Interesterification Kinetics of Triglycerides and Fatty Acids with Modified Lipase in *n*-Hexane

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ABSTRACT: The kinetics of lipase-catalyzed interesterification of triglycerides and fatty acids in organic media was studied. First, the lipase Saiken 100, *Rhizopus japonicus,* was modified by surfactant to form an enzyme precipitate in aqueous solution, which was well dispersed in organic solvents. This modified lipase catalyzed the interesterification of tripalmitin and stearic acid. The enzyme has 1,3-positional specificity and does not distinguish between stearic and palmitic acids. The kinetic model developed to describe the interesterification reaction system is based on mass balance of two consecutive second-order reversible reactions. The reaction rate constant, k, was determined by solving the differential rate equations of the reaction system and by expressing the value of k as a function of concentrations of the substrates with time. The model gave satisfactory results. The best value of the specific reaction rate constant k* that fits all experimental data was $1.2 \cdot 10^{-5}$ [L^2/(mmol • mg biocatalyst • h)] under the reaction conditions in this study.

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KEY WORDS: Acidolysis, biocatalyst, fatty acids, interesterification kinetics, modified lipase, palmitic acid, reaction rate constant, stearic acid, triglycerides.

Interesterification is a process that is used to modify the structure and composition of oils and fats to improve their physical and nutritional properties. In this process, a triglyceride reacts with a fatty acid (acidolysis), an alcohol (alcoholysis), or an ester (transesterification) to produce a new triglyceride mixture (1,2). Conventionally, the interesterification reaction is promoted by sodium metal or sodium alkoxide to catalyze acyl migration between triglyceride molecules and to produce randomly distributed fatty acyl residues among the triglyceride molecules (3). In recent years, the use of lipases as biocatalysts for interesterification reactions has become of great industrial interest for the production of useful triglyceride mixtures. This is mainly because of the specificity of these enzymes with respect to glyceride positions and fatty acid types. For example, highly valued specialty fats, such as cocoa butter and human milk fat substitutes, can be obtained by exploiting lipases with 1,3-positional specificity (4–6).

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Normally, water concentration is of crucial importance in determining the chemical equilibrium of reactions performed in organic solvent systems. High water concentration in the reaction system shifts the chemical equilibrium toward hydrolysis, and reduced water concentration shifts the equilibrium toward interesterification. The optimal concentration of water is lowered so that the occurrence of undesirable hydrolysis products, such as di- and monoglycerides and glycerol, is minimized, but is sufficiently high that the enzyme retains its activity. Water concentration requirements for different enzymes vary considerably, and, typically, for interesterification reactions of triglycerides and fatty acids, 1–4% supplemented water is required (7–10).

Although a tremendous number of quantitative experimental data dealing with lipase-catalyzed interesterification of triglycerides and fatty acids has recently appeared in the literature, little has been reported on the kinetics of the reaction system. However, several kinetic models, based on different mechanisms, have been proposed to describe the degree of conversion of substrate in the reaction system (11,12). These models involve two to six parameters, depending on the reaction mechanism proposed. These parameters were determined by fitting the calculated results of each model with the experimental data by a trial-and-error method. Another model to analyze the kinetics of the interesterification reaction of triglycerides and fatty acids that has appeared in the literature is based on a two-step reaction mechanism—initial hydrolysis and subsequent re-esterification (13). This model is based on a proposed Ping-Pong Bi-Bi enzymatic mechanism, and it consists of five parameters for the hydrolysis reaction and nine parameters for the re-esterification reaction. The lipases used in earlier kinetic studies were either crude, dried cells, or powdered enzymes mixed with stabilizers. Mass transfer limitations had to be considered in the first study but were neglected in the second (14).

In this paper, the lipase-catalyzed interesterification of tripalmitin (PPP) and stearic acid in *n*-hexane was chosen as a model system. To improve the dispersibility of lipase in the organic system, the enzyme was modified by a surfactant to form an enzyme precipitate in water. This surfactant-modified lipase, prepared according to our previous study, catalyzed predominantly the interesterification reaction under low-water content conditions (15–18). For the sake of simpli-

fication, a simple kinetic model with only one parameter was developed. This model is based on a material balance involving three consecutive second-order reversible reactions.

