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LIPASE-CATALYZED POLYESTER SYNTHESIS

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ABSTRACT

The use of lipase as biocatalyst in polyesterification of aliphatic diacids or their derivatives, and diols in an organic solvent has been discussed. We have demonstrated that bis(2-chloroethyl) esters of succinic, fumaric, and maleic acid, and bis(2,2,2-trifluoroethyl) sebacate and -dodecanedioate can be polymerized by lipase-catalyzed polytransesterification. Maleate was isomerized to fumarate even under mild reaction conditions, resulting in poly(1,4-butyl fumarate). In order to obtain a high mass-average molar mass of the polyester, solid *Mucor miehei* lipase was found to be the best lipase and diphenyl ether the best solvent of several investigated. There was no clear relationship with the log *P* value of the solvent and the polyesterification activity of lipase. The highest degree of polymerization (DP = 184) of poly(1,4-butyl sebacate) with a mass-average molar mass of 46,600 $g \cdot mol^{-1}$ was obtained in polytransesterification of bis(2,2,2-trifluoroethyl) sebacate and 1,4-butanediol using a programmed vacuum profile. However, a mass-average molar mass as high as about 42,000 $g \cdot mol^{-1}$ (DP = 167) was also obtained with free sebacic acid when vacuum was employed to remove the water formed during esterification. The mass average molar mass of the polyester increased with an increase in the relative quantity

of lipase up to 1 g per 1.5 mmol of diacid, with an increase in the molar mass of the aliphatic diol up to 1,5-pentanediol, and with an increase in the concentration of substrates up to 0.83 M.

INTRODUCTION

Recently the interest in the applications of biocatalysis in organic syntheses has rapidly increased [1–3]. For example, lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) have been employed in the modification of fats and oils both to provide novel materials of improved characteristics and to upgrade inexpensive raw materials to valuable products [4–6]. Although lipase is an enzyme which catalyzes the hydrolysis of glycerol esters in water solutions, the discovery by Klibanov [7] that crude enzyme powders can efficiently catalyze synthetic reactions in organic solvent systems opened up entirely new possibilities for the use of lipase in esterification and transesterification [8–11]. Lipase catalyzes the following esterification and transesterification reactions:

Esterification

$$R_1COOH + R_2OH \longleftrightarrow R_1COOR_2 + H_2O$$

Transesterification:

Acidolysis:

$$R_1COOR_2 + R_3COOH \longleftrightarrow R_3COOR_2 + R_1COOH$$

Alcoholysis:

$$R_1COOR_2 + R_3OH \longleftrightarrow R_1COOR_3 + R_2OH$$

Interesterification:

$$R_1COOR_2 + R_3COOR_4 \longleftrightarrow R_1COOR_4 + R_3COOR_2$$

Aminolysis:

$$R_1COOR_2 + R_3NH_2 \longleftrightarrow R_1CONHR_3 + R_2OH$$

Of particular interest is the possibility to produce biodegradable polyesters by lipase biocatalysis, an interesting alternative in the climate of ever-increasing global environmental concerns [12–14]. Lipase biocatalysis in an organic solvent minimizes problems arising from poor solubility of some organic substrates and products in water. Carrying out the reaction at a relatively low temperature and pressure markedly reduces most undesirable side reactions.

In biopolymerization the stability of the enzyme, interference by the by-products, and insolubility of the high molar mass product limit the degree of poly-

merization (DP) and, until recently, only oligomers of a DP of less than 10 were reported in lipase-catalyzed polyester synthesis [15]. Wallace and Morrow [10] were the first to obtain a polyester of a relatively high mass average molar mass of 14,900 $g \cdot mol^{-1}$ in porcine pancreatic lipase-catalyzed polymerization of bis(2,2,2-trichloroethyl) adipate with either 1,4-butanediol or 1,6-hexanediol. They introduced two marked improvements: polytransesterification instead of polyesterification, thus avoiding water formation during the reaction, and a halogenated acyl donor bis(2,2,2-trichloroethyl) adipate, minimizing the trend toward a reverse reaction [13]. Further, if the alcohol R_2OH or water formed is removed during polymerization, the reaction proceeds forward toward completion. The method has been since adopted by a number of other research groups [10, 12, 14–19].

In the present work both polytransesterification and polyesterification (alcoholysis) were applied for linear polyester synthesis. We shall discuss in the following a number of important aspects in enzymatic polyester synthesis and demonstrate that a high degree of polymerization can be obtained both with derivatized and underivatized diacids.

