

Synthesis and Characterization of the Poly(ethylene glycol) Grafted Unsaturated Microbial Polyesters

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Abstract

Pseudomonas oleovorans was grown with either 10-undecenoic acid alone or the equimolar mixture of octanoic acid and 10-undecenoic acid to obtain unsaturated poly(3-hydroxy alkanoates) (PHA)s; poly(3-hydroxy-10-undecenoate) (PHU) and poly(3-hydroxy-octanoate-co-3-hydroxy-10-undecenoate) (PHOU), respectively. The addition of bromine to olefinic double bond, by reacting the unsaturated PHA with bromine in homogeneous solution in dark, was readily carried out. The brominated PHA was reacted with polyethylene glycol (PEG) in the presence of a base to obtain PHA-g-PEG graft copolymers. The polymers were characterized by ¹H NMR and FTIR spectroscopy, gel permeation chromatography (GPC), differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA).

INTRODUCTION

Polyhydroxyalkanoates (PHAs) are naturally occurring polyesters that are produced by a wide variety of microorganisms [1-3]. PHA is suitable for a broad range of applications in medicine, pharmacy, and industry due to its biocompatibility and biodegradability [4-6]. *A. Eutrophus (Wautersia eutropha)* produces PHAs with short chain alkyl substituents whether grown on short or long chain carbon sources [1]. *Pseudomonas oleovorans*

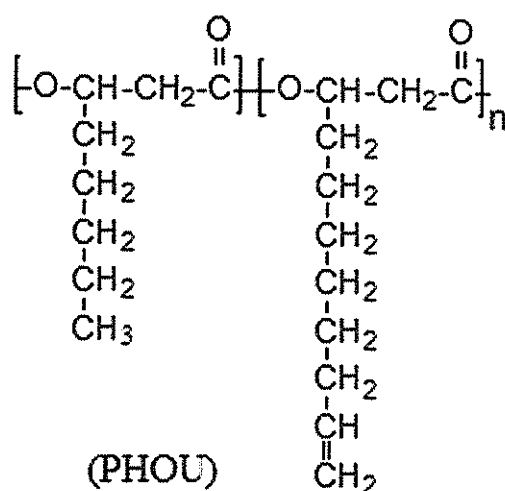
produces PHAs when grown on carbon sources with at least six carbons, and the PHAs so produced contain relatively long pendant alkyl groups (side chains). In addition to producing PHAs with long alkyl groups, *P. oleovorans* can also utilize many functionalized organic substrates to produce PHAs with terminal functional groups in the side chain. In this manner, the production of PHAs with various functional groups including phenyl, chloride, fluoride, bromide, cyano, aryl, alkyl, ester, branched alkyl, and vinyl groups has been achieved [7-13]. The presence of functional groups in PHAs provides sites for chemical modifications and can affect the physical properties of polymers such as their hydrophobicity and solubility [14-21]. A particularly useful type of functional PHAs is the one containing

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unsaturated groups in the side chain. Unsaturated PHAs can be produced from fatty acid-containing substrates obtained from unsaturated natural oils [22-24]. When *P. oleovorans* is grown on 10-undecenoic acid, PHU containing unsaturated side chains is also obtained [25-27]. An unsaturated copolyester, poly(3-hydroxyoctanoate-co-3-hydroxy-10-undecenoate) PHOU, as shown in the following structure, can be obtained from the equimolar mixture of sodium octanoate and 10-undecenoic acid [28]:



Structure of PHOU.

PHOUs containing unsaturated units have potential for a wide range of applications including biodegradable elastomers, hydrogels and adhesives. To achieve specific physical properties, the unsaturated units can be converted into other functional groups, such as epoxy, carboxylic acid and hydroxyl groups [29-33]. PHAs must be chemically modified to produce a water-soluble PHA (amphiphilic polymer) to be used in medically relevant applications requiring water solubility. Recently, diethanol amine modified PHOU resulting in a water soluble polymer at a pH below its pK_a due to protonation of the nitrogen atom has been reported [34]. Amphiphilic block copolymers have attracted special attention in both fundamental and applied research because of their unique chain

