Synthesis of Novel Graft Polyhydroxyalkanoates

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Summary: Graft copolymers were synthesized by direct condensation of methoxy-poly(ethylene glycol) (MePEG) or methoxy-poly(lactic acid) (MePLA) onto a reactive polyhydroxyalkanoate (PHA) backbone in organic solvent. Side carboxylic groups of the PHA were coupled with end hydroxyl groups of MePEG or MePLA in the presence of N,N'-dicylohexylcarbodiimide (DCC). Graft copolymers were characterized by ^{1}H NMR spectroscopy and size exclusion chromatography (SEC). NMR spectra of PHA-*g*-PEG and PHA-*g*-PLA showed the presence of significant amounts of PEG and PLA, respectively. No noticeable unreacted PEG or PLA were detected in SEC chromatograms. Grafting of hydrophilic polymers chains as PEG or biodegradable oligomers as PLA onto PHA backbone will generate polyesters with a more rapid water uptake and faster biodegradation rates. These PHA polymers conjugates could be interesting for bioactive agent delivery systems.

Keywords : graft polyester; PEG; PLA; polyhydroxyalkanoate

Introduction

Poly(3-hydroxyalkanoates) (PHAs) are polyesters produced by a wide variety of microorganisms [1]. Among bacteria able to accumulate PHA, *Pseudomonas sp.* GP01 has been widely investigated due to its capacity to synthesize polymers with medium length side chains [2]. *P. oleovorans* has the particularity to produce polyesters from a large spectrum of carbon substrates, including n-alkanes and alkanoic acids [2-5]. More interestingly this strain can accumulate polymer with functional end groups in side chains, such as Cl, Br, nitrile, and double bounds [6-10]. Poly(3-hydroxyoctanoate-co-3-hydroxyundecenoate) (PHOU), has been produced [11] from sodium octanoate and 10-undecenoic acid mixtures,. The presence of functional groups in PHAs provides sites for chemical modification, which can affect their physical properties. PHAs are biopolymers with considerable interest based on their biodegradability, biocompatibility and other physical properties that range from

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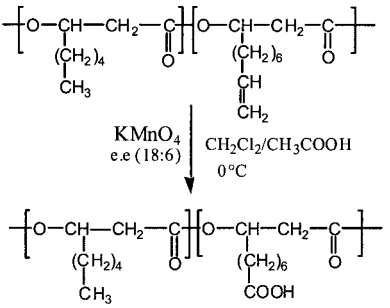
thermoplastic to elastomeric according to the macromolecular composition. PHAs have been currently recognized as suitable material for controlled drug release and biomedical devices [12]. However, their actual use as biomaterials is limited by their low hydrolytic degradation. The hydrolysis of PHAs could be controlled by introduction of hydrophilic units or degradable units. In order to improve degradation of PHAs two modifications have been proposed: on the one hand, increasing the hydrophilicity of the polymers will be result in a faster water uptake and swelling of the polymer matrix, promoting the hydrolysis of PHA. On the other hand, grafting hydrolyzable PLA chains onto hydrophobic PHA backbone will generate polyesters with more rapid degradation. We report here an investigation of new PHA polymer conjugates consisting of hydrophobic PHA backbone to which hydrophilic PEG or hydrolizable PLA are chemically bound by direct esterification.

Results and Discussion

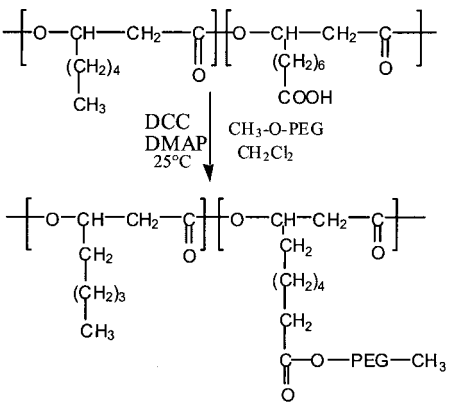
We have previously reported the production of PHAs with unsaturated repeating units (25%) using sodium octanoate and 10-undecenoic acid mixtures as carbon source [11]. As shown in scheme 1, unsaturated groups of PHOU were oxidated with potassium permanganate in presence of a crown-ether. In comparison with our previous reports [13,14], purification and yields were improved. After oxidation, crude product oxidation was transformed in nanoparticles using the nanoprecipitation-solvent evaporation method in aceton. The resulting nanoparticles suspension was transfered into a regenerated cellulose ester dialysis membrane and dialyzed by using deionized water for 48 hours with periodic medium changes. After dialysis, the solution was lyophilized overnight. As indicated by ^{1}H NMR spectroscopy, oxidation was complete and crown-ether has totally disappeared. Poly(3-hydroxyoctanoate-*co*-9-carboxy-3-hydroxydecanoate) ($PHOD_{COOH}$) was obtained in good yields (larger than 90%). For the preparation of grafted PHOD, we employed the carboxylic side groups for esterification reaction with PEG or PLA. To avoid cross-linking reaction commercial methoxy-terminated PEG's (M_n = 350 and 2000 $gmol^{-1}$) were used. The Carboxylic chain end of PLA (M_n = 600 $gmol^{-1}$) was first protected by esterification with (trimethylsilyl)diazomethane. Complete protection was confirmed by ^{1}H NMR.



