similarly, more poly(ester urethane)s based on P3/4HB and PHBHH*x* were synthesized in one-pot bulk polymerization using hexamethylene diisocyanate as a coupling agent in the presence of tin(II) 2-ethylhexanoate as the catalyst (Figure 7).[36] Through the combination of PHAs with different side-chain lengths, the P3/4HB/PHBHHx poly(ester urethane)s with low crystallinities could be obtained. These poly(ester urethane)s were more cell compatible compared to pure PLA, PHB, P3HB4HB, or PHBHHx.

All these results supported the potential application of PHA-based poly(ester urethane)s as hydrophobic wound-healing materials.[36] When PHBHH*x* was replaced by poly(3-hydroxyhexanoate-*co*-3-hydroxyoctanoate) (PHHxHO) in the synthesis, the obtained poly(ester urethane) copolymer films became more hydrophobic. Moreover, the physical properties of these copolymers could be modified as described earlier.[37] In a similar approach, another poly(ester urethane) block copolymer had been synthesized by use of PHO-diol with a number-average molecular weight of 2400 as the amorphous soft segment, PHB-diol with a number-average molecular weight of 2600 as the crystalline hard segment, and L-lysine methyl ester diisocyanate (LDI) as the linking unit. A reaction of these components in the presence of a tin catalyst resulted in a good yield with a high molecular weight of about 34 kDa. The polymer showed excellent thermoplastic properties and good mechanical properties.[43]

Saad and co-workers synthesized a series of semi-crystalline PHB/PCL/1,6-hexamethylenediisocyanate (HDI)-segmented poly(ester urethane)s with different compositions and different block lengths in a one-pot polymerization process.[34] Poly(ester urethane)s with high molecular weight PCL soft segments

(molecular weight greater than 2200 kDa) exhibited greater phase separation than their low molecular weight analogues. This is due to the thermodynamic immiscibility of PHB and PCL segments. The weight fraction and block lengths of PHB in the final product plays a dominant role in determining the thermal and mechanical properties of the PHB/PCL poly(ester urethane)s.[34] Similarly, HDI and toluene diisocyanate (TDI) were used to conjugate PHB-diol and PCL-diols (molecular weight of about 2000 Da).[51] Reaction conditions were tested under different solvents and catalyst systems. Results found that dibutyltin dilaurate (DBTDL) and tin(II) 2-ethylhexanoate show good catalysis properties for the reaction. However, the use of 1,4-dioxane leads to polymers of lower average molecular weight of the block copolymers than 1,2-dichloroethane due to the poorer solubility of the reactants in 1,4-dioxane. The use of the two different coupling agents also lead to different degradation behavior of the copolymer films. For TDI, the hydrolytic degradation of PHB/PCL poly(ester urethane)s is due to the hydrolysis of the ester and urethane bonds, while the hydrolysis of PHB/PCL poly(ester urethane)s using HDI was mainly generated from the scission of their ester bonds.[51] Poly(butylene adipate)-diol (PBA-diol) has also been used as the soft segment in PHB/PBA poly(ester urethane)s.[35] Another type of poly(ester urethane) having P3/4HB and PCL was synthesized by bulk polymerization using HDI as the coupling agent.[39] The obtained P3/4HB/PCL poly(ester urethane)s can be used to form films and coatings. The presence of 4HB in the materialism proves platelet adhesion and blood coagulation, as well as cell compatibility, making its potential candidate for wound healing applications.[39]

Block Copolymerization of PHAs with Poly(ethylene glycol). Poly(ethylene glycol) (PEG) is a biocompatible polymer that has been widely used for biomaterials research. Block copolymerization of PHAs with PEG has been adopted as a promising method to manipulate PHA mechanical and physical properties. The intrinsic hydrophobicity and slow degradation rate of natural PHAs can also be amended through this approach. Various coupling reactions have been applied to link PHAs-diols and PEG in different formation. Table 1 lists the typical examples of PHAs/PEG based block copolymers and their properties.

