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Lipase-catalyzed synthesis and characterization of polymers by cyclodextrin as support architecture

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ABSTRACT

Diesters and diols were successfully converted into aliphatic polyesters by enzymatic lipase *Candida* sp.99-125 catalysis, with β-cyclodextrin acting as supporting architecture (in a similar way as chaperone proteins). No organic solvents were used. The polytransesterification was a much greener process, being solvent-free and without metal residues. Lipase *Candida* sp.99-125 showed a high catalytic activity for bulkpolymerization of diesters and diols with various numbers of methylene groups in their chains. β-Cyclodextrin encircled the linear polymer chain and maintained the chain in a proper configuration to avoid its coagulation. Lipase initiated the polymerization and β-cyclodextrin threaded onto the polymer chain to control the structure for producing high molecular weight polyesters. From a combination of diesters and diols, polyesters with a high molecular weight of 62,100 Da were obtained at 70 °C. The corresponding polyesters showed an excellent thermal stability till 350 °C and had a strong ability to crystallize with up to 72% crystallinity, contributing to their high storage modulus.

1. Introduction

Biodegradable polymers have been extensively used in the field of biomedical application such as tissue engineering and control drug delivery because of their good biocompatibility, non-toxicity and environmental acceptability (Gu, Wang, Zhang, & Ren, 2008; Liu, Jiang, Zhang, & Saltzman, 2009; Matsumura, 2002; Wang, Zhou, Sun, & Huang, 2010; Ye, Kennedy, Li, & Xie, 2006;). Aliphatic polyesters with good thermal stability and mechanical strength are a remarkable group of the biodegradable materials family, which can be used as thermoplastics and biocompatible medical materials (Jiang, 2008).

Enzyme-catalyzed synthesis of polyesters provides a good example to achieve green polymer chemistry, which is a new synthetic strategy and an eco-friendly process for the synthesis of useful polymers (Kobayashi, Uyama, & Kimura, 2001). Synthesis of aliphatic polyesters with lipase catalysis is achieved by means of ring-opening polymerization (Cai et al., 2010; Målberg, Finne-Wistrand, & Albertsson, 2010; Srivastava & Albertsson, 2005; Srivastava & Albertsson, 2007; van As et al., 2005; van Buijtenen et al., 2007), condensation polymerization (Jiang, Liu, Xie, & Gross, 2007; Kaar, Jesionowski, Berberich, Moulton, & Russell, 2003; Olson, Gratton, DeSimone, & Sheares, 2006; Olson & Sheares, 2006) and copolymerization (Fehling, Klein, Vosmann, Bergander, & Weber, 2008; Hagstrom, Nordblad, & Adlercreutz, 2009; Liu, Jiang, Decatur, Xie, & Gross, 2011; Palsule & Poojari, 2010; Tsujimoto, Uyama, & Kobayashi, 2002; Takwa, Simpson, Malmstrom, Hult, & Martinelle, 2006).

Polycondensation is a precise and effective way to obtain homopolymers with tailored properties. Aliphatic dicarboxylic acids and diols are the most convenient substrates for synthesis of linear polyesters. However, phase separation was found in the reaction mixture due to solid dicarboxylic acids with low solubility in diols under mild reaction temperatures during the enzyme-catalyzed process. As a result, the substrates were difficult to diffuse to the catalyst particles. Polyesterification between the two monomers was tedious and it took 7–15 days to yield the desired polyesters. Thus, organic solvents such as diphenyl ether were supplied into the reaction mixture to promote diffusion among the reactants during polyesterification (Uyama & Kobayashi, 2006). Polytransesterification with a mono-phase reaction mixture instead of polyesterification was adopted by replacing dicarboxylic acids with dicarboxylic acid derivatives (such as *bis*(2,2,2-trifluoroethyl) sebacate) as the acyl donor. Polytransesterification could avoid phase separation, yielding the corresponding aliphatic polyesters molecular weight of 46,000 Da with *Mucor miehei* lipase catalyst in the presence of diphenyl ether at 168 h (7 days) (Linko, Wang, & Seppala, 1995a; Linko, Wang, & Seppala, 1995b). Nevertheless, polytransesterification still had disadvantages

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because an undesirable alcohol with halogen atoms would be produced during the process and released to the air, leading to environmental pollution. Then, diethyl succinate was applied for polytransesterification with diols to avoid the poisonous halogen alcohol (Azim, Dekhterman, Jiang, & Gross, 2006). Reducing the amount of the organic solvents has been attempted. The polyesters molecular weight was only 3300 Da in the absence of organic solvents by vacuum process with *Candida antarctica* Lipase B catalysts, compared with 38,000 Da obtained in the presence of diphenyl ether. Accordingly, these previous methods are typically not eco-friendly due to the toxicity of diphenyl ether. Organic solvents should moreover be removed completely from the polyesters before they were applied for biomedical applications.

