Post Polymerization of Saturated and Unsaturated Poly(3-hydroxy alkanoate)s

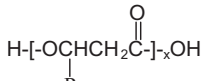
Haydar Erduranlı,[1] *Baki Hazer,**[1] *Mehlika Borcaklı*[2]

Summary: Chemical modification is useful to diversify poly(3-hydroxyalkanoate)s, for medical and industrial applications. In this manner, transesterification reactions of poly(3-hydroxy butyrate) were carried out under reflux condition of 1,2-dichlorobenzene in the presence of 1,4-butane diol, poly(ethylene glycol) bis(2-aminopropyl ether) with molecular weights of 1000 and 2000, poly(ethylene glycol)methacrylate or glycerol at 180 °C. Addition reactions of bromine and –SH groups of 3-mercaptopropionic acid to the double bond of poly(3-hydroxy-10-undecenoate) were also carried out. Functionalized poly(3-hydroxyalkanoate)s were characterized using ^{1}H NMR, FTIR, GPC and thermal analysis techniques.

Keywords: biopolymers; block copolymers; chemical modification; poly(3-hydroxyalkanoate)s; transesterification

Introduction

Bacterial polyesters, also referred to as microbial polyesters and poly(3-hydroxyalkanoate)s, PHAs, are stored as intracellular granules as a result of a metabolic stress upon imbalanced growth due to a limited supply of an essential nutrient and the presence of an excess of a carbon source.[1–3] Functionalization of polymers is a useful methodology for the generation of new materials with wide ranging applications.[4] Therefore the physical and mechanical properties of microbial polyesters need to be diversified and improved for biomedical and industrial applications.[5–10]



PHA

where R is an alkyl group: R = –CH_3, poly(3-hydroxybutyrate), PHB; R = –CH_2CH_3, poly-(3-hydroxyvalerate), PHV; R = –$(CH_2)_4CH_3$, poly(3-hydroxyoctanoate), PHO, and R = –$(CH_2)_5CH_3$, poly(3-hydroxynonanoate), PHN. The variability of bacterial PHAs that can be directly produced by fermentation is very large with about 150 different hydroxy-alkanoic acids including hydroxyl, olefin, phenyl, methyl, bromide, nitro and epoxide groups.[11–21] The chemical modification of PHAs involves grafting reactions and graft/block copolymerization, chlorination, crosslinking, epoxidation, hydroxyl and carboxylic acid functionalization of the PHAs.[22–37] In the chemical modification of PHB, hydroxyl functionalization of PHB is important. The PHB-diol is obtained by the transesterification reactions of PHB with diethylene glycol.[38,39] An amphiphilic triblock copolymer can be synthesized by coupling two chains of methoxy-PEG-monocarboxylic acid with a low molecular weight telechelic hydroxylated PHB (PHB-diol) chain in the presence of 1,3-N,N′-dicyclohexylcarbodiimide.[40]

This work refers to hydroxyl functionalization and PEGylation of PHB in one step under reflux conditions. Bromo and carboxyl derivatives of poly(3-hydroxy-10-undecenoate), PHU are also described. PEG-b-PHB-b-PEG amphiphilic triblock copolymers have especially attracted

[1] Zonguldak Karaelmas University, Department of Chemistry, 67100 Zonguldak, Turkey
[2] TUBITAK-MAM Food Sci. Technol. Res. Inst. Gebze, 41470 Kocaeli, Turkey

special attention in both fundamental and applied research because of their unique chain architecture and physical properties.[41]

Materials and Methods

Materials

PHB and PHU were microbially synthesized from *Alcaligenes eutrophus* fed with oleic acid and from *Pseudomonas oleovorans* fed with 10-undecenoic acid, respectively, according to the procedure cited in the literature.[42] 1,2-Dichlorobenzene (DCB), 1,4-butanediol, poly(ethylene glycol) bis (2-aminopropyl ether) of average MW 1000 g/mol (PEG1KNH2) and MW 2000 g/mol (PEG2KNH2) were gifts from Huntsman Co. (Switzerland). Poly(ethylene glycol)-methacrylate 526 g/mol (vPEGOH), 3-mercaptopropionic acid, bromine, dichloromethane, benzene, carbon tetrachloride and toluene were supplied by Sigma-Aldrich (Germany).