MATERIALS AND METHODS

Materials. Crude lipase Saiken 100, *Rhizopus japonicus*, was purchased from Nagase Biochemicals Ltd. (Osaka, Japan). The enzyme preparation contained 24.5 wt% protein (determined by a model FP-428, Nitrogen Analyzer; Leco Corporation, St. Joseph, MI) and 75 wt% lactose [determined by high-performance liquid chromatography (HPLC)] as a stabilizer. PPP, di- and monopalmitin, tristearin (SSS), di- and monostearin, 1-palmitoyl-3-stearoyl glycerol, and tripentadecanoin, all with better than 99% purity, were purchased from Sigma (St. Louis, MO). 1,2-Dipalmitoyl-3-stearoyl glycerol (PPS) and 1,3-distearoyl-2-palmitoyl glycerol (SPS), both with better than 98% purity, were a gift from Unilever, Colworth Laboratory (Colworth, England). Stearic acid, palmitic acid, and glycerol with more than 99% purity were purchased from Wako Pure Chemical Ind. (Tokyo, Japan). Ethanol, *n*-hexane, pyridine, acetone, acetonitrile, *tris*(hydroxymethyl)aminomethane, and N,O-*bis*(trimethylsilyl)trifluoroacetamide (BSTFA), all of analytical-grade, were also obtained from Wako Pure Chemical Ind. Trimethyl chlorosilane was obtained from GL Sciences, Inc. (Tokyo, Japan), and sorbitan monostearate from Kao Chemicals (Tokyo, Japan).

Enzyme modification. The enzyme was lipophilized according to a method reported in our previous work (15). Crude lipase Saiken 100 (3 g) was dissolved in 1 L *tris*(hydroxymethyl)aminomethane buffer (pH 5) and stirred magnetically at 4°C [the buffer solution was prepared by dissolving 0.61 g *tris*(hydroxymethyl)aminomethane in 1 L deionized water, and the pH was adjusted to 5 by adding appropriately 3 M HCl solution]. Sorbitan monostearate (0.75 g) dissolved in 20 mL ethanol was added dropwise to the stirred enzyme solution. The mixture was sonicated for 15 min, and the precipitate was collected by centrifugation, frozen at −20°C, and then freeze-dried at below 1 Torr pressure and below −20°C temperature in a Kyowa RL-20NA (Kyowa Shinku Co., Ltd., Tokyo, Japan) freeze dryer. The solid collected after freeze drying (*ca.* 0.75 g) has a yellowish color. The protein content in the resultant modified lipase was determined to be 11 wt% with a Nitrogen Analyzer (model FP-428).

Interesterification reaction. The interesterification reaction of PPP and stearic acid catalyzed by modified lipase was performed in a 60-mL glass flask with Teflon-faced septa. Reactions were initiated by adding 30 mg of modified lipase into 55 mL *n*-hexane that contained 0.55 g PPP (12.4 mmol/L) and 0.55 g stearic acid (35.1 mmol/L). The water content in the reaction system, after addition of the substrates and the modified lipase in *n*-hexane dried over molecular sieves, was about 25 mg/L. The temperature of the reaction system was controlled at 40°C by immersion in a water bath. The reaction system was magnetically stirred at 800 rpm. Samples (0.5 mL) were periodically withdrawn from the reaction system and filtered through disposable Millipore filters (pore size 0.5 μm). All experiments, unless stated otherwise, were carried out under these conditions.

Analytical methods. The course of the interesterification reaction was followed by determining the variation of the concentrations of substrates and all possible products with time. Samples (0.5 mL) were withdrawn from the reaction solutions, filtered as described, placed in screw-cap vials, and the *n*-hexane was evaporated by a stream of N_2 gas at 60°C. After evaporation of *n*-hexane, the samples were treated according to whether they were to be analyzed by gas chromatography (GC) or HPLC.

