

Chemo enzymatic Synthesis for Poly3-hydroxypropionate in Solvent-free System

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Abstract. Recently much aliphatic polyester has been synthesized by enzyme-catalyze. However for the expensive or uneasy acceptable materials few were commercial viable. In this paper, a new chemo-enzymatic method was used to synthesize the poly3-hydroxypropionate through the initial material ethylene oxide and carbon monoxide. The influence of reaction temperature, reaction time and the dosage of enzyme to the conversion and molecular weight were studied. When reaction at 45°C and 3% Novozyme435 (relative to the total weight of monomer) after 120h the conversion were 77.6%.

Introduction

A wide variety of microorganism can accumulate Polyhydroxyalkanoates (PHAs) as intracellular carbon and energy storage compounds. This kind of polymer called fifth generation thermoplastic materials[1]being extensively studied for their potential applications as biodegradable and biocompatible materials in both the industrial and medical fields[2], e.g. the high molecular weight polyesters may replace for polyolefin in some applications, on the other hand, the polyester of low-molecular weight of a few thousands may has applications in medicine for drug delivery systems as biocompatible plasticizer polymers.[3,4] This kind of polyester can be made by chemical method, but the use of chemical catalysts for these reactions requires harsh reaction conditions(e.g. high temperature or strict exclusion of moisture)[5,6,16]and the metal catalysts may be problematic for certain product end uses especially for medical use.[7]

Lipase can catalyze esterification reactions at mild temperatures[8-10].These characteristics motivated their study as catalysts for polyesterifications.Recently,enzymatic designing and synthesizing of environmentally acceptable polymeric materials has become an effective method. Unless chemical catalysis lipase catalyzed condensation polymerizations are metal-free and enzymes are derived from natural resources. Enzymatic synthesized of polymer by ring-opening polymerization [11, 12] and condensation transesterification has been reported. Kline and coworkers synthesis of a linear polymer from divinyl adipate and an equimolar of triol/glycol by 1wt% enzyme in 50°C for 24h(Mw3000-14000Da)[13]. Kumar et al copolymerized adipic and sorbitol in bulk using Novozyme-435 10% relative to monomers after 48h give a water soluble polymer (Mw17030) and adipic 1,8-octanediol and sorbitol were also copolymerized for a water insoluble polymer in the molar ratio 50:35:15(Mw1.17×10⁵).[14]Mahapatro and coworkers use Novozyme435(1wt%relative the total weight of monomer) catalyzed adipic acid and 1,8-octanediol copolymerization in vacuum ,after 24h a copolymer Mn nearly 15000 was received[15]. Kulshrestha et al use Novozyme-435(10wt%relative to total monomer weight)at 80°C in vacuum catalyzed adipic acid bis(hydroxymethyl)butyric acid and 1,8-octanediol copolymerization when the monomer ratio of bis(hydroxymethyl)butyric acid from 9-45% after 42h a linear polyester Mw2300-21900 was accepted[16].

From the above documents, enzyme can resolute polymerization efficiently. But the expensive materials make these processes non-viable commercially, to fit the commercial production demand the materials must be cheap and easily obtainable [17]. From this aim we describe a chemo enzymatic route to synthesize a novel polymer poly3-hydroxypropionate in solvent-free system.

Materials and Methods

Materials. Lipase acrylic resin from *Candida Antarctica* (Novozyme435 obtained from Sigma10000u/g) 3-hydroxypropionate methyl Ester, chloroform (Analytical degree), methanol (Analytical degree). All materials are used as received.

General Procedure for Lipase-Catalyzed Condensation Polymerizations of 3-Hydroxypropionate Methyl Ester. Typically, Novozyme435 (3wt% relative to the total weight of monomer) 30mg, 3-hydroxypropionate methyl ester 100mmol were added in a 25ml vessel and the vessel were capped with a rubber septum. The reaction vessel was then placed into a constant preset temperature oil bath on a magnetic stirrer at 200rpm; vacuum was applied to facilitate removal of the product methanol. The reaction was terminated by adding excess chloroform, and stirring for 15min, removing Novozyme435 by filtration (glass fritted filter, medium porosity), and stripping the chloroform by rotary evaporation. Then methanol (4°C) was added to precipitate the polymer.

Molecular Weight Analysis. Number- and weight average-molecular weights (M_N and M_w , respectively) were determined by gel permeation chromatography (GPC). The GPC analyses were performed at room temperature using a Waters HPLC System equipped with a model 510 pump, a model 717 auto sampler, and a model 410 refractive index (RI) detector, and a column set consisting of Polymer Laboratories PL 104 Å and 500 Å columns in series. THF (HPLC-grade) was used as the eluent at a flow rate of 1.0 mL/min. Narrow disparity polystyrene standards purchased from Polymer Laboratories with molecular weights ranging from 900K to 580 were used to calibrate the system. The refractive index signal acquisition, system calibration and relative molecular weight calculations used Water Empower software from GPC Option.