PHB−PEG diblock copolymers containing high molecular weight PHB with monomethoxy-PEG (mPEG) were synthesized under pyrolysis reaction.[53,54] PHA−PEG diblock copolymers were recently reported by functionalizing the carboxylic acid group in PHA with alkyne function, allowing the coupling reaction of alkyne-terminated PHAs and azide-terminated PEG in high efficiency to afford the well-defined PHA−PEG diblock copolymer structure.[55,56]

Amphiphilic PEG−PHB−PEG triblock copolymers were sysnthesized by coupling two chains of methoxy-PEG-monocarboxylic acid with a low molecular weight PHB-diol chain in the presence of DCC (Figure 8). A group of PEG−PHB−PEG triblock copolymers with PEG chain lengths of ∼2000 and ∼5000 Da were used. In each group, triblock copolymers having PHB as the middle segment with the block lengths from 500 to 5500 were used in the synthesis, corresponding to the PHB contents ranging from 8 to 59 wt %.[45] PEG−PHB−PEG triblock copolymers with high PEG content were water soluble and formed micelles with PHB as the core and PEG as the corona.[57]

Interestingly, at certain PEG−PHB−PEG polymer concentration, the region-selective channel structure of the solution

Table 1. List of PHAs/PEG-based Copolymers and Their Mechanical and Thermal Properties

PHA/PEG block copolymers	examples	Mn ($\times 10^5$ g/mol)	T_g (°C)	T_m (°C)	mechanical properties	reference
di/triblock copolymer	PHA	3−15	−40 to 1	130−160	*E*: about 1.5 GPa. σ_{max}: about 3 MPa. ε_{max}: about 5%.	52
	PHB−PEG	2.6−7.3	nd	PEG: 47−56. PHA: 122−152.	nd	53,54
	PHBV−PEG	17.3−23.1	−7 to −9	PEG: 38−54. PHA: 132−153.	nd	55
	PHBHHx−PEG	7.2	0	PEG: 50. PHA: 120.	nd	55
	PHOHHx−PEG	9.9−11.1	−33 to −32	PEG: 53−55.	nd	55,56
	PEG−PHB−PEG	4.5−13.39	nd	PEG: 23−57. PHA: 140−153.	nd	45,57
multiblock copolymer	PHB−*ran*−PEG	17.5−60.4	−49 to −33	PEG: 17−26. PHA: 133−138.	*E*: 4.6−55.0 MPa. σ_{max}: 2.7−7.5 MPa.ε_{max}: 74.3−114.8%.	41,58,52,44
	PHB−*alt*−PEG	7.8−18.9	nd	PEG: 15.7−41.3. PHA: 117.6−149.3.	nd	46
	PHBV−PEG	11.6−22.9	nd	PEG: 46.5. PHA: 134.0.	σ_{max}: 0.11−0.21 cN dtex^{-1}. ε_{max}: 11.9−21.9%.	59
	PHBHHx−PEG	64−97	−61.4 to −12.7	PEG: 20.6−59.2. PHA: 89.6−101.3.	*E*: 32−187 MPa.σ_{max}: 3.7−7.7 MPa. ε_{max}: 4.5−875%.	47
	P3/4HB−*ran*−PEG	17.1−40.1	−24.1 to −18.8	94.6−121.0	nd	60
	P3/4HB−*alt*−PEG	14.3−25.3	−21.4 to −14.9	94.0−121.3	nd	50,60
	PHB−*ran*−PEG−*ran*−PPG	30.0−50.6	nd	nd	η: 0.05−0.2 mPa s at 5 °C;43−55 Pa s above 30 °C.	48,61,62
	P3/4HB−*alt*−PPG−PEG−PPG	35.1−57.8	−47.4 to −24.2	110.7−143.2	*E*: 27.3−141 MPa.σ_{max}: 4.3−6.4 MPa. ε_{max}: 87.4−354.2%.	63

[a]nd: not determined. E: Young's modulus. σ_{max}: tensile strength. ε_{max}: elongation at break. η: visicosity.

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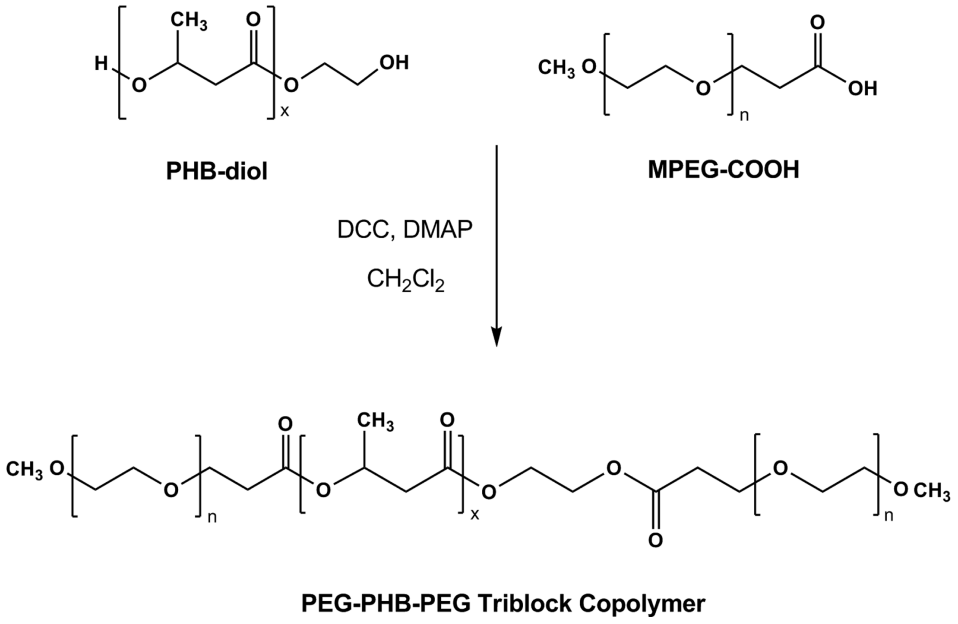

