Lipase-Catalyzed Esterification of Glycerol and Oleic Acid

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ABSTRACT: The enzymatic synthesis of glycerides from glycerol and oleic acid in organic solvent was studied, and the optimal conditions for glyceride synthesis by lipases were established. Of the commercially available lipases that were investigated, *Candida rugosa* lipase and porcine pancreas lipase resulted in the highest extent of esterification. Iso-octane and hexane were particularly useful organic solvents in glyceride synthesis. The water content in the reaction mixture was of primary importance. For *C. rugosa* lipase and porcine pancreas lipase, the optimal water contents were 5 and 1%, respectively. *Candida rugosa* lipase and porcine pancreas lipase manifested contrasting positional specificities in glyceride synthesis.

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KEY WORDS: Esterification, lipase, organic solvent.

Esters of glycerol with FA occur naturally in fats and oils, and they are widely used for edible and industrial purposes. The modification of fats and oils and the production of glycerides from glycerol and FA promise the development of new oil and fat compounds that possess new properties in comparison with the original materials. Conventional esterification of glycerol to produce MG, DG, and TG by chemical catalysts requires high temperatures and leads to dark-colored products and undesired by-products (1).

Using enzymes to catalyze these reactions is superior to using conventional chemical methods owing to their mild reaction conditions, high catalytic efficiency, and inherent selectivity, which result in much purer products. The use of lipolytic enzymes to catalyze the esterification reaction has been investigated by many workers (2–8). Lipases are present in high activity in the reserve tissue of many oilseed plants (9). Some lipases catalyze only the hydrolysis reaction of fats and oils, whereas others show catalytic activity on both the hydrolysis and esterification reactions.

In this study, the synthesis of glycerides from glycerol and oleic acid in organic solvent by lipases was studied, and the effects of process parameters such as organic solvents, water content, glycerol content, and temperature were investigated.

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EXPERIMENTAL PROCEDURES

Materials. Nonspecific *Candida rugosa* lipase and 1,3-positional specific porcine pancreas lipase (PPL), oleic acid, and glycerol were purchased from Sigma Chemical Co. (St. Louis, MO). All solvents used in this work were of analytical grade and were obtained from Merck (Darmstadt, Germany).

Esterification reaction. Reaction mixtures for glyceride synthesis from glycerol and oleic acid consisted of: glycerol, 2 g (about 20 mmol); oleic acid, 0.5 g (about 2 mmol); water, 150 μL; and *n*-hexane, 3 mL. Reaction mixtures were placed in 50-mL Erlenmeyer flasks with silicone-capped stoppers to prevent evaporation of the reactants. The reaction was started by the addition of 0.1 g lipase in the form of dry powder. The suspension that resulted was agitated on an orbital shaker at 200 rpm at 30°C. At various times during incubation, 0.2 mL of the reaction mixture was withdrawn and analyzed by TLC and GC.

Estimation of the degree of synthesis. The reaction was stopped by addition of 20 mL of an acetone/ethanol mixture (1:1, vol/vol), and FFA was titrated with 0.1 N NaOH. The degree of synthesis (%) represents the percentage of initial FA consumed in the reaction mixture.

Identification of reaction products. Reaction products were extracted from each reaction mixture with diethyl ether and identified by TLC. A boric acid-impregnated silica gel plate was prepared and developed in chloroform/acetone/methanol (95:4.5:0.5, by vol). Spots of each lipid were visualized by spraying the plate with a 12.5% (wt/vol) ethanol solution of phosphomolybdic acid (Sigma Chemical Co.) and heating it. Fractions corresponding to each lipid type were scraped from the plates and derivatized for GC analysis.

GC analysis. Each lipid class separated by TLC was methylated. The methyl esters of FA were dissolved in *n*-hexane for analysis. Analysis was performed in a gas chromatograph (Shimadzu model 14A; Shimadzu, Tokyo, Japan). The chromatograph was fitted with a J&W Scientific (Folsom, CA) DB-5ht column (15 m × 0.25 mm i.d. × 0.1 mm film thickness) and an ionization detector. Helium was chosen as a carrier gas at a flow rate of 38 mL/min. The injection port and FID temperatures were both 250°C, and the column temperature was 210°C. The amounts of product formed were determined by comparison with internal standards.