Transesterification Reactions

PHB was transesterified by 1,4-butane diol, poly(ethylene glycol) bis (2-aminopropyl ether) of average MW 1000 g/mol (PEG1KNH2) and MW 2000 g/mol (PEG2KNH2), and poly(ethylene glycol)-methacrylate 526 g/mol (vPEGOH526) under reflux condition in their 1,2-dichloro benzene solutions. As an example, 3 g of PHB, 3 g of 1,4-butane diol, and 0.02 g of stannous octanoate were mixed in 20 mL of 1,2-dichlorobenzene and refluxed for 6 h at 180 °C. The solvent was evaporated and the residue was redissolved in chloroform. PHB-diol was precipitated in a mixture of methanol and water (1/1 v/v) and dried under vacuum overnight at 40 °C. PHB-b-PEG1KNH2 and PHB-b-vPEGOH526 block copolymers were precipitated from light petroleum benzene. Polymer yields of the products ranged between 30 and 70%.

Addition Reaction of Bromine to the Double Bonds of PHU

Stock solution of bromine (1 mL) in freshly distilled CCl_4 (10 mL) was prepared. 0.5 g of PHU was added in 2 mL of stock solution in a schlenck tube and left for 2 h at room temperature in the dark. Brominated PHU (PHU-Br) was precipitated from 50 mL of methanol and dried under vacuum.

Carboxyl Functionalization of PHU

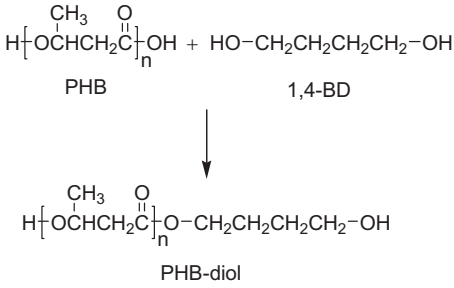
PHU was carboxyl functionalized by using 3-mercaptopropionic acid, MPA, as previously described by Hany et al. [42] For this purpose, MPA (50% w/w excess of equivalency), and 2,2′-azobis(isobutyronitrile), AIBN, 0.1 equiv were dissolved in 10 mL of toluene under argon. The solution was heated at 75 °C for 6 h, cooled to room temperature, and poured into methanol (100 mL). The obtained carboxyl functionalized PHU (PHU-COOH) was dried under vacuum. Polymer was completely soluble and the yield was 60%.

Fractional Precipitations of the Functionalized-PHAs

Fractional precipitations of the functionalized-PHAs were carried out according to the procedure cited in the literature.[43] Vacuum-dried functionalized-biopolyester sample was dissolved in 5 mL of $CHCl_3$. To the stirring solution, MeOH was added dropwise until completion of the first precipitation. After decantation, the upper solvent was followed by addition of MeOH for the second fraction. The same procedure was applied until no more precipitation. Gamma γ values were calculated as the ratio of the total volume of MeOH used for each fraction to the volume of $CHCl_3$. Polymer fractions were dried under vacuum.

Polymer Characterization

FT-IR spectra were obtained using a Jasco FTIR-300 E spectrometer. ^{1}H NMR spectra were recorded in $CDCl_3$ at 17 °C with a tetramethylsilane internal standard using a 400 MHz NMR AC 400 L. The molecular weight of the polymeric samples was determined by gel permeation chromatography (GPC) with a Knauer GPC in $CHCl_3$ solution at 35 °C, at a low rate of 1 mL/min using ChromGate software, a WellChrom Interface Box, RI Detector K-2301, and WellChrom HPLC pump K-501. Polystyrene



Scheme 1.
One step transesterification reaction to obtain PHB-diol.

standards with low polydispersity obtained from Polysciences were used to generate a calibration curve.

Differential scanning calorimeter (DSC) and thermogravimetric analysis (TGA) was performed with a DuPont 2910 to determine the glass transition temperatures (T_g), the melting temperature (T_m), and decomposition temperature (T_d). Samples were heated from −50 to 200 °C in a nitrogen atmosphere at a rate of 10 °C/min.