Figure 8. Preparation of PEG−PHB−PEG triblock copolymers by esterification reaction of carboxylic acid with hydroxyl groups in the building blocks.

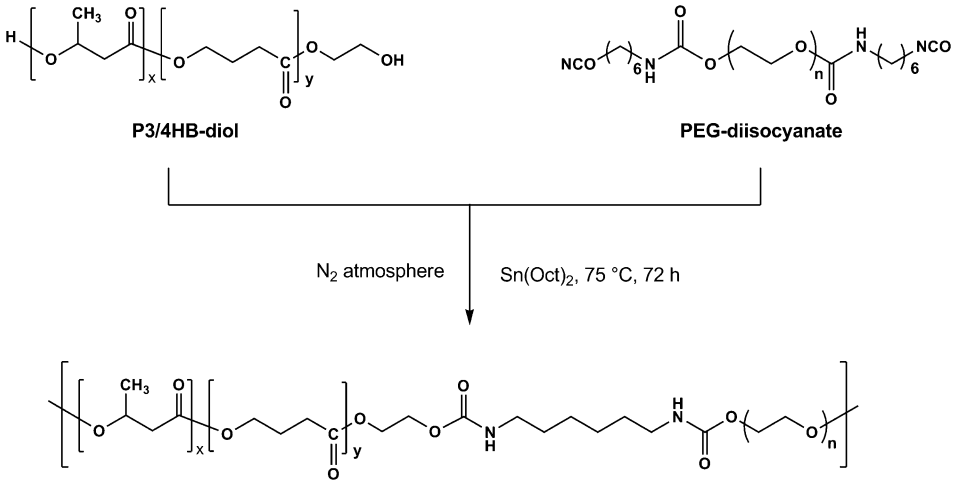

Figure 9. Synthesis of alternating poly(ether ester urethane) block copolymers from P3/4HB-diol and PEG-diisocyanate.

with α-cyclodextrins could trigger phase transformation from sol into hydrogel formation to provide a well-controlled long-term release profile of the encapsulated molecules without burst release.[64,65] PEG−*alt*−PHB multiblock copolymers were synthesized by using telechelic carboxylated PEG (PEG-diacid) instead of methoxy PEG monocarboxylic acid during the esterification reaction, and PEG−*alt*−PHB multiblock copolymers with low molecular weight distributions were obtained. This multiblock copolymer aggregates, forming interesting surface patterns on surfaces.[46]

On the other hand, various approaches of synthesizing PHA−PEG multiblock copolymers have been reported. Polymer properties such as solubility, amphiphilic balance, surface wettability, and mechanical properties can be adjusted. Among the PHA-based poly(ether ester urethane)s block copolymers, PHB and PEG segments as the building blocks have been widely investigated since the first report in 2004.[41] A complete investigation was further carried out to understand the effect of block segments on the copolymer properties.[66] The degradation behavior of these polymers were described in a follow up study on these polymers.[67] The copolymer degraded to give PEG fragments, 3-hydroxybutyric acid, and crotonic acid. PEG content in the copolymers was the key to controlling the hydrolytic stability of these copolymers.[67]

Besides PHB, many other poly(ether ester urethane)s have also been studied by including hydrophobic PHBV, P3/4HB, and PHBHH*x* as building blocks.[44,47,50,59,60] These copolymers had interesting properties. For instance, bulk polymerized PHBHH*x*/PEG poly(ether ester urethane)s block copolymers were more resistant to hydrolytic and lipase degradation as compared with those samples prepared by solution polymerization. There was enhanced tissue compatibility of the copolymer films in vivo and superior physicochemical properties.[47] PEG could be used to modulate the solubility of the copolymers. P3/4HB/PEG poly(ether ester urethane) block copolymers containing amorphous P3/4HB (4HB in mole = 36.3%) and PEG were easily dispersed in water at PEG content above 50 wt %. PHA-based poly(ether ester urethane)s with