RESULTS AND DISCUSSION

Effects of organic solvent on the glyceride synthetic reaction. To carry out bioconversions of lipophilic compounds effectively, it is essential to introduce organic solvents into the reaction systems. The use of organic solvents can improve the poor solubility in water of substrates or other reaction components of a hydrophobic nature. Organic solvents produce various physicochemical effects on enzyme molecules, and the effects differ depending on the kinds of organic solvents and enzymes used. Conformational changes in enzymes, when suspended in organic solvents, have been reported to result in alteration of substrate specificity and the affinity of substrates for enzymes (10,11). To select the most suitable solvent for the glyceride synthetic reaction systems, the effect of organic solvents on the catalytic activity of lipase for the esterification was examined (Table 1). For ester synthesis by *C. rugosa*, a higher extent of esterification was observed in iso-octane and hexane as compared with other solvents. With PPL, high activities (82.3–90.7% synthesis) were observed in heptane, hexane, and iso-octane. More polar solvents, such as benzene, chloroform, and acetone, were found to be unsuitable for the synthetic reaction.

Effects of initial water content on glyceride synthesis by lipase. Lipase-catalyzed reactions are reversible and governed by the water content of the reaction mixture. In the esterification reaction, the content of water in the reaction mixture affects the reaction because water is one of the reaction products. A small amount of water is needed to maintain enzyme activity. However, at higher initial water contents, the degree of synthesis gradually declined. Thus, we investigated the effects of the initial water content on glyceride synthesis by lipases.

The time courses of glyceride synthesis by *C. rugosa* lipase and PPL in hexane containing different initial moisture contents are illustrated in Figures 1 and 2. With a moisture content of 5% (vol/vol) in the reaction mixture, *C. rugosa* lipase showed a maximum glyceride synthesis of about 81%. As expected, the percentage of conversion decreased as the initial water content increased. The elimination of water from the reaction mixture derived from the esterification was attempted by the addition of molecular sieve (4A) pellets as dehydrating agents to obtain a higher conversion rate. As shown in Figure 1, the conversion reached 92% by the addition of 1.0 g of molecular sieve. With a moisture content of 1% (vol/vol) in the reaction mixture, a maximum synthesis of 88% was obtained for PPL.

TABLE 1
Activity of the Lipase in Organic Solvent–Aqueous Phase Reactions

	Degree of synthesis (%)	
Organic solvent	*Candida rugosa* lipase	Porcine pancreas lipase
Hexane	82.5	85.8
Heptane	75.3	82.3
Iso-octane	85.7	90.7
Decane	60.4	58.6
Benzene	20.1	10.3
Chloroform	22.8	32.5
Acetone	31.9	0.0

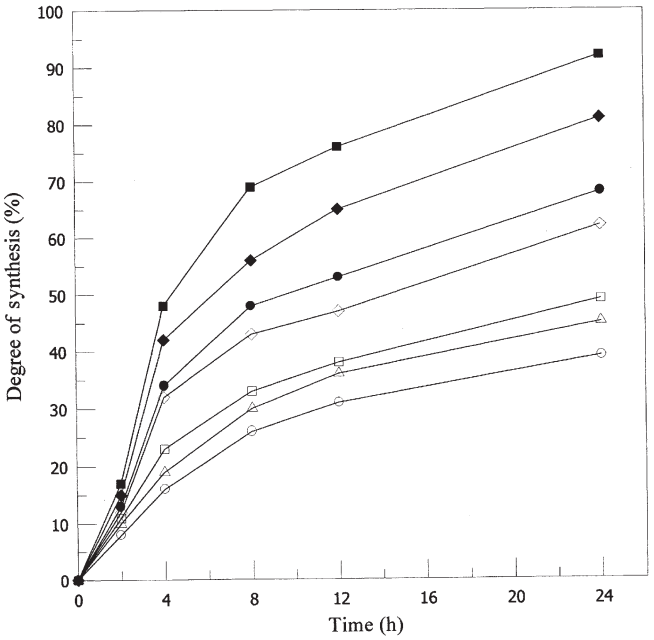

FIG. 1. Effects of water content on glyceride synthesis by *Candida rugosa* lipase. The reaction mixture contained 2.0 g glycerol, 0.5 g oleic acid, 3.0 mL *n*-hexane, and various amounts of water. The amount of enzyme used was 0.1 g. The reaction was carried out at 30°C. (◇) 0% H_2O; (○) 1% H_2O; (●) 2.5% H_2O; (◆) 5% H_2O; (■) 5% H_2O + molecular sieve; (□) 10% H_2O; and (△) 20% H_2O.