EXPERIMENTAL

Chemicals

The bis(2-chloroethyl) derivatives of succinic, fumaric, and maleic acids were synthesized as follows. To a 250-mL round-bottomed flask 0.3 mol of diacid, 1.0 mol of 2-chloroethanol in 0.2 ml of concentrated sulfuric acid, and 60 mL of benzene were added. The flask was fitted with a suitable water separator at the top of a reflux condenser. The mixture was heated under reflux until no further separation of water took place. The reaction mixture was washed with 10% sodium carbonate solution until neutral, followed by washing twice with a saturated sodium chloride solution. The liquid was dried over anhydrous magnesium sulfate, and the benzene and excess 2-chloroethanol were removed by distillation. The chloroethyl esters were purified by vacuum distillation. Bis(2-chloroethyl) succinate was collected at 145–147°C (2 mmHg, 2.67×10^{-2} Pa) as a colorless clear liquid. IR (KBr): ν = 2960, 2940 cm^{-1} ($-CH_2$, $-CH_3$), ν = 1720 cm^{-1} ($-C{=}O$), ν = 1180 cm^{-1} ($C-O-C$), ν = 800, 660 cm^{-1} ($C-Cl$). Bis(2-chloroethyl) maleate was collected at 167–169°C (4 mmHg, 5.3×10^{-2} Pa) as a colorless clear liquid. IR (KBr): ν = 3060 cm^{-1} ($H-C{=}$), ν = 29,650, 2890 cm^{-1} ($-CH_3$, $-CH_2$), ν = 1730 cm^{-1} ($-C{=}O$), ν = 1645 cm^{-1} (*cis* $-C{=}C-$), ν = 1180 cm^{-1} ($-CO-O-C-$), ν = 670 cm^{-1} (*cis* $-CH{=}CH-$). Bis(2-chloroethyl) fumarate was obtained when the reaction mixture was cooled, and a large amount of white solid was precipitated. Pure product could be obtained by recrystallization from a 10:1 (v/v) mixture of hexane and dichloromethane as a white solid with a 62–64°C melting point. IR (KBr): ν = 3060 cm^{-1} ($H-C{=}$), ν = 29,650, 2890 cm^{-1} ($-CH_3$, $-CH_2$), ν = 1730 cm^{-1} ($-C{=}O$), ν = 1670 cm^{-1} (*trans* $-C{=}C-$), ν = 1180 cm^{-1} ($-CO-O-C-$), ν = 975 cm^{-1} (*trans* $-CH{=}CH-$).

Bis(2,2,2-trifluoroethyl) sebacate and -dodecanedioate was synthesized as follows: To a 500-mL three-necked flask equipped with a magnetic stirrer and an addition funnel, 30.0 g (0.3 mol) of 2,2,2-trifluoroethanol, 23.57 g (0.3 mol) of anhydrous pyridine, and 200 mL of methylene chloride were added. The solution

was cooled to 0°C in an ice bath and 36.1 g (0.145 mol) sebacic chloride was added dropwise. The mixture was slowly warmed to room temperature, and the reaction was allowed to proceed for 6 hours after which 100 mL of water was added. The organic phase was washed successively with 3 × 100 mL of 5% aqueous hydrochloric acid, 3 × 100 mL of saturated aqueous sodium bicarbonate, and 3 × 100 mL of water. Water was removed by anhydrous magnesium sulfate. Bis(2,2,2-trifluoroethyl) sebacate was collected by vacuum distillation at 164–165°C (9 mmHg, 1.2×10^3 Pa) as a clear colorless liquid, yield 94.7%, and was checked to be chromatographically pure by HPLC. All other chemicals, obtained from Aldrich-Chemie (Steinheim, Germany), were of analytical grade with a purity of 99.5% or higher. Organic solvents were stored over a 4-Å (0.4 nm) molecular sieve.

Lipases

The lipases from *Pseudomonas fluorescens* (specific activity of 12.0 units·mg^{-1} of solid, 3.1% water), *Mucor miehei* (specific activity of 4.0 units·mg^{-1} of solid, 7.4% water), and *Candida rugosa* (specific activity of 150 units·mg^{-1} of solid, 5.0% water) were obtained as crude powders from Biocatalysts (Pontypridd, UK). The porcine pancreatic lipase (specific activity of 3.9 units·mg^{-1} of solid, 1.7% water) was obtained as a crude powder from Sigma Chemical Co. (St. Louis, USA). Lipases were used without further drying. One unit of activity was defined as that amount of enzyme which catalyzes the release of 1 μmol of free fatty acid from olive oil in 1 minute at pH 7.0, 37°C.