architecture and physical properties [35]. Poly (ethylene glycol) (PEG) is a neutral, nontoxic, water soluble and biocompatible polyether with extraordinary biological properties. PEG is widely used in biomedical applications [36,37]. In this manner, PEG grafting reactions on the hydrophobic PHAs by different methods to obtain new biomaterials were reported [38-40]. In this work, we report the synthesis of a new type of amphiphilic PH(O)U comb-type copolymers with pendant PEG units. For this purpose, PHOU and PHU were quantitatively brominated by bromine addition to the unsaturated side chains as reported previously [41]. Then, the reaction between hydroxyl end groups of PEG and bromide groups of the brominated PHOU and PHU resulted in PHOU-g-PEG and PHU-g-PEG graft copolymers. These novel amphiphilic copolymers were thermally and spectroscopically characterized.

EXPERIMENTAL

Materials

PHU (M_n : 36,000 Da, M_w : 69,000 Da) and PHOU (M_n : 64,000 Da, M_w : 201,000 Da) were obtained as described in our recent work [41]. For this purpose, *P. oleovorans* was grown on 10-undecenoic acid (UA) alone and the equimolar mixture of octanoic acid (OA) and 10-undecenoic acid (UA), respectively. Three different type of PEGs (The numbers indicate the number average molecular weights, M_n , of the PEGs): PEG2000 and methoxyPEG2000 (mPEG2000), bromine, dichloromethane, carbon tetrachloride and toluene were supplied by Sigma-Aldrich (Germany). Chloroform and concentrated HCl were obtained from Riedel-de Haen. Tetrahydrofuran (THF), methanol and sodium hydride were supplied by Merck, Fluka and Aldrich, respectively. They were used without further purification.

Addition Reaction of Bromine to the Double Bonds of PHU and PHOU

Brominated-PHU (PHU-Br) and brominated-PHOU (PHOU-Br) were synthesized by the addition reaction of bromine to the double bonds. The same procedure described in our recent work was applied with a slight modification [41]. For example, stock solution of bromine (1 mL) in freshly distilled CCl₄ (10 mL) was prepared. 2 mL of this bromine solution was added into 10 mL of CCl₄ solution containing 0.5 g of PHU at 5°C with continuous stirring and the mixture was left for 2 h in dark. PHU-Br was precipitated in 50 mL of methanol and dried under vacuum overnight. The same procedure was also applied to the synthesis of PHOU-Br starting with 0.5 g of PHOU.

Synthesis of PHU-g-PEG and PHOU-g-PEG

Halogenated PHA was reacted with hydroxyl end groups of PEGs (mol ratio of PHA-Br to PEG is in the range of 1:1-1:0.2). As an example, the solution of 0.5 g of PHU-Br in THF (10 mL) was added drop wise to the solution containing 0.1 g of NaH and 0.2 g of mPEG-2000 in THF (10 mL) and stirred for 24 hours at room temperature. This solution was poured into 150 mL of petroleum ether which contains 0.02 mL of concentrated HCl. The precipitated graft copolymer was dried under vacuum overnight. Vacuum dried polymer was dissolved in 10 mL of THF, precipitated in methanol for further purification and dried under vacuum overnight at room temperature. Consequently, the PHU-g-mPEG-2000 graft copolymer was obtained. All other copolymers, PHOU-g-mPEG2000, PHU-g-PEG2000, and PHOU-g-PEG2000, were synthesized by using the same procedure.

Polymer Characterization

¹H-NMR spectra were recorded in CDCl₃ with a tetramethylsilane internal standard using a Varian XL 200 device. FT-IR spectra were obtained using a Perkin Elmer Pyris 1 Spectrometer. The molecular

weight of the polymeric samples were determined by gel permeation chromatography (GPC) with SEC measurements in tetrahydrofuran (THF), an Agilent 1100 Series GPC Setup (gel permeation chromatography) was used as an integrated instrument, including a Zorbax PSM 60 S column (5x10²-10⁴ MW Range), Zorbax PSM 1000 S (10⁴-10⁶ MW Range), a UV (254 nm) and RI detector. The eluent was used at 40°C and at a flow rate of 1 mL/min. A calibration curve was generated with four polystyrene green standards provided by EasyCal Agilent Technologies Polymer Standards Service (M.W's: 696500, 50400, 2960 and 162 g/mol).