Results and Discussion

Transesterification Reactions

PHB-diol and PEG (MW: 526, 1000 and 2000)-b-PHB block copolymers have been obtained by transesterification reactions in one step. During this type of reactions, polymer chain scission was unavoidable.

When PHB was boiled with 1,4-butanediol in 1,2-dichlorobenzene, PHB-diol was obtained.

Scheme 1 shows the one step transesterification reaction to obtain PHB-diol. PHB-diol has also characteristic signals of HO-CH_2- group at δ ppm 3.5–4.1 in ^{1}H NMR spectrum. Figure 1 shows the ^{1}H NMR spectrum of PHB-diol. FTIR spectrum of PHB-diol has also indicated the characteristic peak of hydroxyl ends at 3437 cm^{-1}.

PHB-b-PEG1000 and PHB-b-PEG2000 Block Copolymers

The refluxing of PHB and poly(ethylene glycol) bis (2-aminopropyl ether) with MW 1000 and 2000 gave the block copolymers in one step transesterification reactions. According to the equivalency, AB and ABA type of block copolymers could be obtained. Amine ends of the PEG easily react with carboxylic ends of PHB as shown in Scheme 2.

^{1}H NMR spectra of PHB-b-PEG1000 and PHB-b-PEG2000 indicated the characteristic signals of both PHB and PEG. A typical ^{1}H NMR spectrum of the block copolymer is given in Figure 2a. FTIR spectra of the block copolymers also confirmed their chemical structure with the

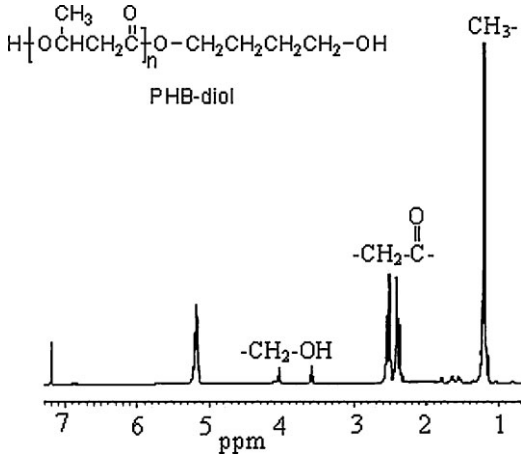
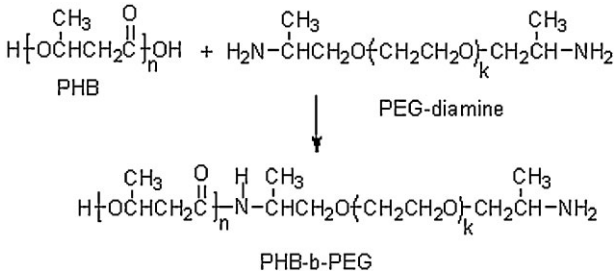


Figure 1.
^{1}H NMR spectrum of PHB-diol.



Scheme 2.
Transesterification reaction of PEG1KNH2 with PHB.