Polymerization

A typical polymerization was carried out as follows: 0.25 g crude lipase powder was added to a reaction mixture of 1.5 mmol of a sebacic acid or its derivative and 1.5 mmol of a 1,4-butanediol as substrates in 2.25 mL of an organic solvent (equivalent to 0.51 M concentration of each substrate) in a 5-mL round-bottomed flask equipped with a magnetic stirrer and a condenser. Substrates were weighed to 0.1 mg accuracy, taking their purity into account. A 1:1 molar ratio of diacid and diol was always used, the most suitable substrate ratio for AA + BB polycondensation to obtain a high molecular weight polyester. The mixture was stirred by magnetic stirrer at 400 rev·min^{-1}, 37°C. In most experiments 5 mmHg (6.7×10^2 Pa) vacuum was exerted for 10 minutes both after 5 and 10 hours. After 20 hours the vacuum was further decreased to 0.15 mmHg (20 Pa) until the experiment was completed. A blank test (without lipase) was also carried out for each experiment, without any observed polymerization. When the reaction was completed, the lipase was filtered off, and a white solid polyester was obtained upon precipitation from methanol.

Analytical Methods

The mass-average molar mass ($\overline{M}_w$) of the polymer was determined by gel permeation chromatography (GPC). GPC analysis was carried out on a Waters 700 Satellite WISP chromatograph (Waters, Division of Millipore, Milford, Massachusetts, USA). Three serially connected μ-styragel columns (0.01, 0.05, and 0.1 μm)

were used. Twenty-one different polystyrene oligomers with known average molar mass from 162 $g \cdot mol^{-1}$ to 1.57×10^6 $g \cdot mol^{-1}$ (Polymer Laboratories, Church Stretton, UK) were used as standards. A calibration curve was constructed by using Baseline 810 software of Waters. Samples consisted of 10 mg of the oligomer mixture in 4 mL of chloroform, of which 200 μL was injected for analysis. HPLC-grade chloroform (Rathburn Chemicals, Walkerburn, Scotland) was used as the eluant at a rate of 1.0 $mL \cdot min^{-1}$. The peaks were detected by a UV detector at 254 nm.

Infrared spectroscopy analysis was performed on a Philips Pye Unicam SP3-100 infrared spectrophotometer (Pye Unicam, Cambridge, UK) equipped with a Philips P3150 computer with a double beam ratio recording mode and scan time of 8 minutes. The sample pellet contained 0.8% of the oligomer mixture in potassium bromide.

^{13}C-NMR spectra were measured with a Varian Unity-400 NMR spectrometer at 100.577 MHz. The proton-decoupled ^{13}C-NMR spectrum was recorded at room temperature in $CDCl_3$ solution of the polyester (0.15 g in 0.7 mL) with a 82° pulse (8.7 μs), 25,000-Hz spectrum width, 1.199 seconds acquisition time, and 2000 scans. ^{13}C chemical shifts were referred to the 77-ppm peak of deuterated chloroform.

Differential scanning calorimeter (DSC) measurements were carried out by PL Thermal Sciences DCS (Rheometer Scientific, formerly Polymer laboratories, Epsom, UK). Nitrogen was used as the sweeping gas, and 6.6 mg samples were heated twice at a rate of 10°C $\cdot min^{-1}$ in order to ensure a similar thermal history. The scanning temperature range was from -100 to 100°C.

RESULTS AND DISCUSSION

Effect of the Type of Lipase

We have previously screened 25 commercially available lipases for the synthesis of butyl oleate [9]. It became clear from the start that the lipolytic activities generally reported gave little information on synthetic performance. From the enzymes of a high esterification activity, *Mucor miehei, Pseudomonas fluorescens*, and *Candida rugosa* lipases were selected for further screening as biocatalysts for the polymerization reactions in various organic solvents. In addition, porcine pancreatic lipase, known to catalyze a number of esterification reactions, was also investigated. Of these, the lipases from *M. miehei* and *P. fluorescens* resulted in the highest degree of conversion in polyester synthesis from 1,4-butanediol and bis(2-chloroethyl) maleate [16] or -succinate [16, 19] in diisopropylether at ambient pressure. In the case of fumarate, *P. fluorescens* lipase was the only lipase active in acetonitrile and diisopropyl ether. Porcine pancreatic lipase showed no activity for any of the three substrates tested. Even at best, a relatively low degree of polymerization and mass-average molar mass were obtained, apparently because no means such as vacuum were taken to eliminate the 2-chloroethanol formed during the reaction. An equilibrium was reached in about 72 hours under the experimental conditions used. Maleate was isomerized to fumarate even under the mild reaction conditions used, resulting in poly(1,4-butyl fumarate).

Inasmuch as the *M. miehei* lipase resulted in the highest mass-average molar mass in the preliminary trials, it was used in most of the subsequent experiments.

