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Biocatalytic Synthesis and Characterization of Copolymers Based on Poly(Ethylene Glycol) and Unsaturated Methyl Esters

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Biocatalytic organic synthesis has proved to be a significant breakthrough in the area of polymer synthesis. Environmentally benign methodology and the use of mild reaction conditions are a hallmark of this approach. We have studied the biocatalytic synthesis of unsaturated polyesters under solvent-less conditions by the condensation copolymerization of dimethyl fumarate and dimethyl maleate with polyethylene glycol (PEG) catalyzed by Novozyme-435 (immobilized Candida antarctica lipase B). The structures of the resulting polymers, poly(ethylene glycol)-co-dimethyl fumarate and poly(ethylene glycol)-co-dimethyl maleate were studied from their ^{1}H and ^{13}C-NMR spectra. The molecular weights of polymers were determined by size exclusion chromatography.

Keywords biocatalytic, unsaturated polyester, polyethylene glycol, *candida antarctica* lipase B, dimethyl fumarate and dimethyl maleate

Introduction

The application of enzymes in the manipulation of chemical entities has been a rapidly expanding area of research that has revolutionized organic chemistry and materials science (1–4). Lipases, in particular have been successfully used to carry out polycondensation reactions for the past decade. Various polyesters are obtained from hydroxyesters, hydroxyacids, diols-diesters, and diols-diacids, by using lipase-catalyzed reactions in organic medium as well as in bulk (solvent free) conditions. Unsaturated polyesters are

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important macromolecules usually prepared by the polycondensation reaction of unsaturated diacids, diesters or anhydrides with diols (5–7). The synthesis of unsaturated polyesters usually involves a bulk reaction at an elevated temperature between diacids, diesters and anhydrides with diols (8, 9). Many side reactions occur during the chemical synthesis of unsaturated polyesters, leading to ill-defined structures. These side reactions include the addition of a hydroxyl group to double bonds commonly known as the Ordelt reaction (10, 11), cis/trans isomerization, transesterification, and dehydration of glycol because of high reaction temperatures. Due to these side reactions, the resultant polyesters are usually highly branched with very low solubility in common organic solvents limiting their utility and post functionalization.

Recently, we have been working on the enzyme-catalyzed polyester and polyamide synthesis using polyethylene glycol as one of the components (12–15). The uniqueness about these enzyme-catalyzed polymerizations is their high selectivity, mild reaction conditions and lack of need for solvent. On reviewing the literature, we found only a handful of reports on enzyme-catalyzed synthesis of unsaturated polyesters using aliphatic diols such as 1,6-hexane diols (16, 17).

Encouraged by the advantages of enzyme-catalyzed polymerization particularly mild reaction conditions, that may help in reducing the side reactions and improve the quality of polymers, we have tried the enzymatic polymerization of unsaturated diesters with PEG. Here, we discuss here the preliminary results for the condensation reaction of dimethyl fumarate and dimethyl maleate with polyethylene glycols.

Experimental

Materials and Methods

Dimethyl fumarate, dimethyl maleate and PEG600 were purchased from Aldrich (Milwaukee, WI). All other chemicals and solvents were of analytical grade and were used without further purification. PEG was dried under vacuum at 60°C for 3 h prior to use.

Characterization

Gel permeation chromatography (GPC) was used to determine the molecular weight and molecular weight distributions, M_w/M_n of polymers using THF as solvent and polystyrene as standard. ^{1}H-NMR, and ^{13}C-NMR spectra were recorded on a 250 MHz Bruker Instrument in $CDCl_3$.

General Enzymatic Polymerization Procedure

In a typical experiment, to a mixture of dimethyl fumarate or dimethyl maleate (1 mmol, 0.144 g), poly(ethylene glycol) (1 mmol, 0.600 g) were taken in a round bottom flask under inert atmosphere, Novozyme-435 (10% of the weight of monomers, 0.075 g) was added. The resulting reaction mixture was stirred at 50°C and vacuum was pulled for a predetermined time. Aliquots were taken out at different time periods to monitor the progress of the reaction by thin layer chromatography. The reaction was quenched by adding chloroform followed by filtering off the enzyme. Chloroform was removed under reduced pressure and the polymer obtained was dissolved in water. The aqueous polymer solution was dialyzed using membrane (MWCO 8000), the resulting solution was freeze-dried to give highly viscous product. The structure of the polymer was

characterized from ^{1}H and ^{13}C-NMR spectra. Molecular weight of the polymer was determined by gel permeation chromatography (GPC).