In our research, we utilized the lipase-catalyzed polytransesterification of aliphatic diesters and diols as reaction substrates in the absence of organic solvents under mild condition, concentrating on yielding aliphatic polyesters of high molecular weights. Also, we employed β-cyclodextrin into the post-polymerization stage. Since the cavities of β-cyclodextrin could encircle the linear polymer chain and play a supplementary role to maintain the chain proper conformation avoiding it coilling, the polymer chain was elongated rapidly (Harada, Osaki, Takashima, & Yamaguchi, 2008). Lipase initiated the polymerization and β-cyclodextrin threaded onto the polymer chain to control the structure for producing high molecular weight polyesters. It was a much greener polymerization process without any environmental pollution. Solvent-free methods were adopted to take place of the use of organic solvents which were toxic. Even the cost of production would be decreased greatly without the high cost organic solvents. It was furthermore an extremely eco-friendly approach that only ethanol was produced during the polytransesterification process. In addition, the reaction mixture of the diesters and diols formed a monophasic liquid so that the problem of phase separation was avoided. Simultaneously, the reaction rate was accelerated efficiently during the polymerization process.

A series of diesters (C1, C4, C6, C10) and diols (C4, C6, C8) were employed to synthesis multiple biodegradable aliphatic polyesters. Polycarbonates synthesized by substrates of C1 had good optical properties, being colorless and transparent, and had been specially used in goggles and bulletproof glass. Polyesters prepared by substrates of C4 and C6 were widely applied in the field of packaging, tableware, bottles of medicines, disposable medical supplies and agricultural film ascribable to their advantage of rapid degradation by microorganisms. As a plasticizer, polyesters produced by substrates of C10 were a kind of largely used industrial polymer additives in the plastics processing to increase the flexibility. They could replace many conventional plasticizers on account of their good thermal stability and mechanical strength.

Various kinds of lipases have been utilized for polymerization until now (Puskas, Sen, & Seo, 2009). The lipase used in our research was from *Candida* sp.99-125 (belonging to *Yarrowia lipolytica*) with high lipase activity (Tan, Zhang, Wang, Ying, & Deng, 2003). The polymerization of aliphatic diesters and diols was performed under mild reaction conditions with lipase *Candida* sp.99-125 as catalyst and β-cyclodextrin as supporting architectures, successfully producing aliphatic polyesters with high molecular weights. The structure, thermal, crystalline, morphology properties and dynamic mechanical analysis of the corresponding polyesters have been investigated comprehensively.

2. The experimental

2.1. Materials

Free lipase from *Candida* sp.99-125 was obtained previously in our laboratory (Tan et al., 2003) and the activity of the crude lipase powder used in this study was 168,000 U/g, which was measured on the basis of an olive oil emulsion method (Gargouri et al., 1986). Diethyl carbonate, diethyl succinate, diethyl adipate, diethyl sebacate, 1,4-butanediol, 1,6-hexanediol, 1,8-octanediol and β-cyclodextrin were of analytical grade, commercially available and used without further purification.

2.2. Measurements

To measure the weight-average molecular weights (M_w) and polydispersity (PDI), gel-permeation chromatography (GPC) analysis was used with a refractive index detector and a styragel (HT6E-HT5-HT3) column. Tetrahydrofuran was treated as the eluent at a flow rate of 0.1 ml/min. M_w and PDI were measured based on the calibration curves obtained using polystyrene as standard.

Both ^{1}H and ^{13}C NMR spectra were recorded through a Bruker AVANCE 400 spectrometer, performed at 400 and 100 MHz respectively in chloroform-*d*. The chemical shifts for ^{1}H and ^{13}C NMR spectra were referenced to internal tetramethylsilane at 0.00 ppm (electron shift) or to the solvent resonance. The results of chemical shifts were listed in Table 1.

Thermogravimetric (TGA) analysis was operated using a NETZSCH TG 209C instrument and scanned at 20 °C/min from room temperature to 600 °C under nitrogen flow.

A NETZSCH DSC 204 F1 instrument recorded the thermal properties of the polymers (Differential scanning calorimetry). The samples were first heated from −100 °C to 100 °C and then cooled to 20 °C. Melting temperatures (T_m) with corresponding transition enthalpies (ΔH_m) were measured from the first heating run at 20 °C/min while crystallization temperatures (T_c) with corresponding transition enthalpies (ΔH_c) were measured from the second cooling run at 10 °C/min. The peak maximum of endotherm and exotherm were taken as T_m and T_c, respectively.

Wide-angle X-ray diffraction (WAXS) was carried out at room temperature by a Rigaku X-RAY Diffractometer (D/max-Ultima III) with a Cu radiation used as X-ray source (40 kV, 40 mA) to measure the degree of crystallinity (χ_c). The diffraction range 2θ was from 3° to 50° and the data collected were evaluated to the degree of crystallinity from the intensity of amorphous and crystalline peaks in the diffractogram, after stripping the background of the diffraction curve. The degree of crystallinity (χ_c) was calculated through the ratio of the crystalline peak intensity to the total diffraction intensity.

A polarizing optical microscope (POM) equipped with Olympus BX51 and a hot stage (Linkam THMS 600) was used to investigate the morphology of the semi-crystalline polyesters. The samples were first heated at 80 °C above the melting temperature for 10 min and then cooled to the selected crystallization temperature of 50 °C at 5 °C/min rate.

The crystal lattice arrangements of the polyesters were detected by transmission electron microscopy (TEM) with a JEOL JEM 2100 apparatus operated at an accelerator voltage of 200 kV. The samples were dissolved in chloroform at a concentration of 1.2 mg/ml and 5 μl of the solution dropped onto a copper grid. The samples were dried in air for 24 h before measurement.