TABLE 1. Effect of the Type of Lipase on Mass-Average Molar Mass of the Polyester from 1.5 mmol Bis(2,2,2-trifluoroethyl) Dodecanedioate or -Sebacate and 1.5 mmol 1,4-Butanediol (0.25 g lipase, 37°C, 400 rpm, 72 h, solvent diphenyl ether, programmed vacuum profile)

Lipase	Poly(1,4-butyl dodecanedioate), $g \cdot mol^{-1}$	Poly(1,4-butyl sebacate), $g \cdot mol^{-1}$
Candida rugosa	1,980	12,800
Mucor miehei	10,550	27,700
Porcine pancreas	7,000	18,900
Pseudomonas fluorescens	7,500	16,900

On polytransesterification of bis(2,2,2-trifluoroethyl) sebacate and 1,4-butanediol with *M. miehei* lipase in diphenyl ether in vacuum, a mass-average molar mass of as high as 27,700 $g \cdot mol^{-1}$ was obtained in 72 hours. This was more than twice that obtained with the *Candida rugosa* lipase, although the latter exhibited a high activity both in hydrolysis and esterification reactions [5, 9]. Also in the polytransesterification of bis(2,2,2-trifluoroethyl) dodecanedioate and 1,4-butanediol in veratrole, *M. miehei* lipase resulted in the highest mass-average molar mass of 10,550 $g \cdot mol^{-1}$, five times higher than that obtained with the *Candida rugosa* lipase (Table 1).

Effect of Lipase Quantity

The relative lipase quantity also affected the mass-average molar mass ($\overline{M}_w$) of the polyester (Table 2). With 1.5 mmol of bis(2,2,2-trifluoroethyl) sebacate and 1.5 mmol of 1,4-butanediol, the highest polyester mass-average molar mass of about 42,000 $g \cdot mol^{-1}$ was obtained in 3 days in diphenyl ether with 0.5 g [per 1.5 mmol of bis(2,2,2-trifluoroethyl) sebacate] of *Mucor miehei* lipase and about 27,000

TABLE 2. Effect of *M. miehei* Lipase Quantity [in g per 1.5 $mmol^{-1}$ bis(2,2,2-trifluoroethyl) sebacate] on Mass-Average Molar Mass of Poly(1,4-Butyl Sebacate) (37°C, 400 rpm. 72 h, programmed vacuum profile)

	Average molar mass	
Lipase quantity, g	Diphenyl ether, $g \cdot mol^{-1}$	Veratrol, $g \cdot mol^{-1}$
0.0625	20,773	
0.125	29,683	8,932
0.25	27,670	10,989
0.50	42,020	17,386
1.0	31,372	26,946

$g \cdot mol^{-1}$ in veratrole with 1 g of *M. miehei* lipase. With further increases in the relative lipase quantity, the viscosity of the solution increased markedly, causing difficulties in mixing. This supports the observation of Knani et al. [11] that mixing has little effect on porcine pancreatic lipase-catalyzed polytransesterification of ω-hydroxyesters in hexane at 69°C in dilute solutions, but in concentrated solutions mixing had an adverse effect on the degree of polymerization.

Effect of Solvent

The type of organic solvent appears to be a very important factor in the lipase-catalyzed enzymatic synthesis, but the solvent effects are still little understood [19]. A considerable amount of work has been carried out in order to clarify the relationships between lipase activity and the properties of the organic solvent used. Of the several physical parameters studied, the Hildebrand solubility parameter δ [20] and log P [21] (the logarithm of the partition coefficient of a given component in the octanol–water two-phase system) have been claimed to be the most informative. Nevertheless, there is no general agreement on how to select a suitable solvent. Table 3 shows, for example, the enzymic oligocondensation of bis(2-chloroethyl) succinate and 1,4-butanediol with two different lipases at ambient pressure in a number of solvents of varying log P, with no clear relationship between the log P value and the maximum mass-average molar mass of the oligomer. According to Laane et al. [21], the catalytic activity of lipase is expected to be low in polar, hydrophilic solvents having a log $P < 2$. We observed that different solvents of a similar log P value could behave quite differently (such as toluene, log $P = 2.5$, and diisopropyl ether, log $P = 2.7$; Table 3), and that the same solvent could have a different effect with different lipases.