^{1}H-NMR Data of Dimethyl Fumarate-PEG600 Copolymer 4 ($CDCl_3$, 250 MHz): δ 2.90 (C-2′H), 3.35 (C-αH), 3.58–3.85 (methylene PEG protons on C-6 and C-7 carbons of the repeating units and C-5, 3.90 (C-βH), 4.20–4.40 (bt, C-4H and C-1′H), 6.9 (s, C-1H and C-2H).

^{13}C-NMR ($CDCl_3$, 62.5 MHz): 65.00 (C-α), 62.21 (C-β), 68.9 (C-5), 70.10–70.83 (PEG), 72.99 (C-4), 133.12 (C-1 and C-2) and 165.78 (COO).

M_n (by GPC): 10,000 Dalton, P_d: 6.7.

^{1}H-NMR Data of Dimethyl Maleate-PEG600 Copolymer 5 ($CDCl_3$, 250 MHz): δ 2.90 (C-2′H), 3.35 (C-αH), δ 3.58–3.85 (methylene PEG protons on C-6 and C-7 carbons of the repeating units and on (C-5), 3.90 (C-βH), 4.25 (t, C-4H and C-1′H), 6.15 (s, C-1H and C-2H).

^{13}C-NMR ($CDCl_3$, 62.5 MHz): δ 54.00 (OCH_3 end group), 64.65 (C-α), 62.71 (C-β), 69.10 (C-5), 70.53–70.10 (PEG), 72.99 (C-4),131.1 (C-1 and C-2), and 165.78 (COO).

M_n (GPC): 9400 Dalton P_d: 8.1.

Experimental Procedure for the Terpolymer (6) Synthesis: Three monomers, PEG600 (1 mmol, 0.600 g), dimethyl fumarate (0.5 mmol, 0.072 g) and dimethyl maleate (0.5 mmol, 0.072 g) were mixed together in a round bottom flask, Novozyme-435 (10% of the weight of monomers, 0.075 g) was added, and the resulting reaction mixture was stirred at 50°C under vacuum for 24 h. The reaction progress, in terms of disappearance of starting material, was monitored by TLC. The reaction was quenched by adding chloroform, followed by the enzyme removal. Chloroform was removed under reduced pressure and the polymer obtained was redissolved in water. The aqueous polymer solution from the filtrate was dialyzed using a membrane (MWCO 8000). After dialysis, the solution obtained was freeze-dried. The structure of the polymer obtained was characterized from its ^{1}H and ^{13}C-NMR spectra. Molecular weight of the polymer was determined by gel permeation chromatography (GPC).

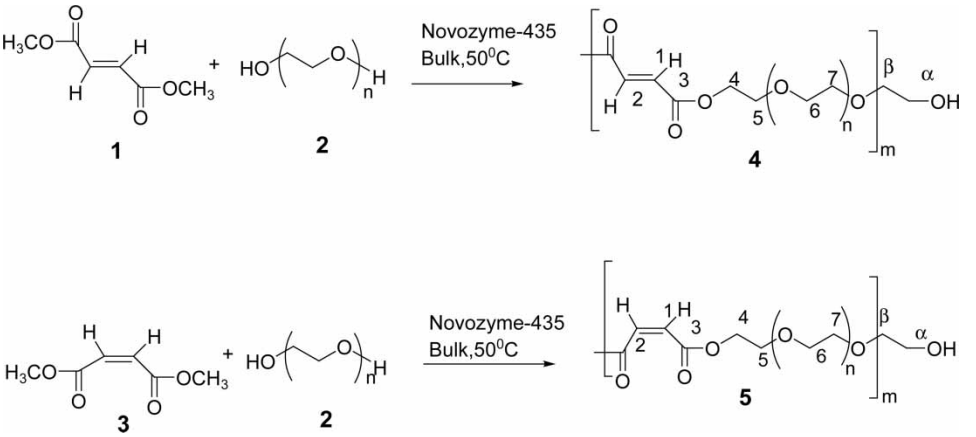
^{1}H NMR Data of Terpolymer 6 ($CDCl_3$, 250 MHz): δ 3.58–3.87 (methylene PEG protons on C-6 and C-7 carbons of the repeating units and on C-5, C-α and C-β), 4.20–4.40 (t, C-4H), 6.25 (s, C-1H and C-2H cis), 6.9 (s, C-1H and C-2H trans), 2.9 (see discussion).