Differential Scanning Calorimeter (DSC) and Thermo-gravimetric Analysis (TGA) were performed with a Dupont 2910 to determine the glass transition temperatures (T_g), the melting temperature (T_m) and the decomposition temperature (T_d).

RESULTS AND DISCUSSION

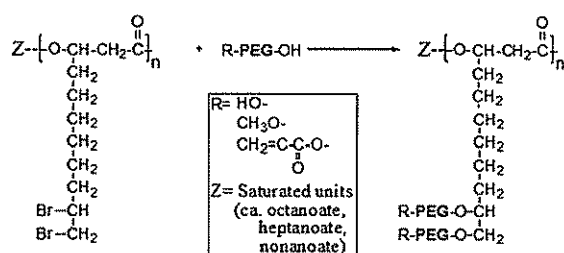
Synthesis of PH(O)U-g-PEG2000 and PH(O)U-g-mPEG2000

Several different approaches have been taken to prepare PEG-g-PHA cross linked amphiphilic graft copolymers via free radical mechanism [38-40]. We describe grafting of PEG chains onto brominated-PHA using a Williamson-ether-synthesis-like reaction. While this reaction was widely used for making macromolecules (i.e. convergent synthesis of Frechet-type dendrimers relies on this reaction) [42] it has not been used for grafting PEG chains onto brominated PHAs.

For this purpose, double bonds of the PH(O)U samples were first quantitatively brominated. Then the brominated PHA was reacted with PEG. The amount of brominated PHA added was in equimolar ratio to PEG. In equimolar amounts of the starting materials gave PHOU-g-PEG partially cross-linked

graft copolymers. When the molar ratio of PHA-Br to PEG was used 1:0.2, PEG-g-PHA amphiphilic graft copolymers were also obtained, which were completely soluble in chloroform and methanol. Number average molecular weight of the obtained PHOU-g-PEG amphiphilic graft copolymer was found to be 7012 g/mol (MWD: 2.655). ^1H and ^{13}C NMR spectra of this product confirmed the copolymer structure having 55 mol% of PEG (The spectra were not shown).

In this manner, some PEG moieties such as methacryloyl oxy poly(ethylene glycol) (vPEG-OH), methoxy poly(ethylene glycol) (mPEG) and poly(ethylene glycol) (PEG) were used to obtain PHU-g-PEG2000, PHU-g-mPEG2000, PHOU-g-PEG-2000 and PHOU-g-mPEG2000 amphiphilic graft copolymers. Scheme 1 summarizes typical PEG grafting reactions to obtain graft copolymers.



Scheme 1. A typical PEG grafting reaction on the brominated PH(O)U.

Molecular weights of the amphiphilic copolymer samples were collected in Table 1.

Table 1. GPC results and copolymer analysis of the amphiphilic comb type graft copolymers.

| PHA code | M_n $\times 10^3$ | $M_w \times 10^3$ | MWD | PEG content in copolymers (mol%) |
|-----------------|------------------------|-------------------|------|--|
| PHU-Br | 4.7 | 2.0 | 4.29 | - |
| PHOU-Br | 3.3 | 2.0 | 5.88 | - |
| PHU-g-PEG2000 | 8.3 | 1.6 | 1.96 | 76 |
| PHU-g-mPEG2000 | 8.2 | 1.6 | 1.91 | 60 |
| PHOU-g-PEG2000 | 7.5 | 1.7 | 2.22 | 75 |
| PHOU-g-mPEG2000 | 9.4 | 2.0 | 2.10 | 67 |

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All graft copolymer samples had two to four times higher M_n than those of precursors. This can be attributed to copolymer formation and the fact that hydrolysis of the polyester does not occur under these reaction conditions while the ester groups of the PHAs can easily be hydrolyzed under acidic conditions [43].