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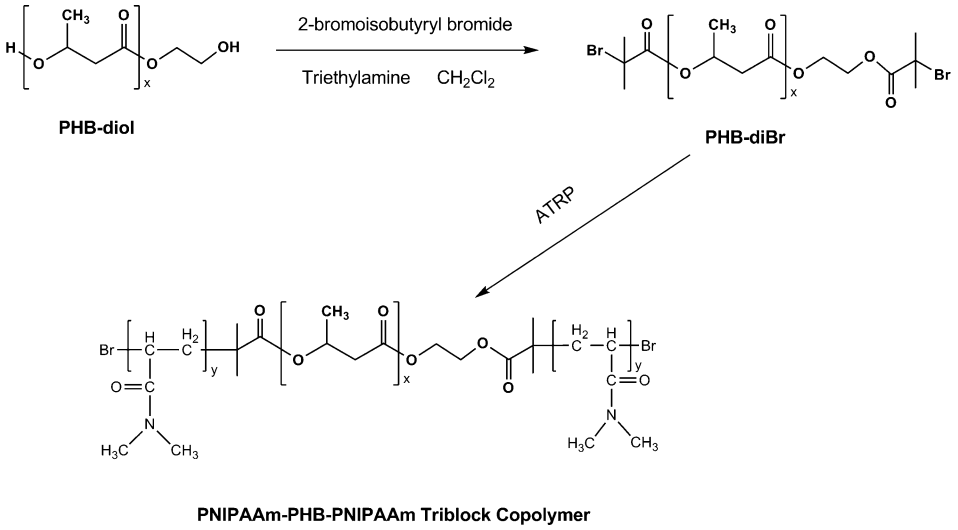

Figure 10. Synthesis of PNIPAAm−PHB−PNIPAAm triblock copolymers by ATRP.

new architectures, and a structure−property relationship has provided insights to new block copolymer development. Pan and co-workers reported the synthesis of a series of amphiphilic alternating block polyurethane copolymers based on P3/4HB and PEG (Figure 9).[50] The hemocompatible copolymer films showed different degradation patterns in different media from surface erosion to bulk degradation.[50] Compared to random P3/4HB/PEG poly(ether ester urethane)s, alternating P3/4HB/PEG poly(ether ester urethane)s was more hydrophilic with a greater surface energy, showed an interesting flower-like surface microstructure.[60]

Functionalization of PHAs throught the incorporation of PEG, PPG, and its copolymer PPG-PEG-PPG to form poly(ether ester urethane)s have also been reported, and the newly developed polymers have expanded PHAs into more advanced biomedical applications.[48] When the PHB content was below 11.4 wt %, the copolymers were water soluble. The water-soluble PHB/PEG/PPG poly(ether ester urethane)s formed micelles at very low polymer concentrations. These polymers showed thermogelling behavior and formed gels at polymer concentrations ranging from 2 to 5 wt %.[48,61] These copolymer hydrogels were subjected to a degradation study in phosphate buffer at pH 7.4 and 37 °C for up to 6 months.[62] The polymer degrades first by erosion of the gel, followed by hydrolytic degradation of the ester bonds of the PHB segments.[62] These copolymer hydrogels promoted cell adhesion compared to the hydrophilic Pluronic F127 (PEG−PPG−PEG).[61] On the other hand, alternating P3/4HB/PPG−PEG−PPG poly(ether ester urethane)s copolymers were reported.[63] The obtained copolymers with 4HB segments within promoted platelet adhesion, making these copolymers excellent hemocompatible biomaterials.[63]

The PHAs-diol oligomers could be modified into various functional end groups and further served as a macro-initiator for controlled radical polymerization. The reaction of terminal hydroxyl end groups of PHB-diol with 2-bromoisobutyryl bromide gives an ATRP macroinitiator based on PHB. A temperature sensitive triblock copolymer with poly(N-isopropylacrylamide) (PNIPAAm) blocks flanking a central hydrophobic PHB block (PNIPAAm−PHB−PNIPAAm) was synthesized by ATRP (Figure 10).[68]

The phase transition of these thermosensitive PNIPAAm−PHB−PNIPAAm copolymers were detected at about 30 °C.[68] Furthermore, the hydrophilic to hydrophobic transitions of PNIPAAm chains was used for the nonenzymatic temperature-induced cell detachment in tissue engineering.[69,70] Unlike trypsinization, the detached cells showed strong intercellular associations and formed cell sheets.[69,70] Using similar synthesis method, temperature and pH-responsive triblock copolymers with poly(2-(dimethylamino)ethyl methacrylate) (DMAEMA) blocks flanking the PHB block were prepared by ATRP.[71] The PDMAEMA−PHB−PDMAEMA micelles were used as sustained release drug carriers and released a hydrophobic drug over 20 days. Furthermore, these micelles showed excellent delivery of doxorubicin to cells.[71]