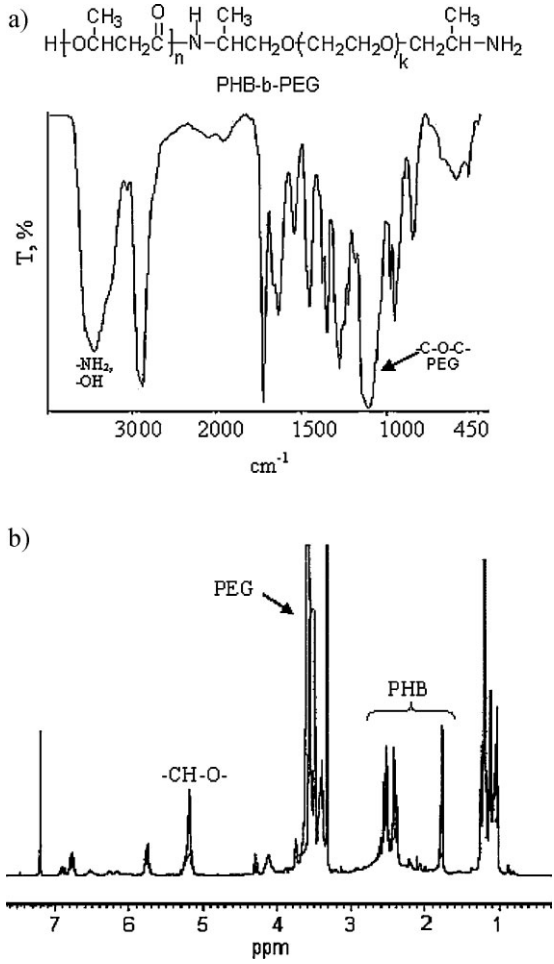
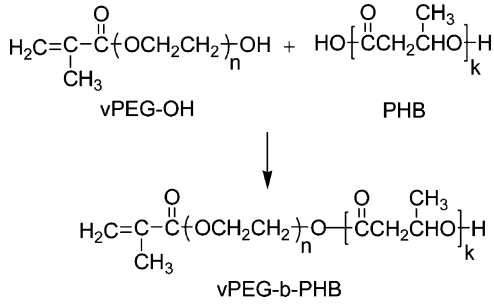


Figure 2.
FTIR (a) and ^{1}H NMR (b) spectra of PHB-b-PEG1000 block copolymer.



Scheme 3.
Transesterification reaction of vPEGOH with PHB.

characteristic signals at 1100 cm^{-1} for etheric groups and of PEG and 1720 cm^{-1} for carbonyl group of PHB.

vPEG-b-PHB Block Copolymer

Transesterification reaction of PHB with poly(ethylene glycol)methacrylate (vPEG-OH) gave vPEG-b-PHB block copolymer as shown in Scheme 3.

^{1}H NMR spectrum of the block copolymer contained characteristic signals of vinyl group (vPEG) at 5.5–6.0 ppm together with PHB signals as shown in Figure 3.

Fractional Precipitation and Molecular Weights

The graft copolymers were fractionally precipitated to determine the γ values of the graft copolymers. γ values change with the block structure, molecular weight of the polymers and the solvent/non-solvent system. Chloroform (solvent)/methanol (non-solvent) system was chosen to be able to make a comparison of all polymers obtained with each other. PHB-(alone) refluxed in 1,2-dichlorobenzene alone for 6 h was precipitated in the γ ranges 1.8–2.4, whereas other functionalized polymers were precipitated in the ranges of 1.9–4.3 which was greater than that of the template. Hydroxyl functionalization and PEGylation shifted the upper γ values as expected. Because the reagents 1,4-BD and PEGs were all soluble in the solvent/non-solvent system, the unreacted residues were already eliminated by remaining in solution during the precipitation procedure. Further purification was also performed by the fractional precipitation. GPC measurements of the fractionally precipitated

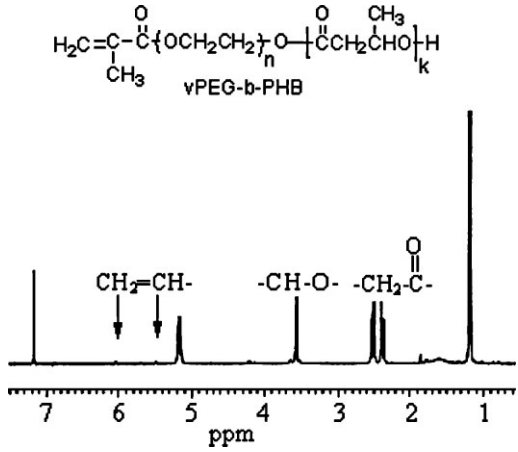


Figure 3.
^{1}H NMR spectrum of vPEG-b-PHB block copolymer.

Table 1.
GPC data of the functionalized PHBs.

