

Lipase-Catalyzed Transesterification of Propylene Glycol with Triglyceride in Organic Solvents

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A mixture of emulsifiers consisting of propylene glycol monoesters and mono- and diglycerides was obtained when triglycerides were reacted with propylene glycol (1,2-propanediol) using lipase as a catalyst. These emulsifiers were synthesized enzymatically to overcome the problems associated with chemical processes. Typically, lipase was added to a reaction mixture containing propylene glycol and acyl donors (tripalmitin, tristearin, and triolein) in various organic solvents containing *tert*-butyl alcohol and molecular sieves. The mixture was incubated in an orbital shaker at a speed of 250 rpm at 40 °C. This study showed that among the eight lipases tested the lipase PS from *Pseudomonas cepacia* was the most effective in synthesizing these emulsifiers by a transesterification process. The anhydrous enzyme and hydrophobic organic solvents were favored for the production of propylene glycol monoesters and mono- and diglycerides. The yields of monoesters and mono- and diglycerides were also affected by temperature, pH memory, propylene glycol/triglyceride ratio, reaction time, and immobilized lipase. The yields of propylene glycol monopalmitate could be increased to 74.6% by the addition of molecular sieves and cosolvents. Lipase immobilization increased the catalytic efficiency up to 5-fold.

Keywords: *Emulsifier mixture; lipase; organic solvent; propylene glycol monoester; monoglyceride; diglyceride*

INTRODUCTION

Propylene glycol (1,2-propanediol) monoesters are good water-in-oil emulsifiers, having low hydrophilic–lipophilic balance values (Stutz et al., 1973). Mono- and diglycerides are both hydrophilic and lipophilic; they are partially soluble in both water and fat (Parker, 1987). These emulsifiers have been approved by the U.S. Food and Drug Administration (FDA) for use in foods and pharmaceuticals (Artman, 1975). Normally they are most often employed in the preparation of cakes, cake mixes, margarines, coffee whitener, and bread (Nash and Brinkman, 1972). Propylene glycol monoesters can be used in combination with mono- and diglycerides to obtain excellent cake batter behavior, resulting in increased cake volume and uniform structure. At present, propylene glycol monoesters and mono- and diglycerides are produced commercially by the transesterification of triglycerides with propylene glycol by using an inorganic catalyst at temperatures >220 °C (Basu Roy Choudhury, 1960; Lauridsen, 1976; Sonntag, 1982). The use of high reaction temperatures results in the formation of dark byproducts with off-flavors. Lipase-catalyzed transesterification reactions offer several advantages over chemically catalyzed reactions, such as milder operating conditions, cleaner products, and reduced waste production (Sreenivasan, 1978; Macrae, 1983a).

In the present work, propylene glycol monoesters and mono- and diglycerides were efficiently synthesized by

transesterification with lipase from *Pseudomonas cepacia* (PS) as biocatalysts in organic media. The effects of acyl donors, temperature, organic solvents, water content, immobilized lipase, addition of *tert*-butyl alcohol as cosolvent, pH memory, and addition of molecular sieves were also investigated.

MATERIALS AND METHODS

Materials. Commercially available lipases were obtained as follows: *Aspergillus niger* (AP-6), *Mucor sp.* (MAP-10), *P. cepacia* (PS), *Rhizopus sp.* (N-conc), and *Rhizopus sp.* (FAP-15) from Amano International Enzyme Co. (Nagoya, Japan); *Candida cylindracea* type VII and porcine pancreatic lipase from Sigma Chemical Co. (St. Louis, MO); *Chromobacterium viscosum* (LP-101-S) from Toyo Jozo Co. (Shizuoka, Japan). Dibutyl ether, *n*-hexane, *tert*-amyl alcohol, *tert*-butyl alcohol, α -terpineol, silical gel 60, silical gel 60G, and molecular sieves (3Å) were obtained from Merck Chemical Co. (Darmstadt, Germany). Propylene glycol, tripalmitin, tristearin, triolein, 1-monomyristoyl-*rac*-glycerol, Celite, and glass beads were purchased from Sigma. DEAE-Sephadex A-25, DEAE-Sephadex A-50, Sephadex G-25, and Sephadex G-50 were purchased from Pharmacia Biotech Asia Pacific Ltd. (Hong Kong).