Hydroxylation of Unsaturated PHAs. The unsaturated groups in the pendant chain of PHAs can be converted into hydroxyl groups by chemical modifications. Lee showed that poly(3-hydroxyoctanoate-*co*-3-hydroxyundec-10-enoate) (PHOU) with reactive unsaturated groups at the end groups of some of the side chains were modified to give hydroxyl groups in the presence of potassium permanganate ($KMnO_4$) resulting in about a 50% degree of hydroxylation.[72] This resulted in a significant enhancement of hydrophilicity of the PHAs. PHOUs with hydroxylation degrees of between 40−60% were completely soluble an acetone−water mixture, methanol, and dimethyl sulfoxide (DMSO). A hydroboration−oxidation reaction using 9-borobicyclononane can also be used to convert the double bonds into hydroxyl groups. However, this resulted in PHAs with low molecular weights and showed contamination with low molecular weight components. These modified PHAs are soft and tacky materials that are difficult to handle compared to the untreated original PHAs.[73,74] These PHAs containing reactive pendent hydroxyl groups can be used to prepare novel graft copolymers with new properties.

INTRODUCTION OF CARBOXYLIC GROUP

Carboxylic groups are functional handles for the binding of bioactive molecules, hydrophilic components, or targeting enzymes. The incorporation of these groups enhances the hydrophilicity of the polymers as well.[74] PHA macromonomers containing a carboxylic acid group at one end and a hydroxyl group at the other end have been reported.[25] Furthermore,

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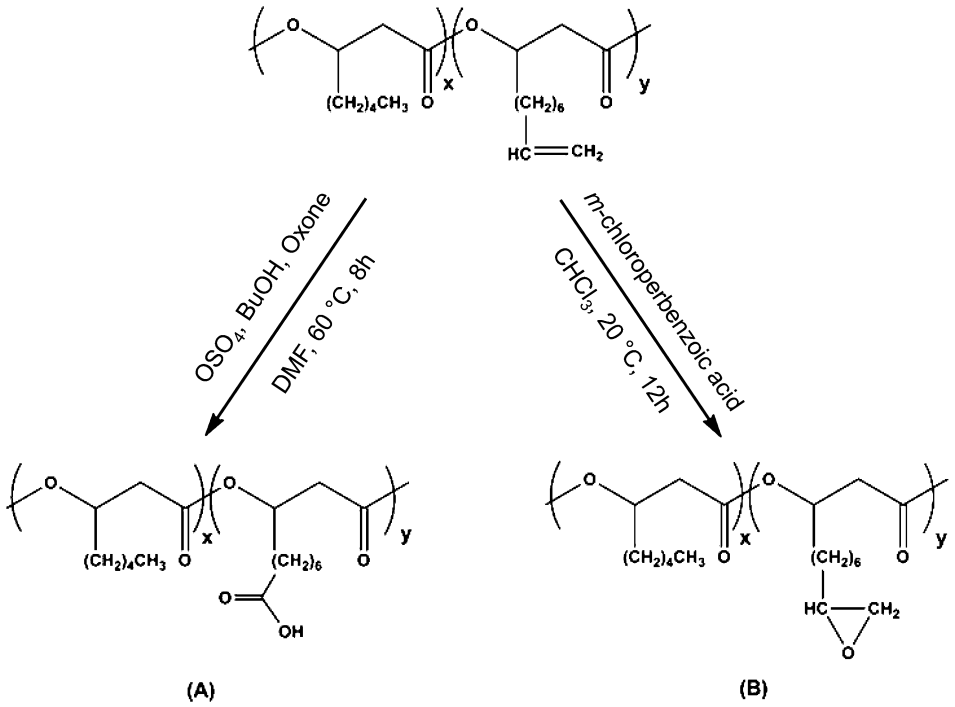

Figure 11. (A) Oxidative conversion of unsaturated groups to carboxylic acids in PHA. (B) Epoxidation of unsaturated groups in PHA.