Scheme 1 : Synthetic route of PHOU oxidation

Esterification in organic solvent with DCC and 4-(dimethylamino)pyridine (DMAP) as catalyst was applied to the reaction of both methoxy-oligomers to produce graft PHAs (Scheme 2). The reaction is carried out at room temperature and under anhydrous conditions to avoid the reaction of DCC with water. Precipitated dicyclohexylurea was filtered out. Purification of PHOD-*g*-PEG was performed similarly to that described above for PHOD. Grafting PEG onto PHOD leads to an amphiphilic copolymer. Nanoparticles can be prepared from the crude product without previous purification. Dialysis process allowed to eliminate unreacted Me-PEG. In the case of PLA, the branched copolymer obtained was hydrophobic, consequently, purification was carried out by precipitation in methanol, because PLA oligomers are soluble in MeOH. PHOD-*g*-PLA has the same features as the PHOU precursor i.d. soluble in a polar solvents and soluble in polar solvents as MeOH or aceton.

To investigate the influence of the reaction conditions, a series of PHOD-g-MePEG350 was prepared by changing the amount of DCC and the reaction conditions (Table 1). The composition of grafted polymer was determined by ^{1}H NMR (Figures 1 and 2). In the NMR spectrum of PHOD-*g*-PEG350, typical signals of PEG and those of PHOD segments were observed. The appearance of a new signal for CH_2 (9) constituted evidence that grafting happened. The composition of graft polymers was estimated by ^{1}H NMR based on integrals of characteristic signals of CH_2 (9) and that of CH (2) groups of PHOD. The same composition values obtained from methyl terminal peak (13) and CH (2) allowed to confirm that unreacted PEG was eliminated.



Scheme 2 : Synthetic route of grafted PHOD

Table 1 : Results of grafting PEG or PLA on $PHOD_{COOH}$

	molar ratio PEG/COOH	molar ratio DCC/COOH	protocol	Reaction time (h)	Yield (%)	COOH conversion (%)
PEG350	1.2	1.2	**I** COOH	3.5	45	68
	1.2	2.4	Preactivated for 1	3.5	11	68
	1.2	2.4	hour	7	26	76
	2.4	2.4		7	gel	gel
PLA	1.2	1.2		3.5	gel	gel
PEG350	1.2	1.2	**II**	3.5	60	76
	1.2	1.2	DCC/PEG/PHOD	7	55	66
	2.4	2.4	Introduced at the	3.5	55	66
PEG2000	1.2	1.2	same time	3.5	52	62
PLA	1.2	1.2		3.5	33	44