Measurement of Lipase Activity. The hydrolytic activities of lipases were measured according to the method described by Rúa et al. (1993).

Transesterification Reaction. Commercial lipase powder (0.08 g) was added to a reaction mixture (2 mL) containing 25 mM triglyceride and 225 mM propylene glycerol in the mixture solvent of *n*-hexane and *tert*-butyl alcohol (9:1, v/v). The reaction mixture was incubated in an orbital shaker at 250 rpm and 30 °C. To study the effect of water content on the lipase-catalyzed synthesis, the enzyme was vacuum-dried using a Savant Speed Vac concentrator (Savant Instruments, Inc., Farmingdale, NY) under a vacuum of 50 mTorr for 24 h. Water was removed from organic media by addition of 3Å molecular sieves (Merck). To study the pH effect on synthesis,

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Table 1. Propylene Glycol Monoester, Monoglyceride, and Diglyceride Formation by Lipase from Different Sources^a

source	trade name or supplier	enzyme units ^b	yield (mM)		
			PGMO ^c	MG ^d	DG ^e
<i>A. niger</i>	Amano AP-6	45.4			trace
<i>C. cylindracea</i> type VII	Sigma	38.1	3.3 ± 0.1 ^f		94.3 ± 1.8 ^f
<i>C. viscosum</i> LP-101-S	Toyo Jozo	59.8	trace		trace
<i>Mucor</i> sp.	Amano MAP-10	2.3	trace		27.8 ± 0.5 ^f
<i>P. cepacia</i>	Amano PS	46.3	94.5 ± 1.8 ^f	34.0 ± 0.7 ^f	165.6 ± 3.2 ^f
<i>Rhizopus</i> sp.	Amano FAP-15	11.5	trace		trace
<i>Rhizopus</i> sp.	Amano N-conc	3.6			trace
porcine pancreas lipase	Sigma	0.7	trace		trace

^a Experimental conditions: 0.05 g of crude lipase powder, 5839 mM propylene glycol, and 587 mM triolein; the suspension was shaken at 30 °C and 250 rpm for 24 h. ^b Enzyme unit was measured by the hydrolysis of *p*-nitrophenyl butyrate as substrate. One unit of enzyme was defined as the amount of enzyme that produced 1 μmol of *p*-nitrophenol/min. ^c PGMO, propylene glycol monooleate. ^d MG, monoolein. ^e DG, diolein. ^f Mean value ± standard deviation.

Table 2. Kinetics of Lipase-Catalyzed Transesterification of Propylene Glycol with Various Triglycerides at Various Temperatures^a

system	formation of propylene glycol monoester (mM)
PGMP ^b	
10 °C	18.0 ± 0.3 ^c
20 °C	44.0 ± 0.8 ^c
30 °C	65.0 ± 1.3 ^c
40 °C	52.0 ± 1.0 ^c
50 °C	32.0 ± 0.6 ^c
PGMS ^b	
10 °C	18.0 ± 0.3 ^c
20 °C	68.0 ± 1.3 ^c
30 °C	89.0 ± 1.7 ^c
40 °C	81.0 ± 1.6 ^c
50 °C	44.0 ± 0.8 ^c
PGMO ^b	
10 °C	84.0 ± 1.6 ^c
20 °C	68.0 ± 1.3 ^c
30 °C	47.0 ± 0.9 ^c
40 °C	52.0 ± 1.0 ^c
50 °C	42.0 ± 0.8 ^c

^a The PS lipase (0.05 g) was added to a reaction mixture (1 mL) containing 50 mM triglyceride and 500 mM propylene glycol in hexane for 12 h. The reaction temperatures were 10, 20, 30, 40, and 50 °C, respectively. ^b PGMP, PGMS, PGMO: propylene glycol monopalmitate, stearate, and oleate, respectively. ^c Mean value ± standard deviation.

the lipase was dissolved in 6 mL of 10 mM mixed Good's buffer solution of different pH values (10 mM each of BICINE, CAPS, sodium acetate, and BIS-TRIS propane were mixed and adjusted to various pH values by either concentrated HCl or NaOH) and then vacuum-dried as described earlier.