In dynamic mechanical thermal analysis (DMTA), polyester samples were molded into rectangular bars of 40 mm (length) × 10 mm (width) × 1.1 mm (thickness). The data were collected by a Rheometric scientific instrument (DMTA V) performed from −150 °C to 70 °C at a heating rate of 5 °C/min and frequency of 1 Hz in tensile mode.

2.3. General procedures for lipase-catalyzed synthesis of aliphatic polyesters

A typical polycondensation of diesters and diols was carried out as follows. Aliphatic diesters (0.05 mol) and diols (0.05 mol)

Table 1
^{1}H NMR and ^{13}C NMR analysis of aliphatic polyesters ($CDCl_3$).

Sample	Chemical shifts (ppm)	
	^{1}H NMR	^{13}C NMR
A-1	1.68 (s, 4H), 4.11 (m, 4H)	29.86, 62.62, 130.24
A-2	1.40 (s, 4H), 1.59 (t, 4H), 4.07 (t, 4H)	25.50, 32.62, 62.75, 130.04
A-3	1.34 (s, 8H), 1.57 (t, 4H), 4.12 (m, 4H)	25.64, 29.34, 32.72, 62.95, 130.21
B-1	1.67 (s, 4H), 2.58 (s, 4H), 4.08 (m, 4H)	25.16, 28.99, 64.12, 172.23
B-2	1.37 (s, 4H), 1.63 (t, 4H), 2.61 (s, 4H), 4.08 (t, 4H)	25.54, 28.45, 29.10, 64.62, 172.34
B-3	1.32 (s, 8H), 1.62 (s, 4H), 2.62 (s, 4H), 4.08 (t, 4H)	25.79, 28.54, 29.10, 29.15, 64.80, 172.38
C-1	1.58 (m, 8H), 2.24 (m, 4H), 4.03 (m, 4H)	25.03, 29.63, 33.84, 64.19, 173.40
C-2	1.37 (m, 4H), 1.65 (m, 8H), 2.32 (t, 4H), 4.05 (t, 4H)	24.40, 25.58, 28.51, 33.90, 64.27, 173.36
C-3	1.32 (s, 8H), 1.61 (t, 4H), 1.66 (m, 4H), 2.32 (t, 4H), 4.05 (t, 4H)	24.41, 25.83, 28.58, 29.11, 33.92, 64.43, 173.40
D-1	1.31 (s, 8H), 1.62 (s, 4H), 1.70 (s, 4H), 2.30 (t, 4H), 4.10 (s, 4H)	24.90, 25.33, 29.08, 34.26, 63.70, 173.78
D-2	1.30 (s, 8H), 1.38 (m, 4H), 1.61, 1.63 (q, 8H), 2.28 (t, 4H), 4.06 (t, 4H)	24.93, 25.60, 28.54, 29.07, 34.30, 64.14, 173.87
D-3	1.29, 1.31 (d, 16H), 1.60 (s, 8H), 2.25 (s, 4H), 4.04 (s, 4H)	24.93, 25.82, 28.59, 29.09, 34.31, 64.28, 173.87

were polymerized in a 100 ml dried and sealed flask placed in a parallel synthesizer connected to a vacuum pump with the pressure controlled by a vacuum regulator. Lipase powder from *Candida* sp.99-125 (2.614 g) was added and the mixture was stirred at 600 rpm and a constant temperature. All the polyesters were synthesized in three stages: the first-stage reaction was an oligomerization carried out under atmospheric pressure for 24 h, converting the monomers to oligomers; followed by a second-stage polymerization in low vacuum of 200 mbar pressure for 24 h and then another continuous third-stage polymerization in high vacuum of 6–10 mbar pressure for an additional 120 h. Before the second-stage polymerization, β-cyclodextrin (0.436 g) was added into the mixture to assist the oligomers maintaining the chain proper conformation and avoiding coagulation. The temperature of three stages polymerization reaction was kept identically.

The insoluble lipase and β-cyclodextrin were removed out by filtration after the last third-stage polymerization reaction. The products were cooled to room temperature and the weight-average molecular weights and polydispersity of the polyesters were determined immediately by GPC.

3. Results and discussion

3.1. Three stages process for polycondensation of diesters and diols

Fig. 1 illustrates the three-stage reaction for condensation polymerization between diesters and diols catalyzed by lipase from *Candida* sp.99-125 with β-cyclodextrin as supporting architecture. Lipase catalyzed polymerization was conducted at 70 °C in absence of organic solvents avoiding any environmental pollution. To investigate the properties of different aliphatic polyesters, various diesters and diols was used. All polycondensations were carried out in the same conditions of three stages reaction: first stage, oligomerization under atmospheric pressure converting the monomers to oligomers; after adding the β-cyclodextrin into the mixture, a second stage-polymerization in low vacuum of 200 mbar; another continuous third stage, polymerization in high vacuum of 6–10 mbar to produce high molecular weight polyesters.

The choice of monomers was determined by the chain length of diesters and diols. A variety of diesters (C1, C4, C6, C10) and diols (C4, C6, C8) were employed to synthesis of different polyesters. Four diesters including diethyl carbonate, diethyl succinate, diethyl adipate and diethyl sebacate were selected to polymerize with three diols involving 1,4-butanediol, 1,6-hexanediol, 1,8-octanediol, respectively. It provided an extremely eco-friendly process to achieve green polymer chemistry for that only ethanol was produced and released during the polytransesterification. The weight-average molecular weights (M_w) and polydispersity (PDI) values of aliphatic polyesters with various chain structures were summarized in Table 2.