Product	M_n/MWD	γ-values
PHB-(alone)	6000/1.36	1.8–2.4
PHB-diol	3100/1.30	2.6–3.1
PHB-b-vPEG360	6800/1.40	1.9–2.9
PHB-b-vPEG526	9200/1.42	1.9–2.9
PHB-b-PEG1KNH2	2700/1.26	3.5–4.3
PHU-Br	14300/2.64	
PHU-COOH	6800/1.46	
PHB-original	151K/2.18	
PHU-original	136K/1.84	

samples all indicated the unimodal GPC curves which can be attributed to the pure graft copolymers freed from the related homopolymers, too. The molecular weights of the functionalized PHB samples are listed in Table 1. Refluxing of the PHB samples gives rise to significant levels of chain scission. Mn of the original PHB was 151,000 Da while functionalized polymers had M_n lower than 10,000 Da.

Thermal Properties

DSC and TGA were used to analyze the thermal properties of the functionalized polymers. The polymers obtained in this work had a lower T_m (141–160 °C) than that of the related original PHB (at around 170 °C) because of their lower M_n's. PHB-diol had a higher T_d (300 °C) than PHB-(alone) (280 °C) according to the rule that an increase in the hydroxy termination in PHB leads to an increase in the thermal stability.[44] The DSC traces of PHB-b-PEG1KNH2, PHB-diol and PHB-(alone) are shown in Figure 4.

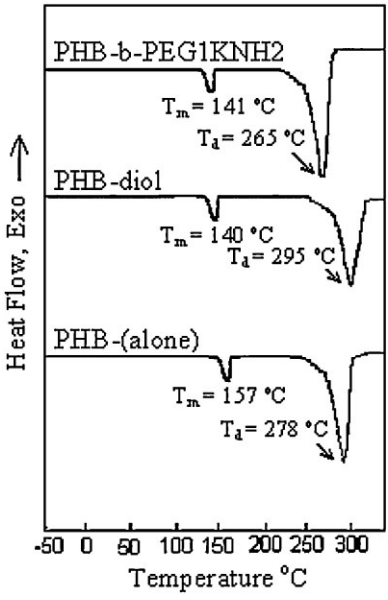


Figure 4.
The DSC traces of PHB-b-PEG1KNH2, PHB-diol and PHB-(alone).

Table 2.
Thermal properties of the functionalized PHB samples.

Product	T_m, °C	T_d, °C
PHB-(alone)	157	280
PHB-diol	141	300
PHB-b-vPEG360	157	300
PHB-b-vPEG526	160	300
PHB-b-PEG1KNH2	141	275

Plasticizer effect of PEG1000 was clearly observed with the lowest T_m and T_d. Thermal analysis results of the functionalized PHB samples are listed in Table 2.

Addition Reactions

PHU-Br

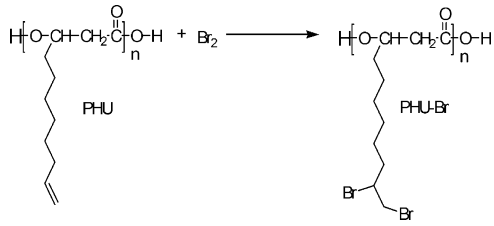
Bromine was added to the double bonds of PHU at room temperature in dark to obtain brominated-PHU (PHU-Br) (Scheme 4).

After the bromination reaction, the signals of the double bonds at 4.9 and 5.8 ppm of PHU in ^{1}H NMR spectrum disappeared. ^{1}H NMR spectra of PHU and PHU-Br are indicated in Figure 5.

PHU-COOH

Inspired by the free radical addition reaction of 11-mercapto undecanoic acid with poly(3-hydroxy octanoate-co-undecenoate) in the literature,[42] PHU was carboxyl functionalized by using 3-mercaptopropionic acid and AIBN under argon. Free radicals generated by the AIBN attack at the double bond of PHU which were terminated by the –SH group of mercaptopropionic acid as shown in Scheme 5.

^{1}H NMR spectrum of PHU-COOH had characteristic at 3.7 ppm for –S–CH_2–, 4.1 ppm for –O–CH– and 10.1 ppm for



Scheme 4.
Synthesis of brominated PHU (PHU-Br).

–COOH. Figure 6 indicates the ^{1}H NMR of PHU-COOH.