Effects of glycerol content on glyceride synthesis by lipase. The effects of glycerol content in the reaction mixture on

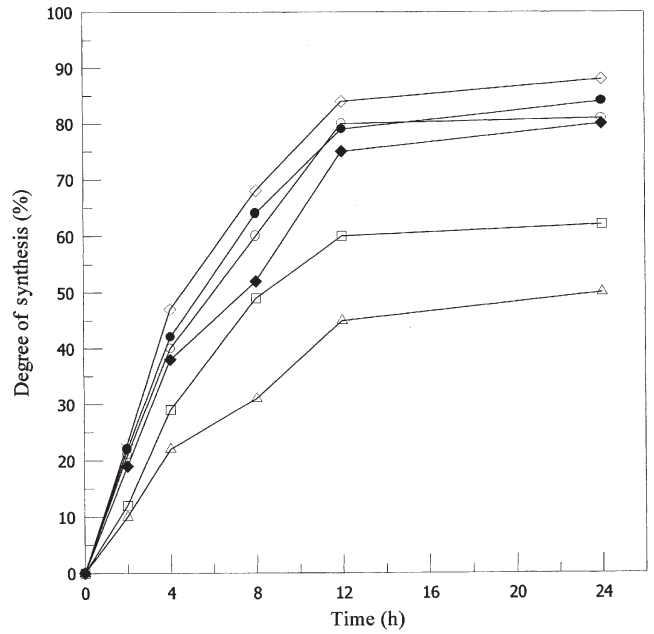

FIG. 2. Effects of water content on glyceride synthesis by porcine pancreas lipase. The reaction mixture contained 2.0 g glycerol, 0.5 g oleic acid, 3.0 mL *n*-hexane, and various amounts of water (from 0 to 20% w/w). The amount of enzyme used was 0.1 g. The reaction was carried out at 30°C. Key as in Figure 1.

TABLE 2
Effects of Glycerol Content on Esterification by Lipases

Glycerol (g)	Degree of synthesis (%)	
	Candida rugosa lipase	Porcine pancreas lipase
0.25	13.5	27.3
0.50	56.2	63.8
1.0	80.3	85.4
2.0	85.5	88.2
5.0	85.0	89.6

TABLE 3
Effects of Temperature on Esterification by Lipases

Temperature (°C)	Degree of synthesis (%)	
	Candida rugosa lipase	Porcine pancreas lipase
0	15.3	9.3
10	21.4	18.1
20	54.5	59.7
30	82.5	85.8
40	72.4	88.2
50	23.6	41.0

esterification by the two lipases were investigated (Table 2). Glyceride synthesis increased with increasing glycerol content. For *C. rugosa* lipase and PPL, glycerol contents of 2 and 5 g were optimal. For PPL, the reaction resulted in a glyceride synthesis of 89.6%.

Effects of temperature on esterification by lipase. The optimal temperature for the glyceride synthetic reaction by *C. rugosa* lipase was 30°C (Table 3). The degree of esterification increased with temperature in the range of 0–40°C with PPL. The optimal temperature for the latter enzyme was 40°C.