Following the same polymerization procedures described above, a series of polyesters formed from diesters and diols had a molecular weight in the range from 4000 Da to 50,000 Da. The polycondensation depended on the methylene length of the diesters and diols. The diesters and diols with longer methylene in chain could be catalyzed more easily. From a combination of diethyl carbonate with 1,4-butanediol, 1,6-hexanediol and 1,8-octanediol, the polymer A-1, A-2 and A-3 were obtained as a viscous liquid, resulting in the molecular weights of several thousand and the yield isolated less than 50%. The poor reaction of diethyl carbonate with three diols indicated that lipase *Candida* sp.99-125 showed low activity in the polycondensation of diesters with one carbon in chain. Diesters in medium length chain of four or six carbons were much easy to polymerize with diols. Except for the polymerization with 1,4-butanediol, diethyl succinate and diethyl adipate were effectively catalyzed by lipase to polymerize with 1,6-hexanediol and 1,8-octanediol, producing the polymer B-2, B-3, C-2 and C-3 with the molecular weights centered between 25,000 Da and 30,000 Da and polydispersity from 2.33 to 4.27. Comparing with the polycondensation of diesters in short and medium length chain, diethyl sebacate in long chain of ten carbons exhibited remarkable polymerization with all three diols. Lipase-catalyzed polycondensation of diethyl sebacate with 1,4-butanediol, 1,6-hexanediol and 1,8-octanediol occurred very quickly at 70 °C in bulk and the polymer D-1, D-2 and D-3 with high molecular weights of 34,100 Da, 27,000 Da, 44,600 Da have been achieved successfully. Among the three diols, 1,4-butanediol as a short chain diol of four carbons was more difficult to be catalyzed by lipase *Candida* sp.99-125 than 1,6-hexanediol and 1,8-octanediol in the polymerization to form high molecular weight polymers. Apart from the polymerization with diethyl sebacate producing a polymer of high molecular weight more than 30,000 Da, the condensation polymerization of 1,4-butanediol with diethyl carbonate, diethyl succinate and diethyl adipate yielded the viscous liquid products A-1, B-1 and C-1 of just 5000 Da around. Thus, in the enzymatic synthesis of aliphatic polyesters described herein, both diesters and diols of medium or long length chains were more convenient to polymerize than their short chains alternatives. Diesters with four, six or ten carbons were the optimal substrates as well as diols with six or eight carbons to prepare the high molecular weight products. Lipase *Candida* sp.99-125 showed excellent catalytic activity in the polycondensation of diethyl sebacate and 1,8-octanediol, forming a polymer D-3 of highest molecular weight of 44,600 Da.