PHAs containing unsaturated groups can be converted to carboxyl groups via oxidation.[15,74] Further improvements of this method was demonstrated when crown ether was added as the phase transfer agent as well as a dissociating agent for $KMnO_4$.[75,76] Higher degrees of conversion were observed, and there was no significant reduction of the molecular weights. For complete oxidation of double bond to carboxylic groups, osmium tetroxide, oxone, and potassium peroxomonosulfate were demonstrated to be effective agents (Figure 11A). The resulting products showed pH dependent solubility in acetone, tetrahydrofuran (THF), and organic−-water mixtures.[77]

The carboxylic acid groups in PHA macromolecules are useful for block/graft copolymer preparation. A series of PHBV, PHBHH*x*, and poly(3-hydroxyoctanoate-*co*-hydroxyhexanoate) (PHOHH*x*) oligomers with a carboxylic acid terminal group was functionalized with an alkyne moiety. Well-defined PHA−PEG diblock copolymers were obtained by the classical "click" reaction with azide-terminated PEG.[55] PHOHH*x*−PEG diblock copolymers self-assembles to micelles under aqueous conditions.[56] PHB-*g*-chitosan and PHB-*g*-cellulose graft copolymers have been prepared by reacting the carboxyl groups in PHB with the amine group of chitosan and hydroxyl groups in cellulose, respectively.[78] Similar work has been done to obtain PHBV-*g*-chitosan or PHO-*g*-chitosan copolymers. Here, PHA content grafted onto chitosan backbone ranged from 7 to 52 wt %.[79] The solubility behavior of the grafted copolymers was dependent on the degree of grafting of PHA. PHA-*g*-chitosan copolymers with different solubilities in 2 wt % acetic acid and in water could be controlled by changing the grafting percentage of PHA. PHB−PEG diblock copolymer can also be prepared by a one-step transesterification reaction using the reaction of carboxylic acid in PHB with respecive end groups in PEG-diamine or PEG.[80]

PHAs with grafted copolymer branches can also be produced by free radical coupling reaction. PEG, polystyrene (PS), and poly(methyl methacrylate) (PMMA) have all been grafted on the main polyester backbone.[15] The reaction of PEG with 4,4′-azobis(4-cyanovaleryl chloride) and 2,2′-azobis(butyronitrile) produces macroazo initiators. These initiators produce free radicals at elevated temperatures. The double bonds on the unsaturated side chains of PHAs are able to react with the PEG radicals giving a graft copolymer structure.[81] Styrene and methyl methacrylate oligomers with peroxide groups in the polymer chain as the active sites can be obtained when the corresponding monomer was polymerized with peroxide initiators. These active polymers were then grafted onto the PHAs with unsaturated side chains using the peroxide radicals.[82] In another approach, the carboxyl acid groups in the PHA side chains were reacted with the hydroxyl group of PEG or PLA oligomers.[83]

Methacrylic PHA can be prepared from PHA oligomers terminated with carboxyl groups. First, natural origin PHBs were subjected to alkaline depolymerization with *tert*-butylammonium hydroxide or KOH containing an 18-crown-6 complexing agent. The chloroform fractions with PHB oligomers were protonated using the acid ion-exchange resin Dowex.[84] These oligomers were reacted with HEMA to afford the methacrylic macromonomer. ATRP copolymerization of methacrylic PHB macromonomer with other methacrylates as comonomers including MMA, ethylene glycol methyl ether methacrylate (MeOEMA), and PEG methyl ether methacrylate (PEGMA) have been demonstrated.[85] Atactic PHB oligomers and its functionalized methacrylate (aPHBMA) have also been reported for PEG-*g*-PHB and PMMA-*g*-PHB graft copolymer preparation.[85−87]

INTRODUCTION OF EPOXY GROUP

An epoxy group could be appended to PHAs through proper chemical modification to achieve specific physical properties. The epoxide function is highly reactive under mild conditions, which can be used for further reactions such as cross-linking, attachment of bioactive substances, and introduction of

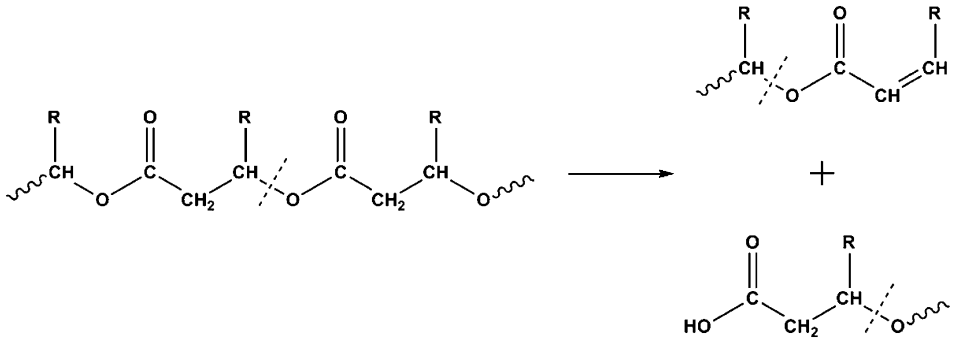

Figure 12. Primary chain scission of PHAs in thermal degradation.