Conclusion

Chemical modification of the PHAs has been very attractive for the scientist to prepare medical and film packing materials.[9–11] PHAs are hydrophobic polyesters and mostly need to gain hydrophilicity for the medical applications. PEG permits control of the balance between the hydrophilic and the hydrophobic parts of the polymers which determines their medical application. Methoxy-PEG-b-PHB block copolymers were obtained by melt conditions to study their morphologies[45] and PEG-b-PHB block copolymers were obtained by the chain extension reactions between PHB-diol and PEG[46] in two steps. In this manner, PEGylation of PHB in a single step was performed in this work via transesterification reaction with either PEG having primary amine ends or methacryloyl polyethyleneglycol which will be useful for further modification reactions.

Brominated PHU was obtained by the bromine addition to the double bonds of the PHU. This reaction was quantitative

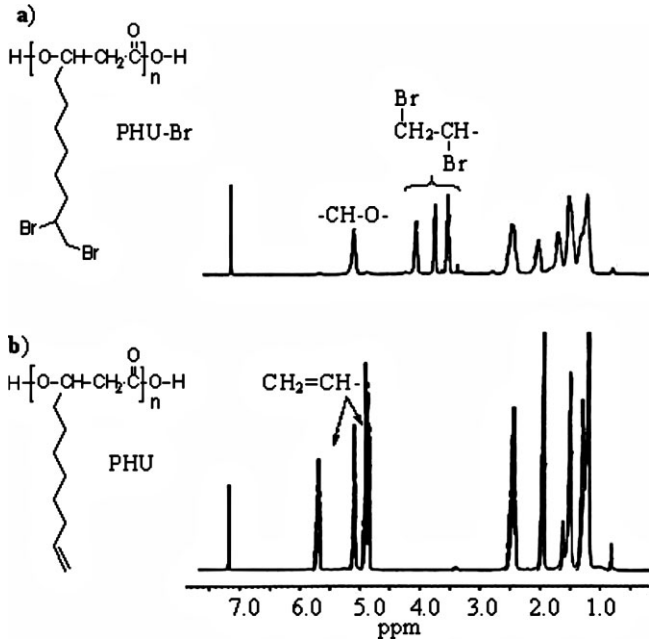
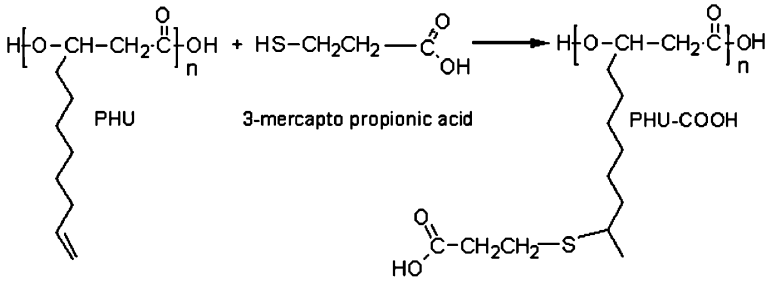


Figure 5.
^{1}H NMR spectra of (a) PHU-Br and (b) PHU.



Scheme 5.
Synthesis of PHU-COOH.

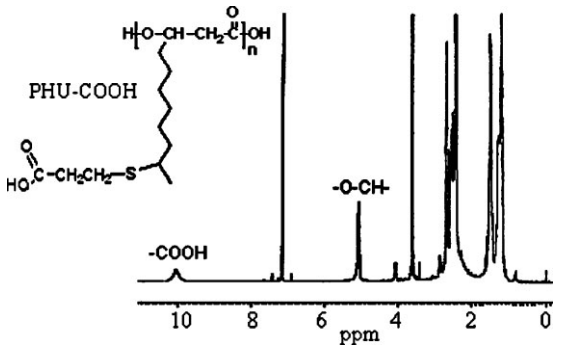


Figure 6.
^{1}H NMR spectrum of PHU-COOH.

and followed by the disappearing vinyl signals in ^{1}H NMR. Carboxylation of the PHU with mercaptopropionic acid instead of mercaptoundecenoic acid[42] and dihydroxylation of PHB with 1,4-butane diol instead of ethylene glycol[27] were evaluated by means of γ-values. During the chemical modification reactions, polymer degradation was unavoidable. Therefore, molecular weights of the functionalized PHAs changed between 3000 and 14000 g/mol. Further functionalization reactions of the modified PHAs obtained in this work are in progress.

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