In practice, the boiling point of the solvent should also be considered, among other factors, if vacuum is to be applied to the system. With halogenated esters as

TABLE 3. Mass-Average Molar Mass of the Oligomers Formed in Enzymic Oligocondensation of 1.0 mmol Bis(2-chloroethyl) Succinate and 1.0 mmol 1.4-Butanediol in Solvents of Varying log P Values (37°C, 260 rpm, 0.1 g lipase, without vacuum)

		Lipase	
Solvent	log P [Reference]	*P. fluorescens* $\overline{M}_w$, $g \cdot mol^{-1}$	*M. miehei* $\overline{M}_w$, $g \cdot mol^{-1}$
n-Hexane	3.5 [21]	851	895
Diisopropyl ether	2.7 [11]	1376	1231
Toluene	2.5 [21]	727	727
Chloroform	2.0 [21]	355	501
Tetrahydrofuran	0.49 [21]	413	674
Acetone	−0.23 [21]	573	725
Acetonitrile	−0.33 [21]	354	736

substrates for polytransesterification, the solvent should have a high enough boiling point to be retained in the reactor during the removal of the alcohol formed from the reaction mixture. Of a large number of solvents tested with a sufficiently high boiling point and widely varying log *P* values, diphenyl ether and veratrole (1,2-dimethoxybenzene) were found to be the most promising solvents for *M. miehei* lipase-catalyzed polymerizations (Table 4). Again, there seemd to be no correlation between the log *P* value and the synthetic activity. Inasmuch as diphenyl ether was found to be generally superior to veratrole, diphenyl ether was used in most experiments. For example, a mass-average molar mass of about 42,000 $g \cdot mol^{-1}$ was obtained in 72 hours in the polymerization of bis(2,2,2-trifluoroethyl) sebacate and 1,4-butanediol in diphenyl ether with 0.5 g *M. miehei* lipase, but only about 27,000 $g \cdot mol^{-1}$ in veratrole with 1 g lipase (Table 2). Morrow [17] found 1,2- and 1,3-dimethoxybenzenes to be better solvents than diphenyl ether for the polymerization reaction of bis(2,2,2-trifluoroethyl) glutarate and 1,4-butanediol catalyzed by porcine pancreatic lipase.

Effect of the Chain Length of Diol

Five different diols, 1,2-ethane-, 1,3-propane-, 1,4-butane-, 1,5-pentane- and 1,6-hexanediols, were each polymerized with bis(2,2,2-trifluoroethyl) sebacate (Table 5). With 1,2-ethanediol a low DP of about 30 was obtained. The DP increased with the chain length of the diol, with 1,5-pentanediol giving the maximum DP of 155, with a mass-average molar mass of higher than 41,000 $g \cdot mol^{-1}$. This is in good agreement with the results of Morrow [17] who observed that an increase in length of the carbon chain of the diol up to about six carbons results in an increase in the average molar mass of the polyester obtained from bis(2,2,2-trifluoroethyl) glutarate with diols in veratrole. He obtained a mass-average molar mass of 19,500 (DP = 104) with 1,4-butanediol after 72 hours.

TABLE 4. Effect of the Choice of Solvent on Mass-Average Molar Mass of Poly(1,4-Butyl Sebacate) from Bis(2,2,2-trifluoroethyl) Sebacate (1.5 mmol of substrates, 0.25 g *M. miehei* lipase, 37 °C, 400 rpm, 72 h, programmed vacuum profile)

Solvent	log *P*	bp, °C	Molar mass, $g \cdot mol^{-1}$
Dodecane	6.6	214	5,900
Hexyl ether	5.0	228	9,800
Diphenyl ether	4.3	259	27,700
Isoamyl ether	4.3	173	9,000
Veratrole	2.2	206	17,400
Triglyme	−0.46	216	7,600

TABLE 5. Effect of Diol on the Mass-Average Molar Mass of Poly(1,4-Butyl Sebacate) from Bis(2,2,2-trifluoroethyl) Sebacate (1.5 mmol of substrates, 0.25 g *M. miehei* lipase, 37°C, 400 rpm, 72 h, solvent diphenyl ether, programmed vacuum profile)

Diol	Average molar mass, $g \cdot mol^{-1}$
1,2-Ethanediol	6,729
1,3-Propanediol	22,071
1,4-Butanediol	27,670
1,5-Pentanediol	41,370
1,6-Hexanediol	38,181

Effect of Substrate Concentration

The mass-average molar mass of the polyester increased with an increase in the substrate concentration up to about 0.83 M bis(2,2,2-trifluoroethyl) sebacate and 0.83 M 1,4-butanediol (Fig. 1). The GPC results showed that there were some low-molar mass oligomers in the product mixture when the concentration of the substrates was low. The peak number and mass percent of the low-molecular oligomers at low substrate concentration was higher than at high substrate concentration. Knani et al. [11] obtained similar results with the polytransesterification of ω-