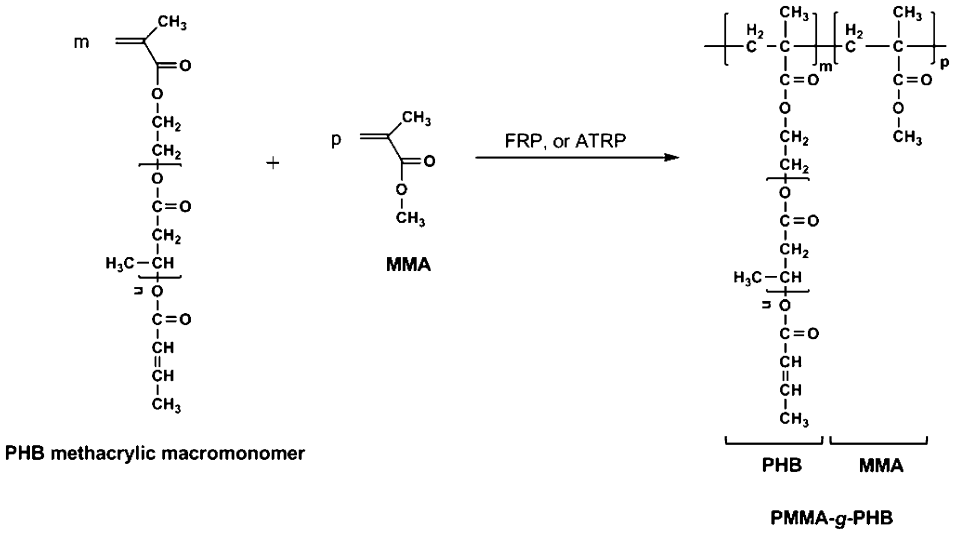

Figure 13. Synthesis of PMMA-*g*-PHB from PHB macromonomer and methyl methacrylate by conventional and controlled free radical polymerization.

ionizable groups. Park showed that the epoxidation of the unsaturated side chains in PHOUs can be carried out with *m*-chloroperbenzoic acid (MCPBA).[88] Under acidic conditions, the epoxidation reactions did not result in a significant change of the macromolecular chains of PHOUs (Figure 11B).[89] Cross-linked PHOUs can be formed by the reaction of epoxidized PHOU with succinic acid anhydride.[90] Such chemical cross-linking improves the polymers' elastic response without the loss of biodegradability.

CHLORINATION OF PHAS

Chlorination of PHAs can be performed by passing chlorine gas through unsaturated PHAs solutions.[91] This gives PHA−Cl with typical chlorine contents of between 5 −40%.[92] By this, thermal properties of the PHA−Cl were dramatically changed in terms of T_g and T_m. Because of chlorination with unsaturated side chains, PHO−Cl increased its T_g to 2 °C from −50 °C (T_g of PHO precursor), and the molecular mobility of chlorinated PHO was highly depending on the chlorine content and the molecular weight of the samples. On the other hand, hydrolysis of the PHAs during the chlorination process occurred. Molecular weights of the chlorinated samples were reduced to half or a quarter of the original values. PHA quaternary ammonium salts, sodium sulfate salts, and phenyl derivatives can be obtained from these chlorinated PHA derivatives. Friedel−Crafts reactions between benzene and PHA−Cl also give cross-linked polymers.[15,92]

FUNCTIONAL GROUPS INTRODUCED BY THERMAL DEGRADATION OF PHAS

The pyrolysis of a polymerized β-lactone leads to the formation of an α/β unsaturated acid.[4] When PHB is pyrolyzed, crotonic acid can be obtained in high yield. The formation of *trans*-crotonic acid and its oligomers is were due to a stereoselective *cis*-elimination.[93] The energetics and mechanisms of the elimination reactions have been analyzed at length.[94] The functional groups in PHA oligomers obtained after thermal degradation are highly dependent on the conditions. At moderately low temperatures (170−200 °C), an oligomer with a molecular weight of about 500 to 10,000 Da is obtained. These oligomers typically contain one unsaturated end group that is predominantly a *trans*-alkenyl end group and a carboxylic end group (Figure 12). During the first few hours of reaction, the thermal degradation of PHB and PHBV follows a kinetic model of random scission. Auto acceleration of the pyrolysis was subsequently observed, and this was attributed to a progressive increase in rate constant due to an autocatalytic effect.[29] Kopinke and co-workers suggested that the unsaturated group conjugated with the ester groups enhances the scission rate of the neighboring ester linkages.[29,93] At temperatures of up to 300 °C, monomeric, dimeric, trimeric, and tetrameric volatile products were observed along with a great decrease in the molecular weight of the PHA. Crotonic acid and β-butyrolactone were also observed during PHB thermal degradation at these temperatures.[95] At even higher