Product Analysis. At various time intervals, 8 μL of the reaction mixture was withdrawn and injected to a gas chromatograph (Hitachi model G-3000; Hitachi, Tokyo, Japan). An Rtx-65TG fused-silica capillary column of 30 m × 0.25 mm i.d. (Restek Corp., Bellefonte, PA) was used. Hydrogen gas was the carrier gas at a flow rate of 1.4 mL/min. The injection port and flame ionization detector temperatures program was as follows: 190 °C, raised at 6 °C/min to 215 °C, raised at 32 °C/min to 360 °C, and 7 min hold. The product compositions (fatty acids, monoglycerides, diglycerides, triglycerides, monoesters and diesters) were quantitated by an integrator with 1-monomyristoyl-*rac*-glycerol as internal standard.

RESULTS AND DISCUSSION

Among the eight commercial lipases tested, nonspecific *P. cepacia* (Amano PS) lipase showed the best catalytic efficiency and specificity for enzymatic synthesis of propylene glycol monoesters and mono- and diglycerides (Table 1). It is interesting to see that *Mucor miehei* lipase IM-20, which was shown to be the best

Table 3. Effect of Propylene Glycol/Tristearin Ratio on the Synthesis of Propylene Glycol Monostearate^a

propylene glycol/tristearin ratio	PGMS ^b (mM)
1	33.0 ± 0.6 ^c
2	41.5 ± 0.8 ^c
5	20.0 ± 0.4 ^c
10	6.5 ± 0.1 ^c

^a The lipase (0.08 g) was added to a reaction mixture (2 mL) containing different ratios of tristearin and propylene glycol in hexane at 30 °C for 4 h. ^b PGMS, propylene glycol monostearate. ^c Mean value ± standard deviation.

Table 4. PS Lipase-Catalyzed Transesterification of Propylene Glycol with Tripalmitin in Various Ratios of *tert*-Butyl Alcohol and *n*-Hexane Mixture at 30 °C^a

system	time (h)	product composition (mM)		
		PGMP ^b	MG ^c	DG ^d
<i>tert</i> -butyl alcohol	24	30.0 ± 0.6 ^e	15.0 ± 0.3 ^e	0.6 ± 0.0 ^e
<i>n</i> -hexane/ <i>tert</i> -butyl alcohol (3:2, v/v)	24	40.0 ± 0.8 ^e	10.0 ± 0.2 ^e	2.5 ± 0.0 ^e
<i>n</i> -hexane/ <i>tert</i> -butyl alcohol (9:1, v/v)	24	56.0 ± 1.1 ^e	5.0 ± 0.1 ^e	3.5 ± 0.1 ^e
<i>n</i> -hexane/ <i>tert</i> -butyl alcohol (49:1, v/v)	46	4.8 ± 0.1 ^e	2.4 ± 0.0 ^e	1.5 ± 0.0 ^e

^a The PS lipase (0.08 g) was added to a reaction mixture (2 mL) containing 25 mM tripalmitin, 225 mM propylene glycol, and 0.0361 g of 3 nm molecular sieves in various ratios of *tert*-butyl alcohol and *n*-hexane mixture. ^b PGMP, propylene glycol monopalmitate. ^c MG, monopalmitin. ^d DG, dipalmitin. ^e Mean value ± standard deviation.

enzyme for propylene glycol monoesters of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Liu and Shaw, 1995), had little activity under the present reaction conditions. This may be due to different fatty acid specificities by the different enzymes. The PS lipase-catalyzed transesterification of propylene glycol with different triglycerides was very efficient. Because the enzyme was insoluble in the reaction mixture, it was easily recovered for reuse. The yields of propylene glycol monooleate (PGMO), monoolein, and diolein were 94.5, 34, and 165.6 mM, respectively, from 587 mM triolein reacting with 5839 mM propylene glycol, catalyzed by 50 mg of lipase at 30 °C for 24 h (Table 1).