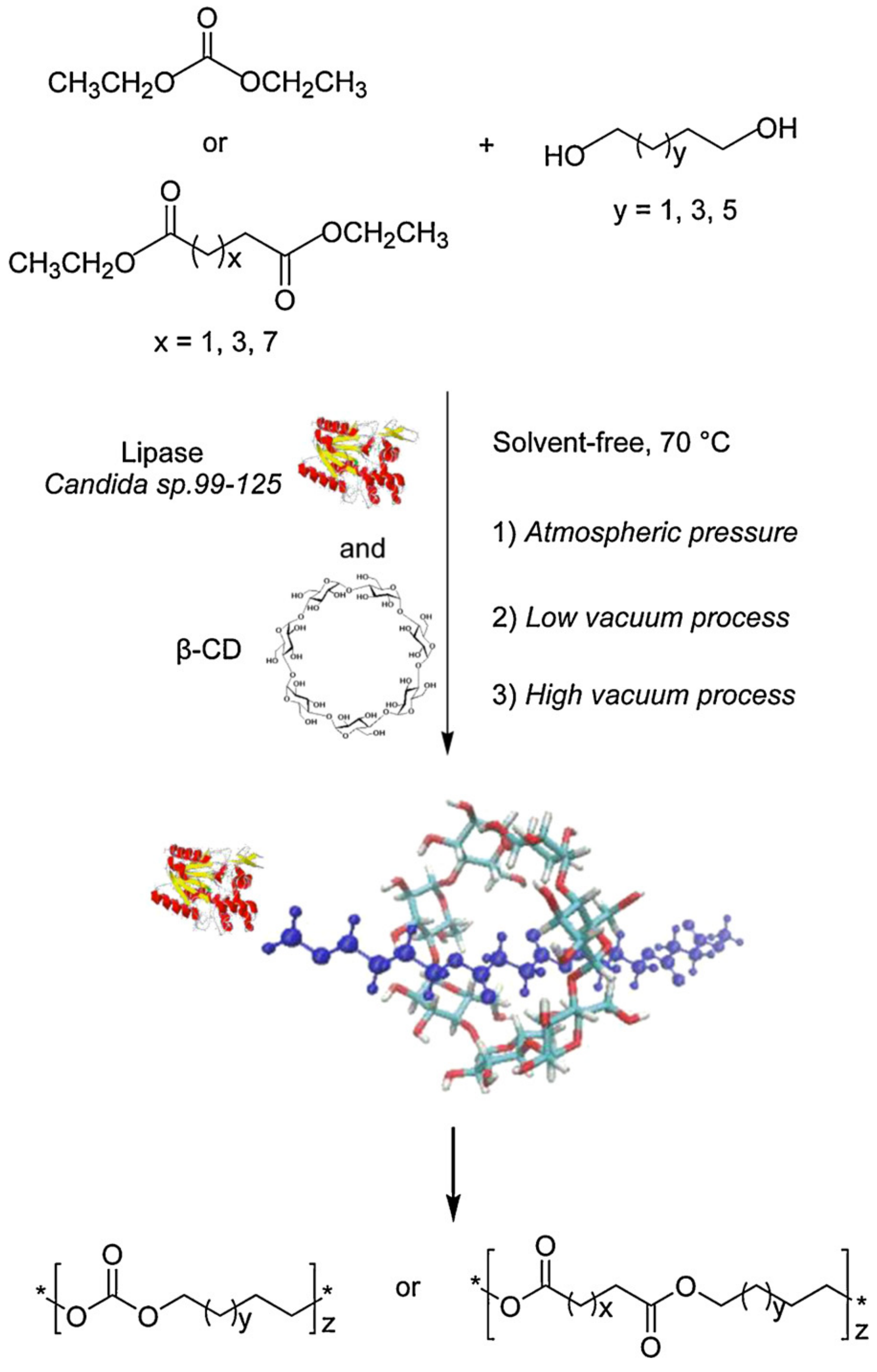

Fig. 1. Third stages reaction for polycondensation of diesters and diols catalyzed by lipase from *Candida sp.*99-125 with β-cyclodextrin as supporting architectures.

3.2. Rapid elongation of the polymer chains with β-cyclodextrin as supporting architectures in the post-polymerization

The polymer chain growth was monitored during the third-stage polymerization of diethyl sebacate and 1,4-butanediol. The polycondensation of 1:1 diester/diol molar ratio was conducted at 70 °C catalyzed by lipase *Candida* sp.99-125 in high vacuum of 6 mbar with β-cyclodextrin as supporting architectures. The results of molecular weight and polydispersity versus the third-stage polymerization time were showed in Fig. 2. Lipase initiated the polymerization and β-cyclodextrin control the structure for producing high molecular weight polyesters. After the monomers were completely consumed converting to oligomers in the first-stage polymerization, the polymer chain formed a random coil conformation in an amorphous state and had a lower mobility. Thus, β-cyclodextrin was added into the mixture before the second-stage polymerization and threaded onto the polymer chain in their cavities to control the structure for preventing the polymer chain from coagulating. Apparently, β-cyclodextrin functioned similarly as a chaperone protein in a biological system which assisted proteins folding and retained the functional state of proteins. β-cyclodextrin encircling the linear polymer chain was crucial to maintaining the propagating state of the polyester. Thus, the polymer chain was extended with a higher mobility in the β-cyclodextrin channel, which was essential for producing high molecular weight polyesters. Accordingly, further polymerization occurred rapidly and continuous chain growth was obviously observed during the 192 h polymerization

Table 2
Molecular weight and polydispersity of aliphatic polyesters synthesized via polycondensation of diesters with diols[a].

Sample	Monomer		M_w[b] (Da)	PDI[b]	DP	Yield (%)
	Diester	Diol				
A-1[c]	Diethyl carbonate	1,4-Butanediol	5000	1.06	24	21.4
A-2[c]	Diethyl carbonate	1,6-Hexanediol	5100	1.06	22	47.2
A-3[c]	Diethyl carbonate	1,8-Octanediol	4900	1.05	19	35.0
B-1[d]	Diethyl succinate	1,4-Butanediol	5300	1.05	20	63.7
B-2[d]	Diethyl succinate	1,6-Hexanediol	27,300	4.27	93	65.4
B-3[c]	Diethyl succinate	1,8-Octanediol	29,400	4.19	92	71.0
C-1[c]	Diethyl adipate	1,4-Butanediol	5300	1.09	18	61.6
C-2[d]	Diethyl adipate	1,6-Hexanediol	25,500	2.33	80	74.3
C-3[d]	Diethyl adipate	1,8-Octanediol	25,100	3.36	72	76.0
D-1[c]	Diethyl sebacate	1,4-Butanediol	34,100	1.79	98	53.5
D-2[e]	Diethyl sebacate	1,6-Hexanediol	27,000	3.46	72	81.1
D-3[c]	Diethyl sebacate	1,8-Octanediol	44,600	2.83	110	66.1

[a] Conditions: the reactions were catalyzed in bulk by lipase from *Candida* sp.99-125 at 70 °C in molar ratio of 1:1 diester/diol with β-cyclodextrin as supporting architectures.
[b] Measured by GPC.
[c] 6 mbar during third-stage reaction.
[d] 8 mbar during third-stage reaction.
[e] 10 mbar during third-stage reaction.

period of the third-stage reaction (Fig. 2). At the adopted intervals of 6, 12, 40, 120 and 192 h, the corresponding poly(butylene sebacate) had molecular weight values of 9800, 12,100, 24,300, 34,100 and 62,100 Da, respectively. Hence, the polymer chains grew rapidly and the highest molecular weight of 62,100 Da was achieved when the third-stage polymerization was terminated at 192 h.