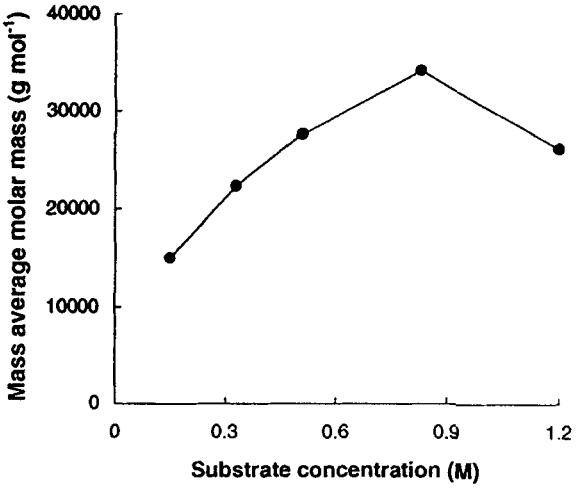

FIG. 1. Mass average molar mass of poly(1,4-butyl sebacate) as the function of substrate concentration (0.25 g *M. miehei* lipase, 37°C, 400 rpm, 72 hours, solvent diphenyl ether, programmed vacuum profile).

hydroxyesters catalyzed by porcine pancreatic lipase in hexane at 69°C. The average molar mass of the polymer increased with an increasing concentration right up to the limit of no solvent being added. Our results showed that if the concentration of both substrates was as high as 1.2 M, the solution became very viscous, interfering with efficient mixing and resulting in a decrease in the average molar mass.

Effect of Vacuum

When bis(2,2,2-trifluoroethyl) sebacate was polymerized with 1,4-butanediol at ambient pressure to form poly(1,4-butyl sebacate), the mass-average molar mass of the polyester obtained in 72 hours was only 7800 $g \cdot mol^{-1}$ or less, as compared with the 27,700 $g \cdot mol^{-1}$ obtained in a vacuum system for the removal of the 2,2,2-trifluoroethanol formed during the transesterification. Under optimal conditions the polymerization both with sebacate and dodecandioate progressed with time up to 168 hours (7 days), at which time a maximum average molar mass of 46,410 $g \cdot mol^{-1}$ (DP = 184) or 34,750 $g \cdot mol^{-1}$, respectively, was obtained (Fig. 2). This compares favorably with the about 40,000 $g \cdot mol^{-1}$ reported by Morrow [17] on the polytransesterification of bis(2,2,2-trifluoroethyl) glutarate with 1,4-butanediol in veratrole, catalyzed by porcine pancreatic lipase for 432 hours (18 days). The high molar mass polyester was purified by precipitating from methanol, and the white solid was shown to be linear poly(1,4-butyl sebacate) by ^{13}C NMR.

No polymerization took place without lipase. In all cases the removal of water or alcohol formed by vacuum was very important in order to obtain a high average molar mass product. Nevertheless, during the early stages of polymerization care should be taken to prevent the removal of low-molar-mass reactants. In the present work, two 10-minute periods at about 5 mmHg (6.7×10^2 Pa) pressure during the

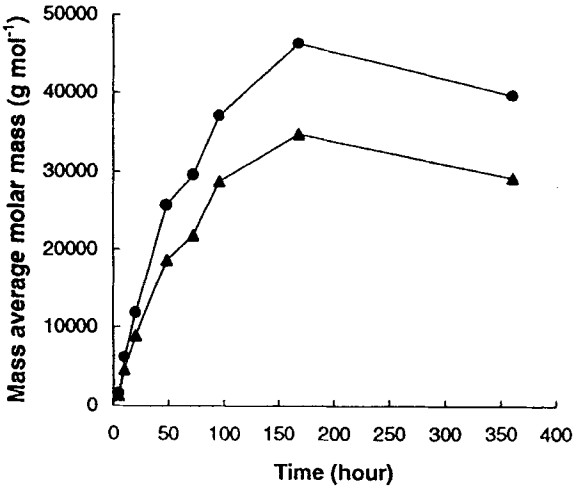

FIG. 2. Time courses of lipase-catalyzed synthesis of poly(1,4-butyl sebacate) (●) and poly(1,4-butyl dodecandioate) (▲) (1.5 mmol substrates, 0.25 g *M. miehei* lipase, 37°C, 400 rpm, 15 days, solvent diphenyl ether, programmed vacuum profile).

first 20 hours followed by 0.15 mmHg (20 Pa) until the end of the experiment was found optimal.