The temperature effect is shown in Table 2. The yields of propylene glycol monopalmitate (PGMP) and propylene glycol monostearate (PGMS) increased up to 30 °C for 12 h. However, the optimum reaction temperature was 10 °C for PGMO synthesis for 12 h. Because of the oxidation of the unsaturated fatty acid (oleic acid), it is more desirable to carry out the trans-

Table 5. PS Lipase-Catalyzed Transesterification of Propylene Glycol with Tripalmitin in Various Alcohols and *n*-Hexane Mixtures at 30 °C^a

system	time(h)	product composition (mM)		
		PGMP ^c	MG ^d	DG ^d
<i>n</i> -hexane/ <i>tert</i> -butyl alcohol (9:1, v/v)	3	37.5 ± 0.7 ^e	4.0 ± 0.1 ^e	4.0 ± 0.1 ^e
	11	56.0 ± 1.1 ^e	6.0 ± 0.1 ^e	3.4 ± 0.1 ^e
	24	56.0 ± 1.1 ^e	5.0 ± 0.1 ^e	3.5 ± 0.1 ^e
<i>n</i> -hexane/ <i>tert</i> -amyl alcohol (9:1, v/v)	3	33.0 ± 0.6 ^e	3.8 ± 0.1 ^e	4.6 ± 0.1 ^e
	11	52.0 ± 1.0 ^e	5.5 ± 0.1 ^e	4.0 ± 0.1 ^e
	24	58.0 ± 1.1 ^e	5.0 ± 0.1 ^e	2.4 ± 0.0 ^e
<i>n</i> -hexane/3-methyl-3-pentanol (9:1, v/v)	3	33.5 ± 0.6 ^e	4.1 ± 0.1 ^e	3.5 ± 0.1 ^e
	11	55.0 ± 1.1 ^e	6.5 ± 0.1 ^e	2.8 ± 0.1 ^e
	24	56.5 ± 1.1 ^e	5.5 ± 0.1 ^e	3.2 ± 0.1 ^e
<i>n</i> -hexane/ α -terpineol (9:1, v/v)	3	11.0 ± 0.2 ^e	1.1 ± 0.0 ^e	2.9 ± 0.1 ^e
	11	25.5 ± 0.5 ^e	2.7 ± 0.1 ^e	2.8 ± 0.1 ^e
	24	41.0 ± 0.8 ^e	3.5 ± 0.1 ^e	2.6 ± 0.1 ^e

^a The PS lipase (0.08 g) was added to a reaction mixture (2 mL) containing 25 mM tripalmitin, 225 mM propylene glycol, and 0.0361 g of 3 nm molecular sieves in various organic solvent mixtures. ^b PGMP, propylene glycol monopalmitate. ^c MG, monopalmitin. ^d DG, dipalmitin. ^e Mean value ± standard deviation.

Table 6. PS Lipase-Catalyzed Transesterification of Propylene Glycol with Tripalmitin in Various Organic Mixtures at 30 °C^a

system	time (h)	product composition (mM)		
		PGMP ^b	MG ^c	DG ^d
<i>n</i> -hexane	7	7.5 ± 0.1 ^e	1.7 ± 0.0 ^e	2.8 ± 0.1 ^e
	24	8.5 ± 0.2 ^e	1.5 ± 0.0 ^e	2.8 ± 0.1 ^e
	72	11.0 ± 0.2 ^e	2.2 ± 0.0 ^e	2.6 ± 0.1 ^e
<i>n</i> -hexane/ <i>tert</i> -butyl alcohol (9:1, v/v)	7	45.5 ± 0.9 ^e	7.0 ± 0.1 ^e	6.5 ± 0.1 ^e
	24	56.0 ± 1.1 ^e	5.0 ± 0.1 ^e	3.5 ± 0.1 ^e
	72	54.5 ± 1.0 ^e	5.5 ± 0.1 ^e	2.5 ± 0.0 ^e
<i>n</i> -hexane/dibutyl ether (9:1, v/v)	7	9.5 ± 0.2 ^e	1.1 ± 0.0 ^e	3.9 ± 0.1 ^e
	24	10.5 ± 0.2 ^e	0.9 ± 0.0 ^e	4.6 ± 0.1 ^e
	72	13.0 ± 0.3 ^e	1.1 ± 0.0 ^e	4.7 ± 0.1 ^e

^a The lipase (0.08 g) was added to a reaction mixture (2 mL) containing 25 mM tripalmitin, 225 mM propylene glycol, and 0.0361 g of 3 nm molecular sieves in various organic solvent mixtures. ^b PGMP, propylene glycol monopalmitate. ^c MG, monopalmitin. ^d DG, dipalmitin. ^e Mean value ± standard deviation.