Figure 1 shows ^1H NMR spectra of the PEG grafted PHA samples indicating the characteristic signals of C-O-C- ether group of the PEG units at δ ppm 3.5-4.1. These broad signals may also overlap with the signals due to the unreacted bromide groups of the brominated PHAs. Vinyl groups of the vPEG units of the PH(O)U-g-vPEG were observed at 5.8-6.3 ppm. The arising signal at 0.8 ppm in the ^1H NMR spectra of the PH(O)U-g-PEG graft copolymers belongs to the pendant methyl groups of the saturated repeating units [28,41]. After the brominating reaction of PHU and PHOU, the signals of the double bonds at 4.9 and 5.8 ppm completely disappeared as reported before [41]. From the ratio of the integrated intensities of the total bromide signals to the total integral values of the whole spectrum, mol % of double bond content of the polymers were determined to be 18 mol% for PHU and 11 mol % for PHOU.

Figure 2 shows FTIR spectra of the PEG-g-PHU (a) and PEG-g-PHOU (b) samples. All samples had the characteristic signals of the ether groups of PEG units at $1066-1105 \text{ cm}^{-1}$ together with the bands of hydroxyl at $3375-3401 \text{ cm}^{-1}$ and carbonyl moieties at 1738 cm^{-1} .

Thermal Properties

DSC and TGA were used to analyze the thermal properties of the graft copolymers. Figure 3 shows the DSC curves of the PEG-g-PHA samples.

For PHU-g-vPEG360 sample, two glass transitions at -41°C and -16°C and a weak melting transition at

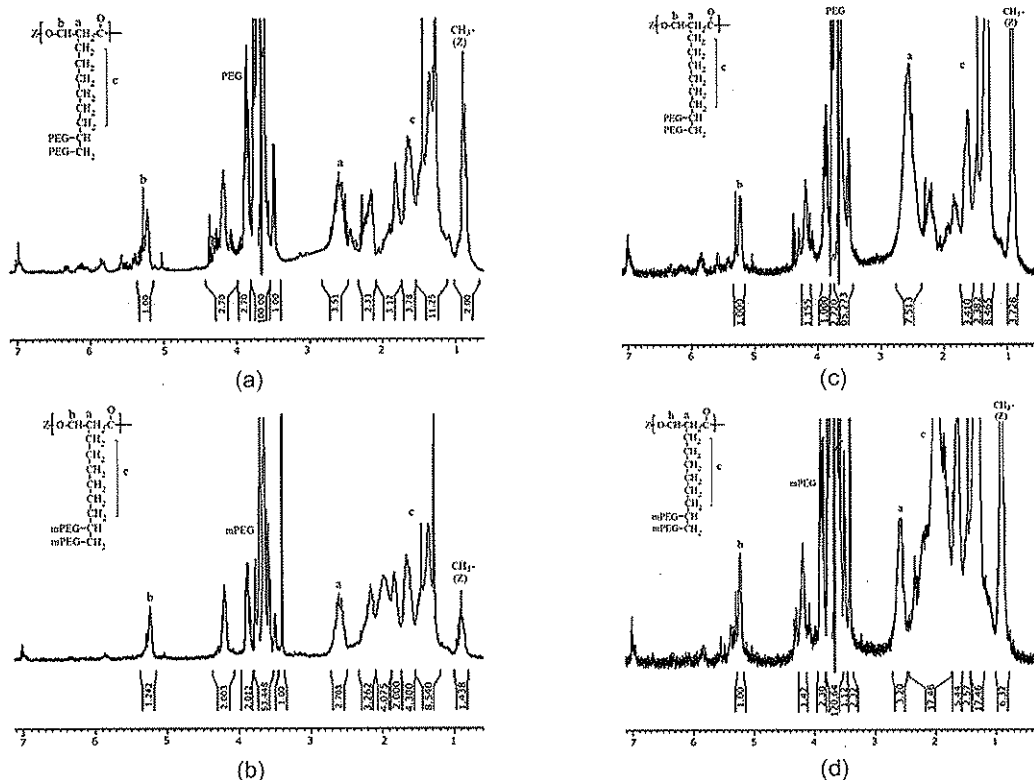


Figure 1. ¹H NMR spectra of (a) PHU-g-PEG2000(Z: saturated repeating units: ca. heptanoate, nonanoate); (b) PHU-g-mPEG2000. (Z: saturated repeating units: ca. heptanoate, nonanoate); (c) PHOU-g-PEG2000. (Z: saturated repeating units: ca. octanoate, heptanoate, nonanoate); (d) PHOU-g-mPEG2000. (Z: saturated repeating units: ca. octanoate, heptanoate, nonanoate).