Polyesterification of Sebacic Acid with 1,4-Butanediol

An interesting result was also that in vacuum *Mucor miehei* lipase had a relatively high activity for the polyesterification of underivatized sebacic acid with 1,4-butanediol in diphenyl ether with a mass-average molar mass of 42,050 $g \cdot mol^{-1}$ (DP = 167) obtained in 7 days (Table 6). This was four times higher than that obtained at atmospheric pressure, and of the same order of magnitude as that obtained in the polytransesterification of the derivatized acid. Only a few earlier reports have been published on the polyesterification of underivatized diacids with diols. Okumura et al. [22] used *Aspergillus niger* lipase for the polyesterification of several diacids in the presence of an excess of a diol, and Ajima et al. [23] used *Pseudomonas fluorescens* lipoprotein lipase solubilized with poly(ethylene glycol) for the polymerization of hydroxydecanoic acid in benzene, but only small molecular oligomers were obtained. Binns et al. [18] reported a molar mass higher than 7199 $g \cdot mol^{-1}$ and a mass-average molar mass of 4645 $g \cdot mol^{-1}$ with immobilized *Mucor miehei* lipase for polyesterification of adipic acid with 1,4-butanediol in hexane.

Characteristics of Poly(1,4-Butyl Sebacate)

For characterization purposes the synthesis of poly(1,4-butyl sebacate), both from sebacic acid (a) and bis(2,2,2-trifluoroethyl) sebacate (b), and 1,4-butanediol was scaled-up 20-fold, using *Mucor miehei* lipase, diphenyl ether as the solvent, 37°C, and programmed vacuum profile. Figure 3 shows that the molar mass distri-

TABLE 6. The Synthesis of Poly(1,4-Butyl Sebacate) from Different Monomers as a Function of Time (1.5 mmol of substrates, 0.25 g *M. miehei* lipase, 37°C, 400 rpm, 2.25 mL solvent, programmed vacuum profile)

	Sebacic acid		Diethyl sebacate		Bis(2,2,2-trifluoroethyl sebacate	
Time	Diphenyl ether $\overline{M}_w$, $g \cdot mol^{-1}$	Veratrole $\overline{M}_w$, $g \cdot mol^{-1}$	Diphenyl ether $\overline{M}_w$, $g \cdot mol^{-1}$	Veratrole $\overline{M}_w$, $g \cdot mol^{-1}$	Diphenyl ether $\overline{M}_w$, $g \cdot mol^{-1}$	Veratrole $\overline{M}_w$, $g \cdot mol^{-1}$
5 h				1,038	6,712	1,884
10 h				867	7,348	1,913
20 h	8,205	1,842	5,673	1,311	11,820	3,984
2 d	20,670	4,368	13,390	4,401	25,650	7,338
4 d	33,681	12,191	19,407	7,040	37,060	12,059
7 d	42,050	23,471	18,193	16,191	46,410	15,950
11 d				21,794		22,278
15 d	41,716	20,442	20,204	18,661	39,670	18,510

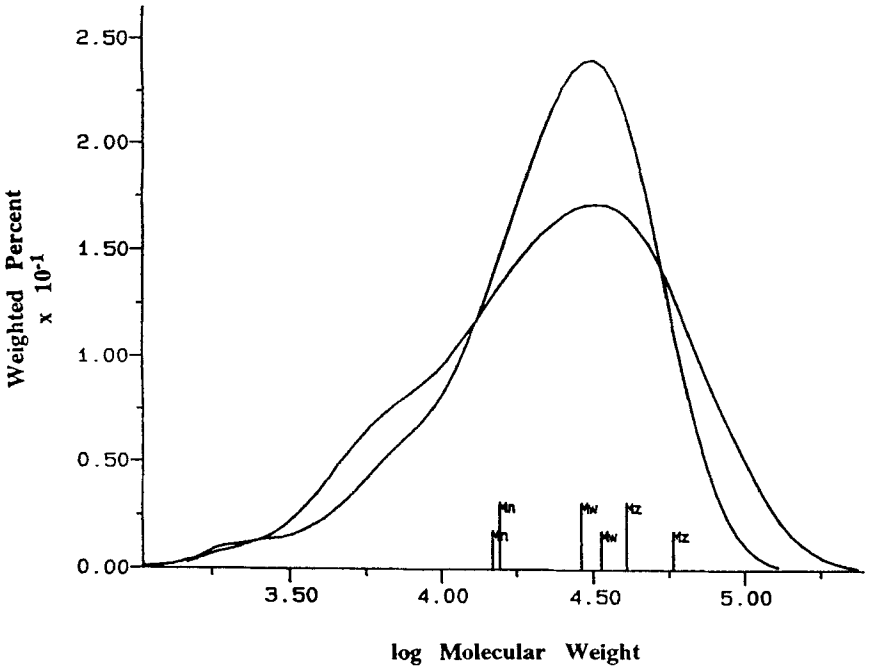

FIG. 3. Molar mass distributions of poly(1,4-butyl sebacate) both from sebacic acid (a) and bis(2,2,2-trifluoroethyl) sebacate (b), and 1,4-butanediol (30 mmol substrates and 7.5 g *M. miehei* lipase, 7 days; other conditions as in Fig. 1).