For GC analysis, the *n*-hexane-free samples were mixed with 1 mL of internal standard solution (6.6 mM *n*-hexadecane in pyridine), 0.2 mL BSTFA, and 0.1 mL trimethyl chlorosilane (TMS). These screw-cap vials were kept in a water bath at 70°C for 30 min to allow silylation of the carboxyl and hydroxyl groups. To determine the concentrations of fatty acids, mono- and diglycerides, glycero, and triglycerides, the samples (after cooling to room temperature) were analyzed in a GC-14AH (Shimadzu, Kyoto, Japan) equipped with a flame-ionization detector. A capillary column (0.25 mm internal diameter, 25 m length and 0.1 μm film thickness; Shinwa Chemical Industries Ltd., Tokyo, Japan) was used. The flow rate of helium carrier gas was 5 mL/min, and pressures of nitrogen and air were 0.6 and 0.5 kg/cm^2, respectively. The injector and detector were maintained at 370 and 400°C, respectively. The column was programmed with an initial temperature of 80°C, which was heated to a final temperature of 360°C at a heating rate of 10°C/min. Then the column was kept at 360°C for 5 min to elute all triglycerides. A split injection ratio (1:10) was used.

For HPLC analysis, the *n*-hexane-free samples were mixed with 1 mL of internal standard solution (10 mg tripentadecanoin dissolved in 10 mL pyridine) and then analyzed by HPLC (model 600E; Waters, Milford, MA; equipped with a Mass Detector, model 750/14; ACS, Macclesfield, England). The column used to separate triglycerides was LiChrospher 100, RP-18 (5 μm) (Cica-Merck, Tokyo, Japan). The mobile phase was a gradient mixture of acetone and acetonitrile. The gradient set point and solvent compositions are given in Table 1. Both analysis results, obtained by HPLC and GC, were in a good agreement.

Water content assay. Water contents in the reaction system were measured with a Karl Fisher Titrator 684 KF (Coulometer; Metrohm, Herisau, Switzerland). After being freeze-dried, the modified lipase contained 0.041 mg H_2O/(mg biocatalyst). The water content in the *n*-hexane was reduced to 10 mg/L by drying over molecular sieves [300 g molecular sieves/(5 L *n*-hexane)]. All substrates were desiccated below 0°C.

Kinetic modeling. The interesterification reaction of PPP and stearic acid, catalyzed by the modified lipase with 1,3-positional specificity, can be analyzed as a homogenous reaction system that consists of two consecutive interesterification reactions and also as one concurrent transesterification reaction. In the first step, stearic acid substitutes for the palmi-

TABLE 1
Solvent Gradient for High-Performance Liquid Chromatography Analysis[a]

Time (min)	Solvent composition[b] A%	B%	Comment
0	55	45	Inject
3	55	45	
11	95	5	
12	55	45	Regenerating column
17	55	45	Next injection

[a]Flow rate was set at 2 mL/min, and the column temperature was kept at 30°C.
[b]A, acetone and B, acetonitrile.

tate residue on the *sn*-1 or *sn*-3 position of the glycerol moiety to form 1,2-dipalmitoyl-3-stearoyl glycerol (PPS) (Eq. 1). In the second step, stearic acid replaces the palmitate residue in the *sn*-1 position of the PPS to produce SPS (Eq. 2). Palmitic acid is released in both steps. A transesterification reaction may also occur between PPP and SPS to produce PPS (Eq. 3).

To describe a simple mathematical model for the aforementioned interesterification reaction system, the following assumptions were adopted and later were experimentally verified: (i) The lipase used has 1,3-positional specificity, and therefore SSS is not produced in the reaction system. (ii) Equations 1, 2, and 3 (below) are reversible reactions. The enzyme used in this study has no fatty acid specificity; it acts concurrently on any acyl group. (iii) There are no losses of enzyme activity during the course of the reaction. Steady-state is achieved after some time, and there is no further change in the compositions of the system after addition of more biocatalyst. (iv) Formation of diglycerides (around 6 wt% of initial concentration of triglycerides) was not taken into account for the sake of simplicity of the model. Monoglycerides and glycerol were not detected under the reaction conditions employed for the kinetic study. (v) Mass transfer limitations in the reaction system were ruled out because of the homogeneity of the system when the lipophilized lipase was used.

The scheme of the interesterification reaction of PPP and stearic acid can be shown as follows:

$$\mathrm{PPP + S} \underset{k}{\overset{2k}{\rightleftarrows}} \mathrm{PPS + P} \qquad [1]$$

$$\mathrm{PPS + S} \underset{2k}{\overset{k}{\rightleftarrows}} \mathrm{SPS + P} \qquad [2]$$

$$\mathrm{PPP + SPS} \underset{k^0}{\overset{4k^0}{\rightleftarrows}} \mathrm{2PPS} \qquad [3]$$

where k represents the interesterification reaction rate constants, k^0 represents the transesterification reaction rate constant, S is stearic acid, and P is palmitic acid. If the biocatalyst used has no specificity toward either stearic or palmitic acid residues, then the production rate of PPP from PPS and P in the reverse reaction of Equation 1 is similar to that of SPS from PPS and S in Equation 2. According to the law of probability, the disappearance rates of the symmetrical substrates, PPP and SPS, have rate constants whose values are twice those for the nonsymmetrical substrate (PPS). A similar argument can be applied for Equation 3.