^{13}C-NMR ($CDCl_3$, 62.5 MHz): δ 54.00 (OCH_3 end group), 66.65 (C-α), 64.71 (C-β), 69.10 (C-5), 70.83 (PEG), 72.99 (C-4), 131.1 (cis C-1 and C-2), 133.12 (trans C-1 and C-2), and 165.78 (COO–).

M_n (GPC): 8600 Dalton P_d:5.6.

Results and Discussion

The enzymatic synthesis of copolymers starting from dimethyl fumarate or dimethyl maleate and poly(ethylene glycol 600) was performed as shown in Scheme 1.



Scheme 1. Novozyme-435 catalyzed synthesis of unsaturated polyesters.

Candida antarctica lipase B was used to catalyze these copolymerization reactions because of its high catalytic activity for ester synthesis, high thermal stability and immobilization on the large surface area material. *Candida antarctica* lipase B catalyzed condensation reactions under solvent-less conditions resulted in the synthesis of copolymers **4** and **5**. In polymerization under the same reaction conditions, but without enzyme (control experiment), the monomers were recovered unchanged after 24 h. Furthermore, no polymer formation was observed by using the deactivated *Candida antarctica* lipase B. These data imply that the present polymerizations proceeded through lipase catalysis.

The structures of the repeating units of the polymers were identified using NMR experiments. In the ^{1}H-NMR spectrum (Figure 1) of copolymers **4** and **5**, a new signal at δ 4.35 and 4.25, respectively for the C-4 protons, indicated the formation of ester linkages between the hydroxyl group of poly(ethylene glycol 600) and methyl ester of fumarate and maleate. A corresponding signal in ^{13}C-NMR spectras appears at δ 72.99. The broad signal at δ 3.58–3.85 in the ^{1}H-NMR spectrum of **4** and **5** was assigned to the main chain protons of poly(ethylene glycol 600) units (C-6 and C-7 protons) with C-5 protons partially merged at the downfield end of this signal. The olefinic protons C-1 and C-2 appeared at δ 6.90 and δ 6.15 for the copolymer **4** and **5**, respectively. In the ^{13}C-NMR spectrum, the signal at δ 165.78 was assigned to the ester carbonyl carbon. The number average molecular weights of the polymers were determined by GPC measurements and found to be 10000 and 9400 for the copolymers **4** and **5**, respectively. During the repeating unit structure analysis of the copolymers **4** and **5** by ^{1}H-NMR analysis, no cis/trans isomerization was observed as is evident by the absence of the olefinic signal for the other isomer (Figure 1a and 1b).

The integration for unsaturated protons C-1H and C-2H in the ^{1}H-NMR spectra of both the copolymers **4** and **5** was less as compared to other protons, suggesting a possible loss of unsaturation during the co-polymerization reactions. These observations are in accordance to the chemical polymerizations for the synthesis of unsaturated polyesters, which usually leads to a Ordelt reaction i.e., the addition of hydroxyl groups to the double bond resulting in the saturation of double bonds (10, 11). It was also noted that the extent of saturation was more in the case of fumarate based copolymer **4** (determined by the ratio of the signal at δ 6.90 for olefinic protons to the signal at δ 4.35 for C-4 protons) as compared to maleate based copolymer **5** (determined by the ratio of the signal