93°C were observed (Figure 3a). Presumably, the low crystallinity led to a weak T_m signal. T_g 's of the PHAs in copolymers were observed at -16°C. T_m 's of the PHAs were observed at 48°C and 51°C. Additionally, T_m 's of the PEG2000 segments were observed at higher temperatures (ca. from 85°C to 93°C). DSC of PHOU-g-vPEG360 was measured but was not shown because vPEG360 did not affect on the DSC thermogram of PHOU-g-vPEG360. Table 2 contains data from the thermal characterization of the different graft copolymers of PH(O)U-g-PEG.

Figure 4 shows TGA thermograms of PH(O)U-graft copolymer samples with different PEG moieties. The thermal decomposition patterns of the polymers with PEG and mPEG followed different patterns indicating some characteristic weight loss steps. The first weight loss step is probably due to the release of low molecular weight decomposition -

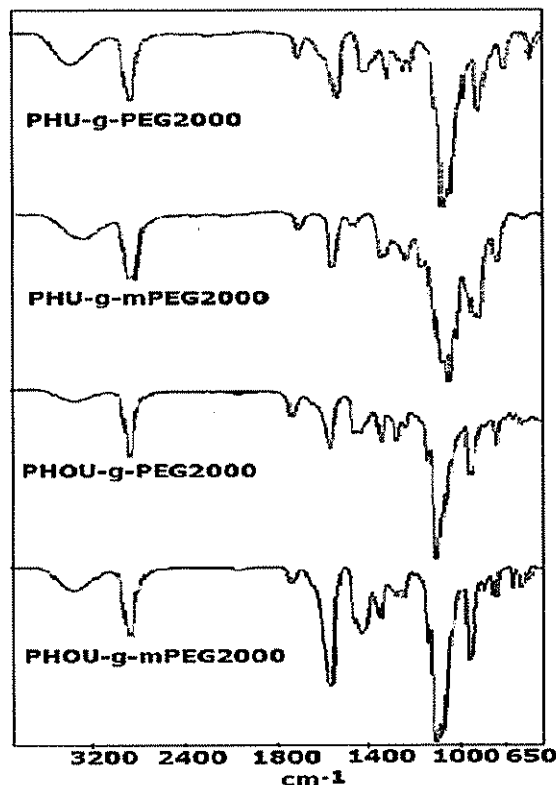


Figure 2. FTIR spectra of copolymers.