For all the poly(butylene sebacate) products shown in Fig. 2, the polydispersity values (PDI) ranged from 1.79 to 2.46. The general trends observed were increased PDI values from 2.2 to 2.46 for polymerization conducted from 6 to 40 h and decreased PDI values of 1.79 and 1.92 as the reaction time was prolonged from 40 to 120 and 192 h. The low PDI values were attributed to the longer polymerization time, which permitted short polymer chains diffusing to catalyst particles to form long polymer fractions with high molecular weight, thereby decreasing PDI values. This was consistent with the previous reports that as the polycondensation proceeded and the molecular weight increased, the polymers resulted in narrow molecular weight distribution (Azim et al., 2006; Jiang, 2008).

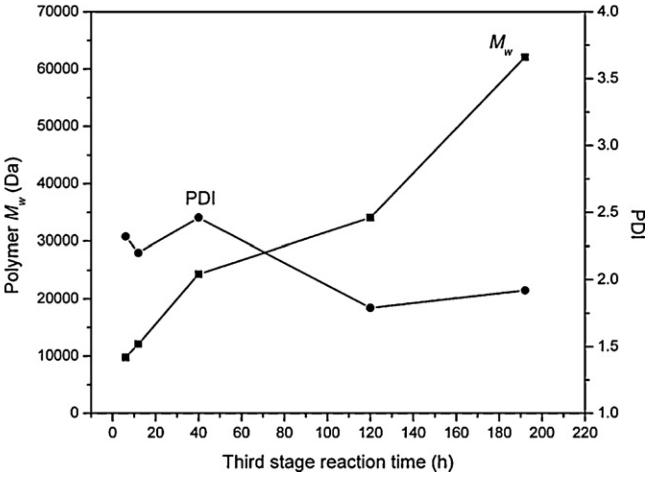

Fig. 2. Effects of reaction time on molecular weight and polydispersity during the third stage polycondensation of diethyl sebacate and 1,4-butanediol at 70 °C under 6 mbar catalyzed by lipase with β-cyclodextrin as supporting architectures.

3.3. *Thermal characterization and crystalline properties*

Thermal and crystalline properties of aliphatic polyesters were investigated comprehensively and the results of product B-2, B-3, C-2, C-3, D-1, D-2, D-3 were summarized in Table 3. Thermal stability of the seven samples mentioned above was studied by thermogravimetric analysis (TGA) and performed at 20 °C/min from room temperature to 600 °C under nitrogen flow. All the polyesters were stable up to 350 °C with weight loss below 3%, exhibiting good thermal stability. The main single weight loss step shifted from 350 °C to 450 °C. The temperature of maximum weight loss rate ($T_{\max}$) was taken as the peak maximum of the derivative curve of TGA trace. The $T_{\max}$ values of all samples appeared in the range from 400 to 430 °C (Table 3). Nevertheless, thermogravimetric analysis revealed that polyesters prepared with a high quantity of methylene groups as starting substrates, had a slightly higher $T_{\max}$ values than that synthesized by short chain monomers. The $T_{\max}$ values of product B-2 and B-3 polymerized with diethyl succinate were centered at 409 °C and 411 °C, and that of product D-1, D-2 and D-3 produced by diethyl sebacate appeared approximately at 424 °C.

The thermal properties of the seven polyesters B-2, B-3, C-2, C-3, D-1, D-2 and D-3 were analyzed by differential scanning calorimetry (DSC). The first heating runs were performed at 20 °C/min from −100 °C to 100 °C, followed by the second cooling scans operated at 10 °C/min rate from melt to room temperature. Melting temperatures (T_m) were taken as the peak maximum of endotherm while crystallization temperatures (T_c) were measured as the peak maximum of exotherm. The results of T_m values with corresponding transition enthalpies (ΔH_m) and T_c values with corresponding transition enthalpies (ΔH_c) were summarized in Table 3. During the heating runs, only a single T_m peak turned out at each endotherm between 50 °C and 70 °C. The glass transition temperature (T_g) of all products could not be observed clearly from the endotherm. Meanwhile, a sharp and intensive crystallization exotherm appeared during the second cooling runs and T_c values were concentrated to an extent of 20–60 °C. In addition, a small undercooling ($T_m - T_c$) of 10–30 °C appeared and the melting enthalpies were high in all polyesters ($\Delta H_m \approx 84–149$ °C). The DSC behaviors indicated that all seven products investigated had a strong ability to crystallize and the polymers were to a large extent converted to crystals at a rapid rate during cooling from the melt, resulting in no T_g values obtained from the endotherm. The results

Table 3
Thermal and crystalline characterization of aliphatic polyesters.

Sample	TGA	DSC				WAXS	DMTA
	$T_{\max}$[a] (°C)	T_c[b] (°C)	ΔH_c[b] (J/g)	T_m[c] (°C)	ΔH_m[c] (J/g)	χ_c[d] (%)	T_g[e] (°C)
B-2	409	28	63	56	84	56	−48
B-3	411	42	86	62	113	69	−41
C-2	420	38	110	55	123	59	−40
C-3	423	46	109	66	122	67	−35
D-1	425	41	102	67	126	70	−32
D-2	424	49	126	62	149	67	−33
D-3	424	51	79	70	117	72	−29

[a] Measured as the temperature of maximum weight loss rate.
[b] Taken as the peak maximum of exotherm.
[c] Taken as the peak maximum of endotherm.
[d] Crystallinity degree from WAXS.
[e] Peak temperature of main relaxation (tan δ) from DMTA.

are in agreement with earlier published data on the calorimetric behavior for Poly(PDL-*co*-butylene-*co*-succinate) which possessed a similar ability of chains to crystallize (Mazzocchetti, Scandola, & Jiang, 2009). Both melting and crystallization temperatures were also correlative to the quantity of methylene groups in the starting substrates. The T_m values increased from 55 °C up to 70 °C and the T_c values shifted from 28 °C to 51 °C by replacing short chain monomers with the starting substrates of long methylene in chains.