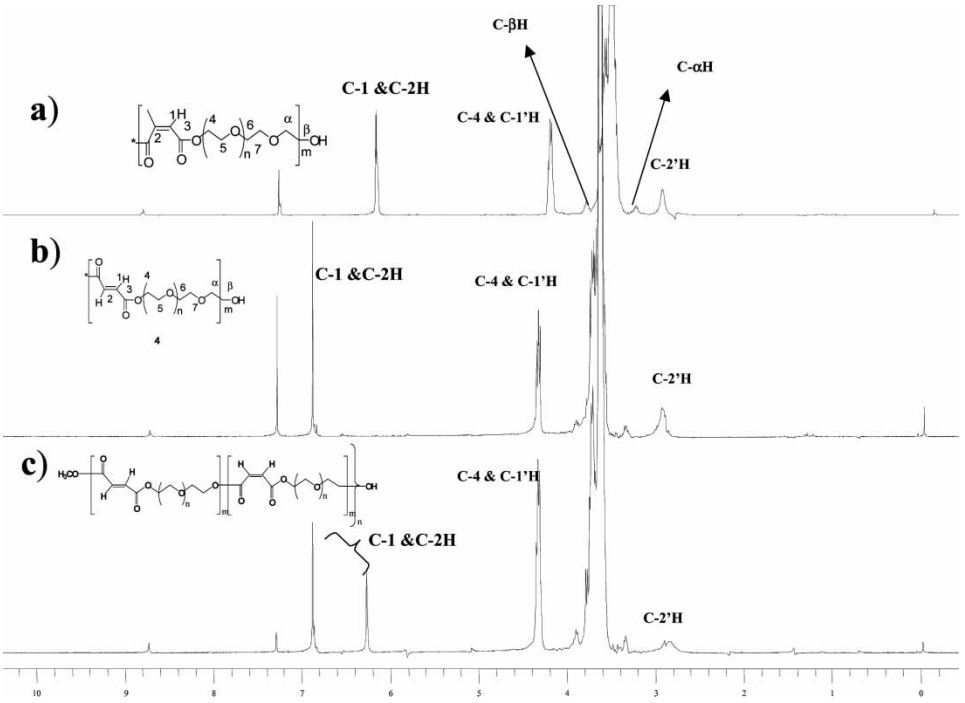


Figure 1. ^{1}H-NMR spectra, a) dimethylmaleate-co-PEG600, **5** b) dimethylfumarate-co-PEG600, **4** and c) terpolymer **6**.

at δ 6.15 for olefinic protons to the signal at δ 4.25 for C-4 protons). The saturation of double bonds in the copolymers **4** and **5** was further confirmed by the appearance of a signal at δ 2.90 and at 4.40 (possibly merged with C-4H signal) for CH_2 (C-2′) and CH (C-1′) respectively, resulting from the saturation of double bonds (Figure 2).

We have also carried out the terpolymerization reaction as shown in Scheme 2 using Novozyme 435. The polymer **6**, obtained by the terpolymerization reaction contains both unsaturated components as is evident from its ^{1}H-NMR spectra due the presence of signals in the olefinic region at δ 6.90 and δ 6.15 for dimethyl fumarate and dimethyl maleate units, respectively. The terpolymer obtained showed a higher ratio of fumarate units as compared to maleate. The terpolymerization also resulted in the saturation of the double bonds in the terpolymer **6** as was observed during the synthesis of the copolymers **4** and **5**.

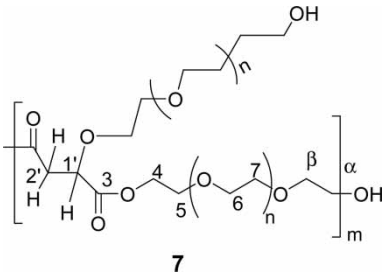


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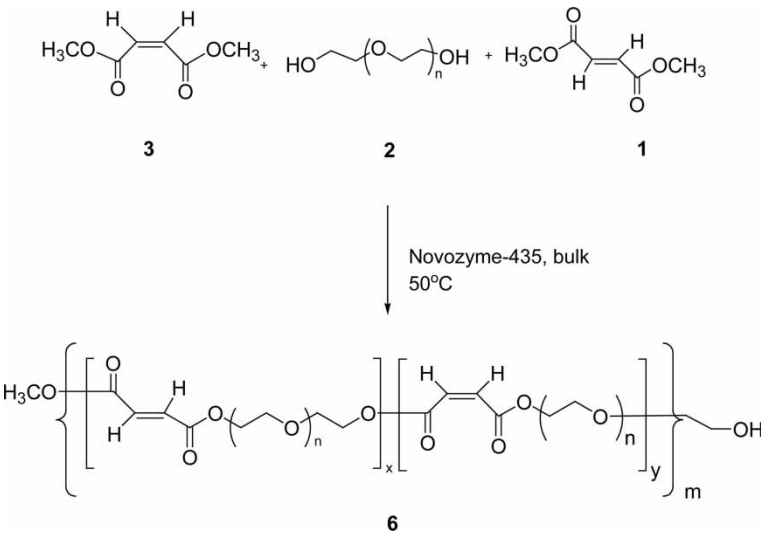
Figure 2. Saturation of the double bonds during enzymatic polymerization reactions.



Scheme 2. Terpolymerization of dimethyl maleate, dimethyl fumarate with PEG600 catalyzed by Novozyme-435.

Conclusions

The enzymatic polymerization of PEG with dimethyl fumarate and dimethyl maleate under solvent-less conditions gave corresponding copolymers through condensation reactions and was also accompanied by the addition of PEG hydroxyl groups across double bonds to some extent. Though no cis/trans isomerization was observed under enzymatic polymerization conditions, the saturation of double bonds remains a problem. The work is under way in our laboratory to minimize or eliminate the saturation of double bonds during enzymatic polymerizations to make it an attractive method for the synthesis of linear unsaturated polyesters.

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