Results

Studies by ^1H and ^{13}C NMR Spectroscopy, Nuclear Magnetic Resonance (NMR). The polyesters were solved in CDCl_3 and characterized using proton (^1H), ^{13}C NMR. ^1H NMR spectra were recorded on a Bruker NMR spectrometer (model DPX300) at 300 MHz. The chemical shifts in parts-per-million (ppm) for ^1H NMR spectra were referenced relative to tetramethylsilane (TMS, 0.00 ppm) as the internal reference.

The information of the polyester about ^1H -NMR scheme1 (Cl_3DC , ppm): $\delta = 3.840$ (OCH₃, S), $\delta = 2.661$ (CH₂, T), $\delta = 4.370$ (CH₂O, T).

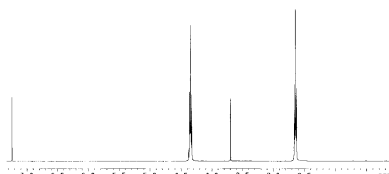


Figure 1. ^1H NMR (400-MHz) spectra in chloroform- d of poly3-hydroxypropionate obtained by using 3% Novozyme435 for 120h at 55°C.

The information of the polyester about ^{13}C -NMR scheme2 (Cl_3DC , ppm): $\delta = 170.3$ (CO), $\delta = 60.1$ (OCH₂), $\delta = 58.2$ (OCH₃), $\delta = 33.6$ (CH₂).

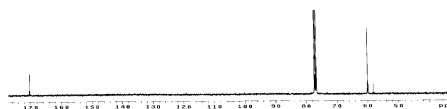


Figure 2. ^{13}C NMR(400-MHz) spectra in chloroform-d of poly3-hydroxypropionate

Synthesis of Poly3-Hydroxypropionate. The mechanism (Figure 3) of this reaction was reported by Nobles and coworkers [18]. First the substrate with the lipase to form an acyl enzyme intermediate. The second step this acyl enzyme intermediate reacted with hydroxyl end of the growing polymer chain. To improve the conversion and molecules weight, in enzymatic catalyzed condensation polymerization reduced pressure was used to facilitate removal of one product methanol [15].

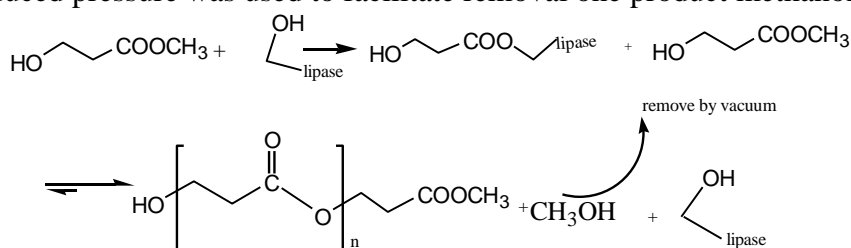


Figure 3. Proposed mechanism of Novozyme435 catalyzed polymerization of 3-hydroxypropionate methyl ester for PHA.

Lipase *Candida* Antarctic lipase Novozym435 catalyzed the condensation polymerization of 3-hydroxypropionate methyl ester to poly(3-hydroxypropionate) under various conditions. The results have been shown in Table 1 to Table 3.

Table 1 The effect of addition amount of lipase on the reaction

Addition amount of lipase	temperature ($^{\circ}\text{C}$)	con (%)
1%	55	67.5
3%	55	79.1
6%	55	97

Table 2 The effect of temperature on reaction

Novozyme435	temperature ($^{\circ}\text{C}$)	con (%)
Novozyme435	35	10.7
Novozyme435	45	77.6
Novozyme435	55	80.1
Novozyme435	65	33.5
Novozyme435	75	ND

Table 3 the effect of reaction time on the conversion

catalyst	temperature ($^{\circ}\text{C}$)	time (h)	con (%)
Novozyme435 (3%)	55	48	20.2
Novozyme435 (3%)	55	120	79.8
Novozyme435 (3%)	55	240	95

Summary

In order to demand the materials must be cheap and easily obtainable, we describe a chemoenzymatic route to synthesize a novel polymer poly3-hydroxypropionate in solvent-free system. In summary, from our research we can observe that the new chemoenzymatic method can be used to synthesize the poly3-hydroxypropionate through the initial material ethylene oxide and carbon monoxide.

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