As discussed above, the aliphatic polyesters had a high crystallinity and it was a very unusual behavior in macromolecules. In order to verify this point, all seven products were subjected to wide-angle X-ray diffraction (WAXS) analysis to confirm the degree of crystallinity (χ_c). The χ_c values calculated from the ratio of crystalline peak intensity to total diffraction intensity were listed in Table 3. The WAXS patterns of all products were similar and exhibited two main reflections at about 21.4° and 24.1°. Except for product C-2, other polyesters presented minor reflections located at different positions in the range of 5–15°. In agreement with the high melting enthalpies derived from DSC analysis, the degree of crystallinity value was higher than 56% in all polymers and sample D-3 with a highest χ_c value was up to 72%. The crystallinity degree of the polymers shown in Table 3 varied in the same trend as the T_m values. The χ_c values would rose to a small extent by making use of diesters or diols with long methylene in chain.

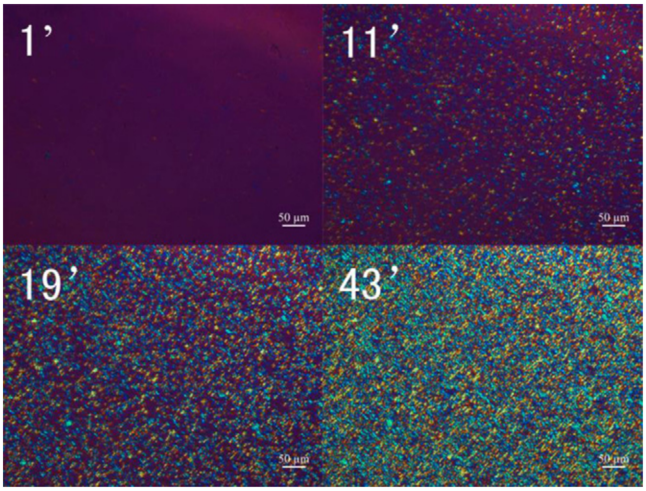

Fig. 3. Polarized optical micrographs of sample D-3 during crystallization from melt to 50 °C. Pictures were taken at the time indicated on each image.

3.4. Morphology features

Morphologies of polymer were investigated by polarizing optical microscope (POM) and transmission electron microscopy (TEM). A polarizing optical microscope equipped with a hot stage was used to record the morphology of the semi-crystalline polyesters. Fig. 3 showed POM images of sample D-3 which was first heated at 80 °C for 10 min and then crystallized at selected temperature of 50 °C for 5 min after cooling at 5 °C/min rate. Pictures were taken at the time indicated on each image. As the cooling of the sample from 80 °C to 50 °C, an increase of spherulite birefringence was observed and apparently it was negative spherulite as seen in the last picture. The polymer had a strong ability to crystallize and was converted to crystallization from starting to termination at a rapid rate within 43 s, which was in accord with the results of no T_g values obtained from DSC analysis. Since the polymers were semi-crystalline and contained a large fraction of crystallization, the crystal morphology of sample D-3 was detected by transmission electron microscopy (TEM) and crystal lattice arrangement was exhibited in Fig. 4.

3.5. Dynamic mechanical thermal analysis

The aliphatic polyesters crystallized rapidly from the melt, resulting small fraction of amorphous phase. The glass transition

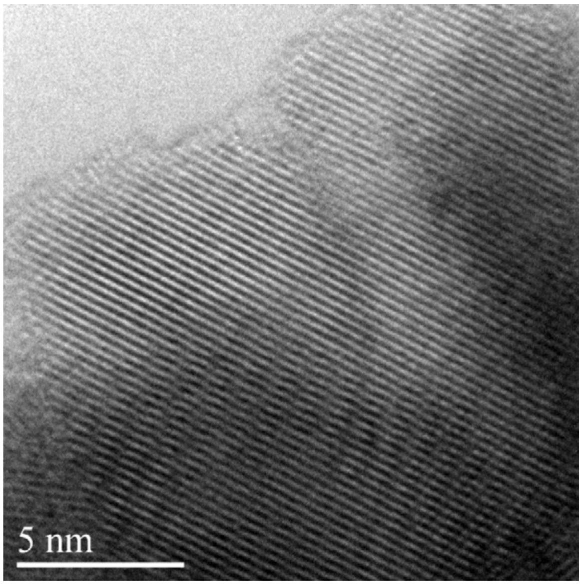

Fig. 4. Transmission electron microscopy (TEM) micrograph of sample D-3.