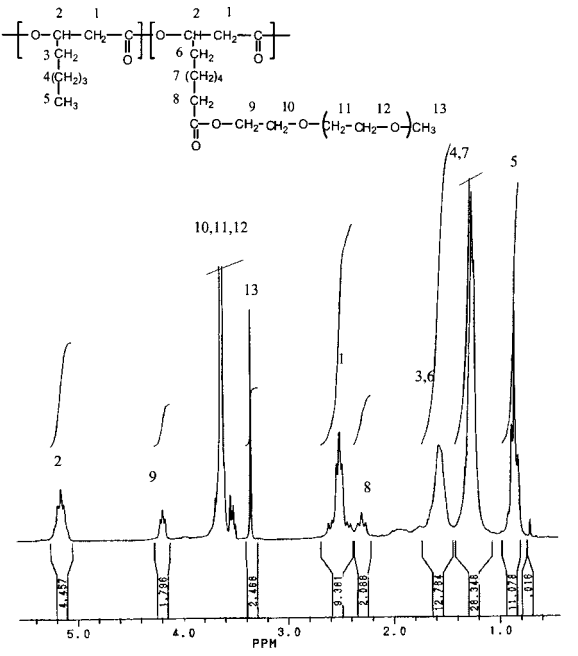


Figure 1 : 200 MHz ^{1}H NMR spectrum of PHOD-g-PEG350 in $CDCl_3$

In the case of PLA (Figure 2), the characterization is similar. The conversion percentage was calculated from CH_3 (11) of PLA and CH_3 (5) of PHOD. Furthermore, the disappearance of the signal at 4.3 ppm of CH in PLA end groups demonstrated, that unreacted PLA was eliminated by precipitation. During the reaction, gelification was observed. Carboxylic group activation by DCC leads to insoluble anhydride formation between carboxylic groups. Gel formation was more important when all reagents were introduced at the same time (protocol I). We found that introducing reagents all at once (protocol II) is the most suitable reaction condition to limit side cross-linking reactions by competition between anhydride formation and esterification reactions. Never complete COOH conversion even with an increasing amount of DCC or reaction time was observed. The best conditions were 1.2:1 DCC/COOH molar ratio, 3.5 hours duration.

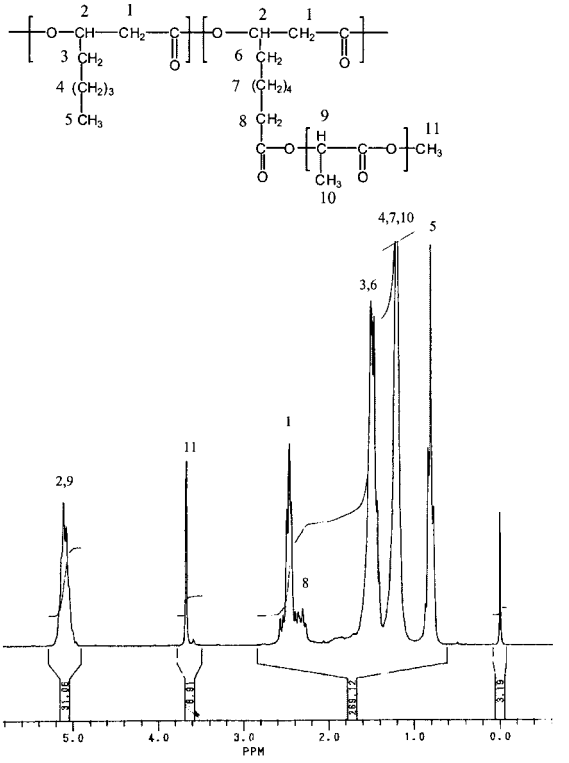


Figure 2 : 200 MHz ^{1}H NMR spectrum of PHOD-g-PLA in $CDCl_3$

The presence of free COOH groups in the resulting graft polymers can explain the lower yield obtained in the PLA. Only partial precipitation in MeOH was observed.

Graft polymers were characterized by SEC analysis using polystyrene standards. SEC chromatograms of grafted PHOD were compared with those of the startings products. After 24 hours of dialysis, copolyesters exhibited unimodal molecular weight distribution i.d. the polymers did not contain unreacted PEG350 or PLA. The obtained molecular weights of copolymers were very close to that of precursor PHOD (Table 2). This result can be explained by the short chain lengths of PEG350 and PLA. In the case of PEG2000, special care was taken to remove unreacted PEG by dialysing for 4 days because graft polymers tended to aggregate in solution and solvated free PEG. Molecular weights of PHOD-*g*-

PEG2000 were under estimated by using polystyrene standards, which might be due to the different hydrodynamic behaviour of PHOD and graft PHOD because of conformational differences.

Table 2 : GPC Characteristics of graft polymers

	Precursor PHOD		Graft PHOD	
	Mn	**Mw**	**Mn**	**Mw**
PHOD-*g*-PEG350	36000	80000	34000	74000
PHOD-*g*-PEG2000	44000	142000	41000	51000
PHOD-*g*-PLA	48000	113000	43000	79000

The physical-chemical properties of these graft polyesters are different compared with PHOD precursor. PHOD is soluble in polar solvents such as DMSO, aceton or methanol and in less polar solvents such as $CHCl_3$ and CH_2Cl_2 whereas the PHOD-*g*-PLA copolyester is only soluble in less polar solvents. While polyesters with a short hydrophilic PEG chain showed an unsolubility in water, longer chain length (40-45 ethylene oxide units) yielded water soluble polymers.

Conclusion

We have prepared new polyesters by grafting PLA or PEG onto reactive PHA backbone. The properties of these new polyesters can be controlled by the substitution degree and the chain length. For using these copolymers in bioactive agent delivery systems it is necessary to adjust carefully polymer degradation properties. It can be supposed that hydrophilic PEG or degradable PLA has an influence on the degradation of the PHOD backbone. In vitro degradation studies are currently under investigation.

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