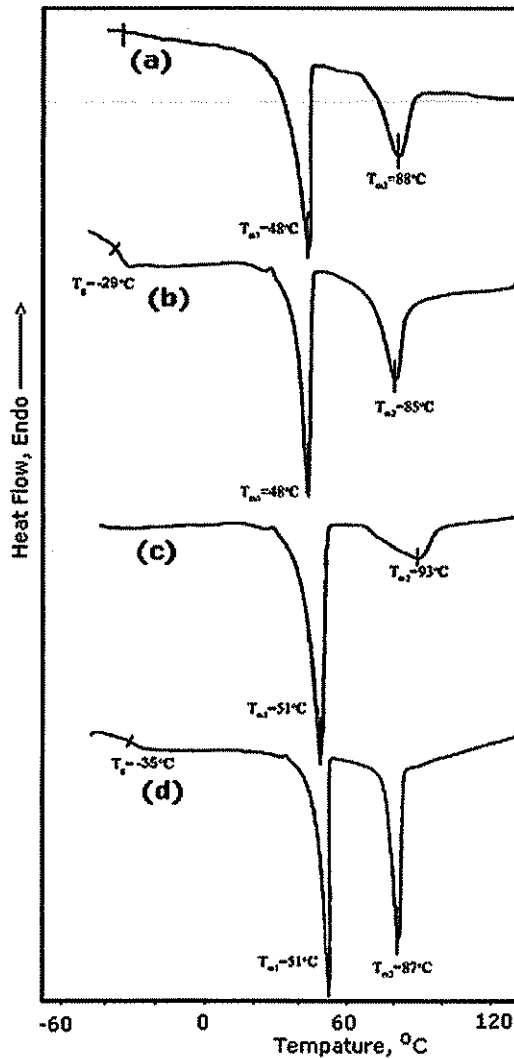


Figure 3. DSC thermograms of the PHA-graft copolymers: (a) PHU-g-PEG2000, (b) PHU-g-mPEG2000, (c) PHOU-g-PEG2000, (d) PHOU-g-mPEG2000.

products from the polyester linkage ($T_d=200-250^\circ\text{C}$) and the second step is due to the decomposition of grafted PEG residue ($T_d=380-420^\circ\text{C}$). Figure 4 also shows TGA thermograms of PHU-Br and PHOU-Br samples. Decomposition temperature of the PHU-Br sample containing 18 mol% of Br ($T_d=380^\circ\text{C}$) was higher than that of the PHU-Br with 11 mol% of Br. As bromide content increases, higher decomposition temperature has been observed.

CONCLUSIONS

Chemical modification of the hydrophobic PHAs has been very attractive for medical applications. For many medical applications, PHAs with hydrophilic character are required. In this work, hydrophilic PEG blocks were simply introduced into the PHA copolymers. This process yields comb-type graft copolymers with a wide structural range, thus controlling the hydrophilic/hydrophobic balance.

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Table 2. Thermal analysis results of the amphiphilic graft copolymers.

| Run No | T _{g1} (°C) | T _{m1} (°C) | T _{m2} (°C) | T _{d1} (°C) | T _{d2} (°C) | T _{d3} (°C) | T _{d4} (°C) |
|-----------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| PHU-g-PEG2000 | -34 | 48 | 88 | 250 | 330 | | |
| PHU-g-mPEG2000 | -29 | 48 | 85 | 250 | 420 | | |
| PHOU-g-PEG2000 | - | 51 | 93 | 270 | 420 | | |
| PHOU-g-mPEG2000 | -35 | 51 | 87 | 240 | 420 | | |
| PHU-Br | | | | 220 | 380 | | |
| PHOU-Br | | | | 100-200 | 280 | | |

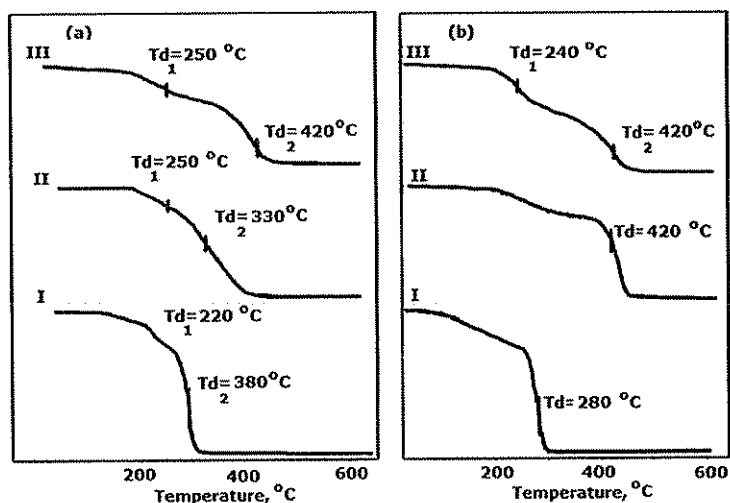


Figure 4. The TGA thermograms of (a): (I) PHU-Br, (II) PHU-g-PEG2000, (III) PHU-g-mPEG2000; and (b): (I) PHOU-Br, (II) PHOU-g-PEG2000, (III) PHOU-g-mPEG2000.

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