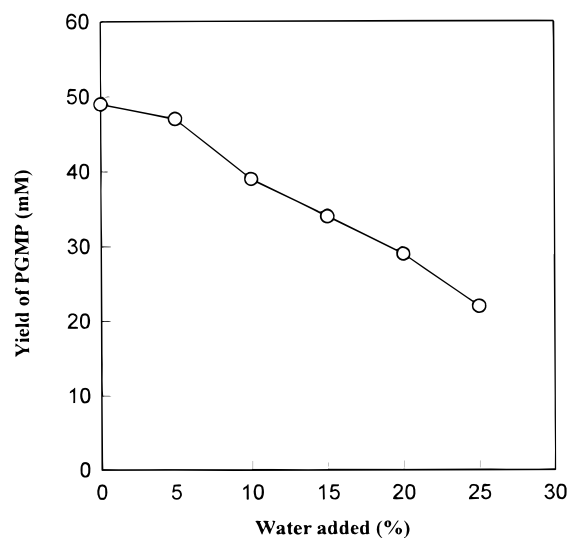
Table 7. Effect of Carriers on Transesterification of Propylene Glycol with Tripalmitin by Immobilized PS Lipase^a

carrier	product composition (mM)		
	PGMP ^b	MG ^c	DG ^d
silica 60	56.0 ± 1.1 ^e	1.4 ± 0.0 ^e	0.8 ± 0.0 ^e
silica 60G	64.5 ± 1.2 ^e	2.4 ± 0.1 ^e	1.1 ± 0.0 ^e
DEAE-Sephadex A-25	0.8 ± 0.0 ^e	0.0 ± 0.0 ^e	0.6 ± 0.0 ^e
DEAE-Sephadex A-50	0.8 ± 0.0 ^e	0.0 ± 0.0 ^e	0.3 ± 0.0 ^e
glass beads	57.5 ± 1.1 ^e	7.5 ± 0.1 ^e	2.6 ± 0.1 ^e
Celite	60.5 ± 1.2 ^e	5.5 ± 0.1 ^e	1.8 ± 0.0 ^e
Sephadex G-25	38.0 ± 0.7 ^e	4.2 ± 0.1 ^e	4.1 ± 0.1 ^e
Sephadex G-50	10.0 ± 0.2 ^e	0.5 ± 0.0 ^e	2.7 ± 0.1 ^e

^a Lipase was immobilized on various carriers as described under Materials and Methods. Reaction conditions: 0.24 g of immobilized enzyme, 25 mM tripalmitin, 225 mM propylene glycol, and 0.0361 g of 3 nm molecular sieves in 2 mL mixture solvent of hexane and *tert*-butyl alcohol (9:1, v/v), shaken at 250 rpm for 20 h at 30 °C. ^b PGMP, propylene glycol monopalmitate. ^c MG, monopalmitin. ^d DG, dipalmitin. ^e Mean value ± standard deviation.

esterification reaction at low temperature. In other words, this is due to the different substrate specificity of enzyme.

The ratio of propylene glycol and tristearin was varied from 1:1 to 1:10 (Table 3). The yields of PGMS increased as the ratio of propylene glycol/tristearin increased up to 2. Higher ratios decreased the yields of PGMS. However, the selective synthesis of mono- and

**Figure 1.** Effect of water on the synthesis of PGMP by PS lipase. The lipase (0.08 g) was added to a reaction mixture (2 mL) containing 25 mM tripalmitin and 225 mM propylene glycol in the mixture solvent of *n*-hexane and *tert*-butyl alcohol (9:1, v/v) with various amounts of added water at 30 °C for 20 h. PGMP, propylene glycol monopalmitate.

diesters of propylene glycol increased when the propylene glycol was in excess.