Rate equations for S, P, PPP, PPS, and SPS in the reaction system can be derived from Equations 1, 2, and 3 as follows:

$$\frac{d[S]}{dt} = -2k[PPP][S] + k[PPS][P] - k[PPS][S] + 2k[SPS][P] \qquad [4]$$

$$\frac{d[P]}{dt} = 2k[PPP][S] - k[PPS][P] + k[PPS][S] - 2k[SPS][P] \qquad [5]$$

$$\frac{d[PPP]}{dt} = -2k[PPP][S] + k[PPS][P] + k^0\left([PPS]^2 - 4[PPP][SPS]\right) \qquad [6]$$

$$\frac{d[PPS]}{dt} = -k[PPS][P] + 2k[PPP][S] - k[PPS][S] + 2k[SPS][P] + k^0\left(4[PPP][SPS] - [PPS]^2\right) \qquad [7]$$

$$\frac{d[SPS]}{dt} = -2k[SPS][P] + k[PPS][S] + k^0\left([PPS]^2 - 4[PPP][SPS]\right) \qquad [8]$$

The total concentration of triglycerides, T, is constant during the interesterification reaction because hydrolysis reactions are assumed to be negligible. Equations 4–8 can be solved analytically under the initial conditions (t = 0) [PPP] = T, [PPS] = 0, [SPS] = 0, [S] = S_0, and [P] = 0, where S_0 is the initial concentration of stearic acid. The concentration change of each component as a function of other concentrations measured in the reaction system with time can be expressed as indicated in Equations 9–13 (see Appendix):

$$[S] = \frac{S_0}{S^*}\left\{S_0 + 2T\exp(-kS^*t)\right\} \qquad [9]$$

$$[P] = \frac{2TS_0}{S^*}\left\{1 - \exp(-kS^*t)\right\} \qquad [10]$$

$$[PPP] = \frac{T}{S^{*2}}\left\{4T^2 + 4TS_0\exp(-kS^*t) + S_0^2\exp(-2kS^*t)\right\} \qquad [11]$$

$$[PPS] = \frac{T}{S^{*2}}\left\{4TS_0 + 2S_0(S_0 - 2T)\exp(-2kS^*t) - 2S_0^2\exp(-2kS^*t)\right\} \qquad [12]$$

$$[SPS] = \frac{TS_0^2}{S^{*2}}\left\{1 - 2\exp(-2kS^*t) + \exp(-2kS^*t)\right\} \qquad [13]$$

where $S^* = S_0 + 2T$.

Equations 9 and 10 can be rearranged for k to give Equations 14 and 15:

$$k = \frac{1}{t}\cdot\frac{-1}{S^*}\ln\left(\frac{[S](S^*) - S_0^2}{2TS_0}\right) \qquad [14]$$

$$k = \frac{1}{t}\cdot\frac{-1}{S^*}\ln\left(\frac{2TS_0 - S^*[P]}{2TS_0}\right) \qquad [15]$$

Equations 14 and 15 represent the interesterification rate constant as a function of time and fatty acid concentration for a given enzyme concentration and initial concentrations of

triglyceride and fatty acid. A specific interesterification reaction rate constant, k^*, can be defined as:

$$k^* = k/E \quad [16]$$

where E is the biocatalyst concentration.

A set of experiments, where the initial concentrations of PPP and stearic acid were kept constant but the biocatalyst concentration (E) was varied, was carried out to determine the value of k^*. The k values were determined at different times before the concentration of steady-state was reached for each enzyme concentration. The average value of k for each experiment was plotted against the biocatalyst concentration. A straight line was fitted to the data by linear regression, and the specific rate constant was calculated from the gradient.