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temperatures of above 500 °C, propene and carbon dioxide are obtained as major products along with carbon monoxide, ketene, and ethanol.[96]

In the primary chain scission of PHAs thermal degradation, the obtained macromers containing one unsaturated end group and a carboxylic end group could be used for block and graft copolymer synthesis of biodegradable and biocompatible specialty macromolecules. Previous reports showed that PHAs with unsaturated crotonate end groups cannot be easily polymerized by free radical polymerizations.[97] The presence of the β-methyl substituent on the unsaturated group induces steric hindrance with the α-substituent.[98] However, the carboxylic acid terminated PHB oligomers produced by thermal degradation can be subsequently modified to give methacrylic PHA macromonomers. The graft copolymers containing PHAs can obtained by copolymerization of PHAs macromonomers with other (meth)acrylic family members. This can be accomplished by free radical polymerization (FRP) or controlled free radical polymerization (Figure 13). Nguyen and co-workers reported graft copolymers with acrylic-type backbones and side chain PHB segments.[99] PMMA-*g*-PHB copolymers were synthesized by copolymerizing of MMA and PHB macromonomers by FRP. The segment blocks were randomly dispersed and nanosized domains were obtained. The PMMA-*g*-PHB copolymers showed relatively uniform microstructures, and the resistance to strain was determined by the extent of crystallinity. However, this method produced a poorly controlled polymer structure with unpredictable molecular weights and high polydispersity. ATRP was utilized to prepare PMMA-*g*-PHB copolymers with better properties (Figure 13).[100] Higher reactivity of PHB macromonomers were observed for MMA at low molar feed ratio. However, the reactivity of the PHB macromonomer decreased compared to MMA, with an increasing proportion of macromonomer in the reaction feed.[100] These graft polymers are possible candidates in acrylic bone cements for orthopedic applications.[101] These copolymers were also incorporated in a commercially available acrylic bone cement.[101]

The synthesis of diblock copolymers from high molecular weight PHB with monomethoxy-PEG (mPEG) under pyrolysis took a long time and gave poor yields. The addition of tin(II) 2-ethylhexanoateas a calatyst in a bulk reaction resulted in yields of up to 77% within an hour. The molecular weights of the resulting PHB-PEG diblock copolymers were approximately 2000 to 7000 Da.[53] Interestingly, these polymers behaved differently from the usual diblock copolymers. First, they did not self-assemble spontaneously in water. Second, once formed from oil-in-water emulsion, the polymers did not self-assemble into micelles because the PHB core was in a folded chain lamella arrangement. This appeared to be the key factor for the poor solubility of this copolymer as this arrangement was considered a frozen polymeric structure. However, these PHB−PEG diblock copolymer could form nanoparticles in selected oil/water suspension and be applied as a drug carrier.[54]

CONCLUSION

The development of PHAs has gained great interest recently, and novel pathways for the biosynthetic PHAs have produced a variety of copolyesters by the combination of different PHA monomers. However, the greatest majority of PHA materials are more hydrophobic and show brittle mechanical properties, which limit their applications as biomedical materials. Chemical modification is an important strategy to enlarge the spectrum of PHAs in the biomedical field. Attempts to modify the PHA properties have been made by introducing different functional groups into PHA polymer structures such as hydroxyl groups, carboxylic acid, epoxy, chlorination, and double bonds. With these chemical reactions, an array of new polymers have been produced to modulate the range of accessible properties of PHAs that cannot be obtained by biotechnological processes. The tailored properties of the newly developed PHA materials in thermal and mechanical proeprties, hydrophilicity, and degradation rate have met some special requirements for specific biomedical applications.

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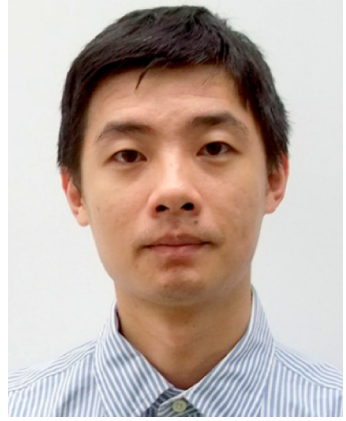
Notes

The authors declare no competing financial interest.

Biographies



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