butions obtained in 7 days with both substrates were nearly identical. In this case the mass-average molar mass ($\overline{M}_w$) for (a) was about 33,600 g·mol^{-1} (DP 134) and for (b) 29,000 g·mol^{-1} (DP 116), the number-average molar mass ($\overline{M}_n$) for (a) 14,800 g·mol^{-1} and for (b) 15,700 g·mol^{-1}, and polydispersity $\overline{M}_w/\overline{M}_n$ for (a) 2.27 and for (b) 1.85. That the polyesters obtained from both substrates were identical was also demonstrated by the ^{13}C-NMR (Fig. 4) and DSC analyses (Fig. 5). The melting temperature was 65°C, and no glass-transition temperature could be observed. The linear polyester structure obtained by ^{13}C-NMR analysis is given in Fig. 4.

$$\{COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOCH_2CH_2CH_2CH_2O\}_n$$
f d b a a e c

Chemical shift (ppm)	a	b	c	d	e	f
Assignment	24.939	25.404	29.150	34.345	63.792	173.983

FIG. 4. Assignment of ^{13}C-NMR spectrum of poly(1,4-butyl sebacate) (DP = 134).

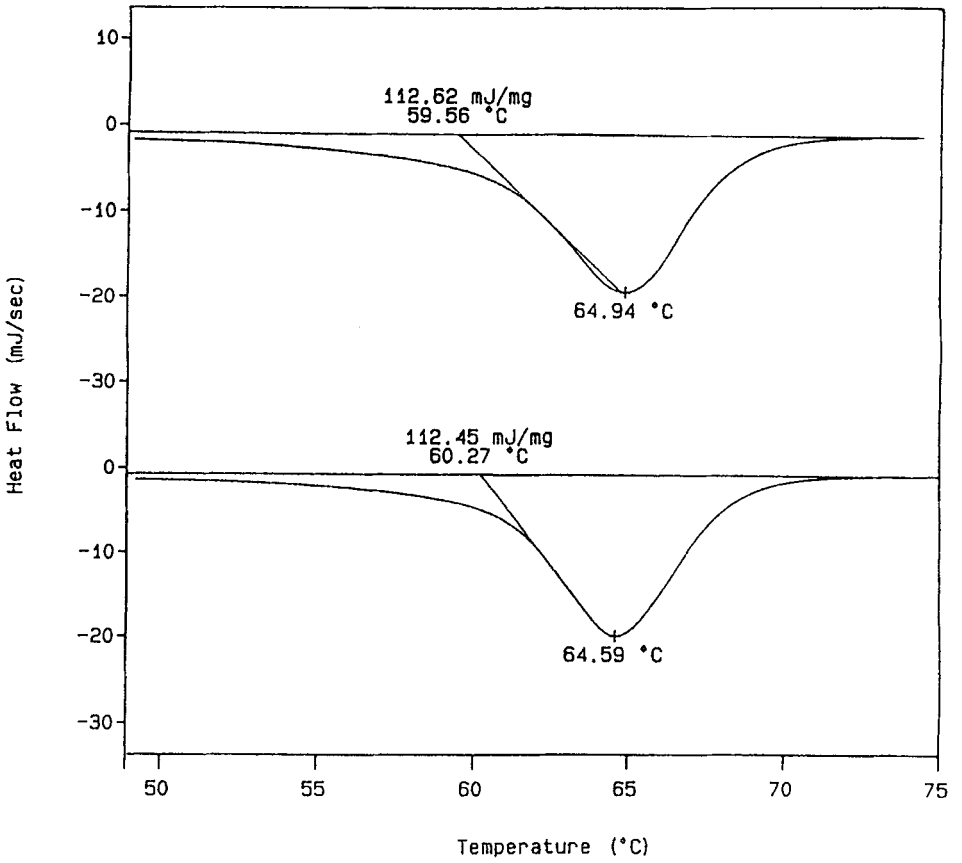

FIG. 5. Differential scanning calorimeter scans of poly(1,4-butyl sebacate) both from sebacic acid (a) and bis(2,2,2-trifluoroethyl) sebacate (b), and 1,4-butanediol (conditions as in Fig. 3).

CONCLUSIONS

We have demonstrated that high molar mass polyesters with characteristics suitable for commercial applications can be produced by lipase-catalyzed polyesterification and polytransesterification of aliphatic diacids or their derivatives, and diols in an organic solvent, with a degree of polymerization as high as 184 and a mass-average molar mass as high as about 46,000 $g \cdot mol^{-1}$. In order to obtain a high degree of polymerization, the water or alcohol formed needs to be removed. The use of lipase as a biocatalyst in polyesterification reactions opens up an interesting alternative for producing biodegradable polymers under mild reaction conditions.

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