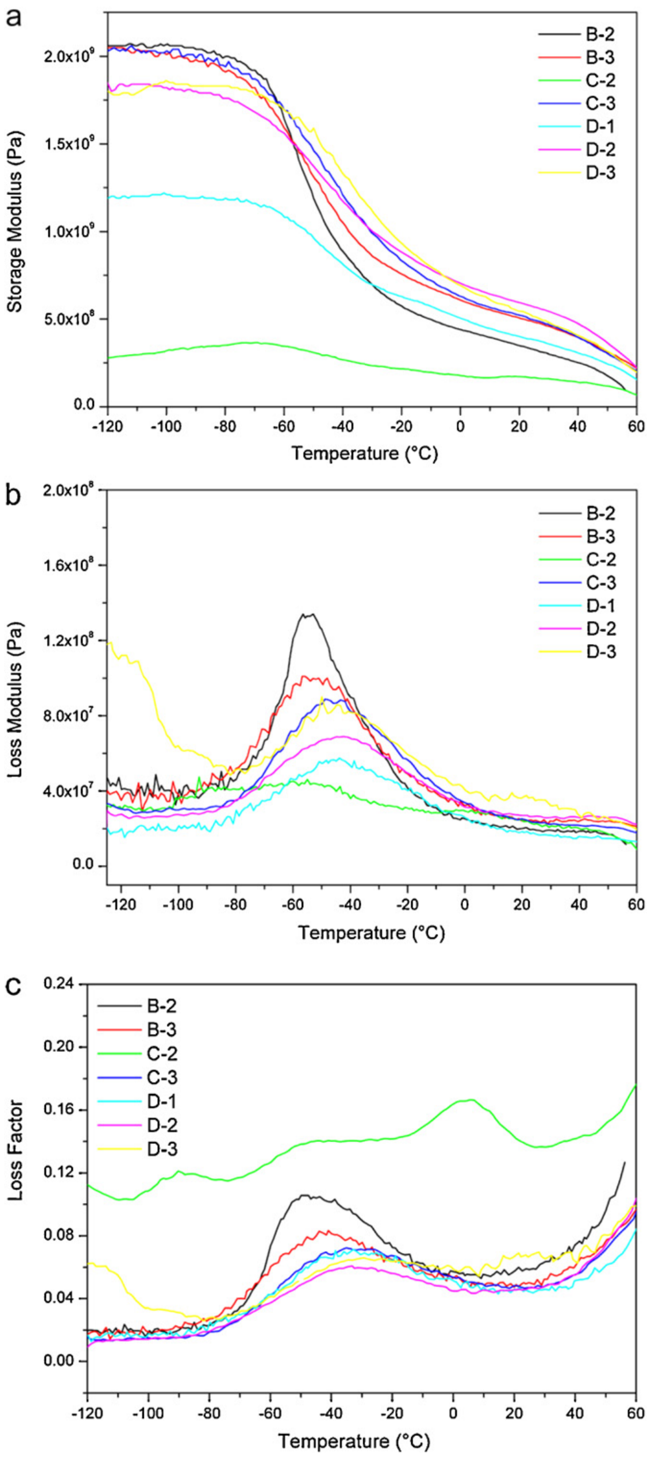

Fig. 5. DMTA curves (1 Hz, 5 °C/min) of aliphatic polyesters. (a) Storage modulus E′, (b) loss modulus E″ and (c) loss factor (tan δ) vs temperature for different polymers.

temperature (T_g) could not be detected in the DSC analysis. Accordingly, dynamic mechanical thermal analysis (DMTA) was employed as a more sensitive method to evaluate the T_g values as well as the mechanical properties for the polymer B-2, B-3, C-2, C-3, D-1, D-2 and D-3. The storage modulus (E'), loss modulus (E''), and loss factor (tan δ) versus temperature curves for the polyesters measured at a frequency of 1 Hz were displayed in Fig. 5(a)–(c), respectively. All samples exhibited a gradual decrease in the storage modulus from glass to rubber and ultimately to melt in Fig. 5(a). A sharp drop was observed in the region corresponding to the glass-transition. Except for product C-2, the storage modulus values of the polyesters were higher than 10^9 Pa, which were mainly ascribable to the high crystallinity degree measured by WAXS. In the temperature between 0 °C and 20 °C, a cold crystallization of polymer C-2 appeared in the storage modulus curves. As the crystallinity of the polymers increased, a slight shift of the loss modulus peak toward higher temperature was shown in Fig. 5(b).

The DMTA curves of all products presented a tan δ peak (α-relaxation) associated with the glass transition in Fig. 5(c). Peak temperatures of main relaxation (α) considered as the glass transition temperature (T_g) were also summarized in Table 3, which were centered in the range from −20 °C to −50 °C. The T_g values rose to higher temperature with increasing crystallinity degree of polyesters. In addition, a secondary relaxation (β) appeared in the DMTA curves of product C-2 and D-3, located at lower temperature about −90 °C. The β-relaxation was ascribable to local motions of long methylene (more than three) in polymer chains. The peak existed in the temperature higher than T_g of sample C-2 curve was due to the cold crystallization.

4. Conclusion

Lipase-catalyzed synthesis of aliphatic polyesters was achieved via a solvent-free method with β-cyclodextrin as supporting architecture in a three-stage polymerization under mild condition, producing the corresponding polymers of high molecular weights. The polymers synthesized by extracellular enzymes were considered to be a new area in precise synthesis of polymers to achieve green polymer chemistry without the use of toxic reagents. The lipase from *Candida* sp.99-125 showed high catalytic activity for bulkpolymerization of diesters and diols with a high quantity of methylene groups in their chains. Lipase initiated the polymerization and β-cyclodextrin, acting similarly to a protein-chaperone, threaded onto the polymer chain in their cavities to maintain the chain proper conformation for producing high molecular weight polyesters. All polyesters had good thermal stability and were highly crystalline contributing to the high storage modulus.

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