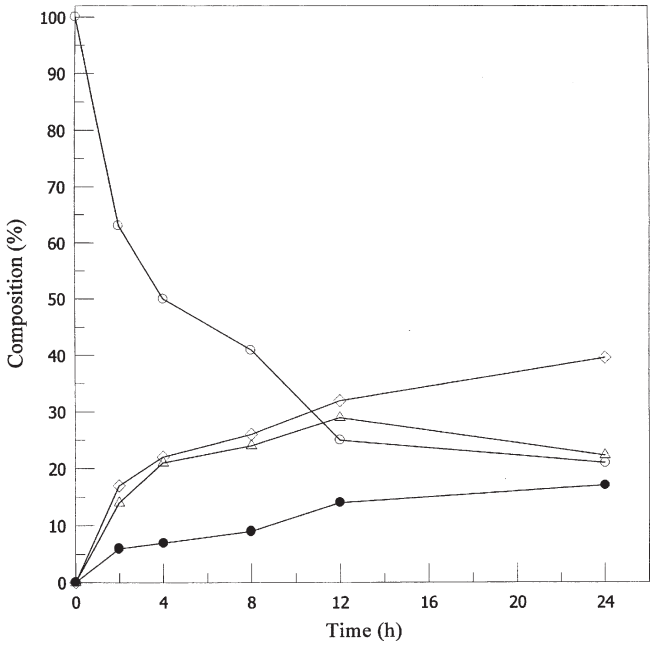

FIG. 3. Composition of glycerides synthesized by *C. rugosa*. The reaction mixture contained 2.0 g glycerol, 0.5 g oleic acid, 3.0 mL *n*-hexane, and various amounts of water (from 0 to 20%, w/w). The amount of enzyme used was 0.1 g. The reaction was carried out at 30°C. (△) MG; (△) DG; (●) TG; (○) oleic acid.

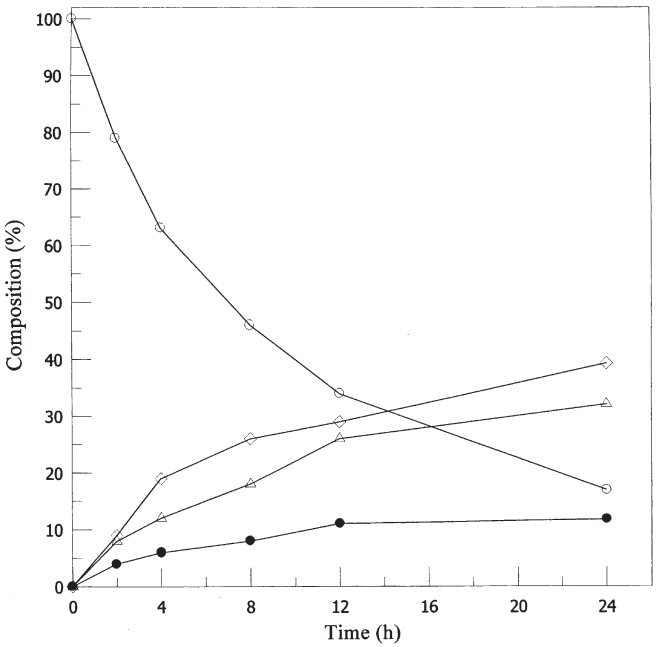

FIG. 4. Composition of glycerides synthesized by porcine pancreas lipase. The reaction mixture contained 2.0 g glycerol, 0.5 g of oleic acid, 3.0 mL *n*-hexane, and various amounts of water (from 0 to 20%, w/w). The amount of enzyme used was 0.1 g. The reaction was carried out at 30°C. (△) MG; (△) DG; (●) TG; (○) oleic acid.

Composition of glycerides synthesized by lipases. Changes in the composition of lipid in the *n*-hexane mixture during the course of the esterification reaction by lipases are detailed in Figures 3 and 4. Initially, almost equimolar MG and DG were produced as FA decreased. The formation of TG was much lower than that of MG and DG. For *C. rugosa* lipase, similar amounts of MG and DG were observed during the first 12 h of the enzyme reaction. At 24 h, the concentration of MG, DG, and TG reached 22.3, 39.6, and 17.1%, respectively, for *C. rugosa* lipase. For PPL, at 24 h, the concentration of MG, DG, and TG reached 32.1, 39.3, and 11.7%, respectively. PPL is 1,3-specific, and production of TG is likely to have resulted from acyl migration to the 2-position in 1- or 3-MG or 1,3-DG, followed by further enzymatic esterification.

From the above results, it can be concluded that glycerides from glycerol and oleic acid may be synthesized easily. MG and DG are the major products formed, and *C. rugosa* lipase was different from PPL with respect to the positional specificity in glyceride synthesis.

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