Organic solvents greatly affected the yields of monoester and mono- and diglycerides. This could be due to conformational changes in the enzymes. Enzymes suspended in organic solvents have been reported to result in alteration of substrate specificity and affinity of substrates for enzymes (Dordick, 1989). Table 4 showed the effect of *n*-hexane/*tert*-butyl alcohol ratio on the synthesis of propylene glycol monoester. It appeared that the yield of PGMP was greatest in an *n*-hexane/*tert*-butyl alcohol ratio of 9:1 than in pure *n*-hexane. The effect of *tert*-butyl alcohol could be due to the change of enzyme specificity, which can lead to selective accumulation of monoester. For propylene glycol monoester and mono- and diglyceride synthesis by lipase PS, a higher extent of transesterification was observed in *tert*-butyl alcohol, *tert*-amyl alcohol, and 3-methyl-3-pentanol as compared to α -terpineol (Table 5). The composition of the mixed organic solvent also affected the yields of propylene glycol monoester and mono- and diglyceride. Organic solvents produce various physicochemical effects on enzyme molecules, and the effects differ depending upon the kind of organic solvents. As shown in Table 6, cosolvent *n*-hexane was the best for propylene glycol monoester and mono- and diglyceride production.

The effects of immobilized catalysts on the transesterification on eight carriers were investigated (Table 7). The immobilization of Amano PS lipase greatly increased the catalytic efficiency up to 5-fold compared to nonimmobilized enzyme. For lipase PS immobilized carriers, silica 60G and Celite were the best. This could be due to a better distribution of the immobilized enzyme that made the transesterification reaction more efficient.

The effect of water content on the synthesis of PGMP is illustrated in Figure 1. Apparently, lipase PS performs best under anhydrous conditions for the transesterification of propylene glycol with tripalmitin. This is consistent with our previous observation that *M. miehei* lipase performed best under lyophilized condi-

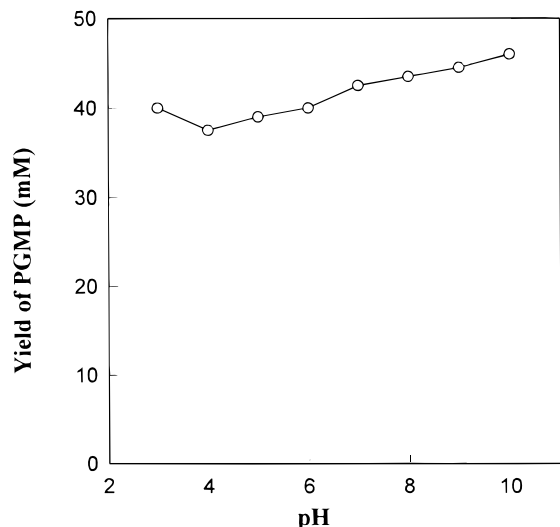


Figure 2. Dependence of PGMP formation on the pH of the aqueous solution from which the PS lipase was lyophilized. Experimental conditions: 0.08 g of the lipase was added to a reaction mixture (2 mL) containing 25 mM tripalmitin and 225 mM propylene glycol in the mixture solvent of *n*-hexane and *tert*-butyl alcohol (9:1, v/v); the suspension was shaken at 30 °C and 250 rpm for 20 h. PGMP, propylene glycol monopalmitate.

tions for the esterification of propylene glycol with either EPA or DHA (Liu and Shaw, 1995) or stearic acid (Shaw and Lo, 1994). Okumura et al. (1979) found that the yield of propylene glycol oleate was poor by lipase-catalyzed reaction in phosphate buffer (high water content), and Berger et al. (1992) also reported that high yields of diol esters were obtained from lipase-catalyzed esterification of silica gel-adsorbed diols with vinyl esters in anhydrous nonpolar organic solvent.

The yield of monoester was also affected by the pH of the aqueous solution from which the lipase was vacuum-dried, a phenomenon named pH memory by Klibanov (1989). As shown in Figure 2, the enzyme was active even at high pH for the lyophilized lipase-catalyzed synthesis of PGMP. This possibly reflected the fact that changes in enzyme conformation resulting from pH changes affected the transesterification reaction of propylene glycol with tripalmitin. From the above results it can be concluded that yield of propylene glycol monoesters and mono- and diglycerides may be easily synthesized by the lipase-catalyzed transesterification of triglyceride from fat or oils and propylene glycol. The

composition of the emulsifiers mixture can be controlled by various reaction conditions.

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