RESULTS AND DISCUSSION

Specificity of the modified lipase and steady-state of the reaction. To check the positional specificity of the modified lipase to catalyze the substitution of fatty acids on each of the three positions of the glycerol moiety, a reaction was carried out and the components in the reaction system were monitored for 8 h. Steady-state was achieved in the reaction system after approximately 5 h. A fresh batch of 30 mg of modified lipase was added after 10 h to the reaction mixture, and the system was stirred for a further 15 h. The profile of all components in the reaction system is shown in Figure 1, which shows that the modified lipase catalyzed predominantly the interesterification of PPP and stearic acid in the presence of low-water content (25 mg/L).

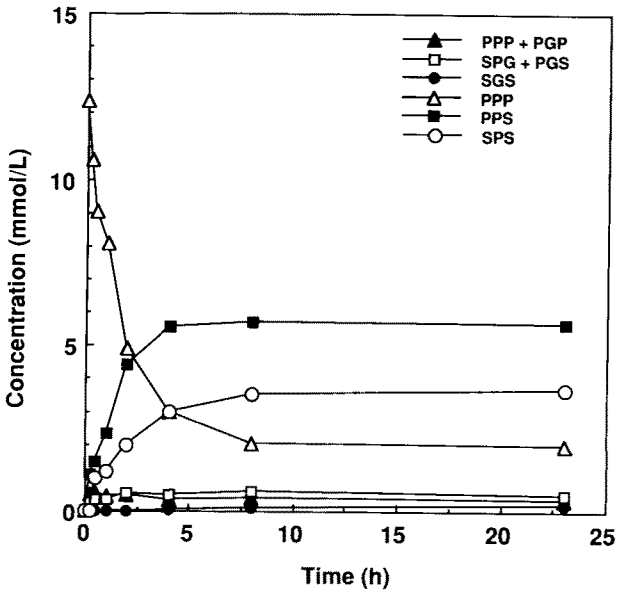

FIG. 1. Triglycerides and diglycerides concentration profiles resulting from the modified lipase-catalyzed interesterification of tripalmitin (PPP) and stearic acid. PPG, 1,2-dipalmitin; PGP, 1,3-dipalmitin; SPG, 1-stearoyl 2-palmitoyl glycerol; PGS, 1-palmitoyl 3-stearoyl glycerol; SGS, 1,3-distearin; PPS, 1,2-palmitoyl 3-stearoyl glycerol; SPS, 1,3-stearoyl 2-palmitoyl glycerol; and SSS, tristearin. The reaction was carried out in 55 mL *n*-hexane that contained 0.55 g PPP, 0.55 g stearic acid, and 30 mg modified lipase. The reaction mixture with 25 mg/L water was magnetically stirred at 800 rpm and thermostated at 40°C.

GC and HPLC analysis showed that the modified lipase can also catalyze the hydrolysis of triglycerides to release diglycerides (e.g., 1,2-dipalmitin, PPG). 1,2-Diglycerides are chemically unstable, and therefore they undergo either spontaneously or enzymatically aided acyl migration to form 1,3-diglycerides (19,20). This isomerization of PPG explains the formation of 1-palmitoyl-3-stearoyl glycerol (PGS), which proceeds *via* a further lipase-catalyzed interesterification reaction with stearic acid to form 1,3-distearin (SGS) after prolonged reaction time. However, the percentage of total diglycerides produced in our system did not exceed 6 wt% of the initial PPP concentration. The degree of hydrolysis for this modified lipase to catalyze interesterification reactions of triglycerides and fatty acids is lower than the hydrolysis activities of other lipases studied to date under similar reaction conditions (21–23). Also, the steady-state for the interesterification of PPP and stearic acid under the conditions described was achieved after approximately 5 h, and that of PPG, 1,3-dipalmitin (PGP) and stearic acid to produce 1-stearoyl-2-palmitoyl glycerol (SPG), PGS and palmitic acid was rapidly established after less than 1 h. Lack of any further changes in the compositions of the reaction system after adding a fresh batch of modified lipase into the system demonstrates that the steady-state corresponds to equilibrium. Furthermore, GC and HPLC analysis showed that SSS was not formed in the reaction system. This result provides further evidence of 1,3-positional specificity of the modified lipase.

The substrate specificity of lipases used to catalyze interesterification reactions is normally dependent on the type of fatty acids to be incorporated into the glycerol moiety, as well as on the types of acyl groups of the glycerol backbone. For example, the chainlength of fatty acids and their degree of saturation significantly affect the extent of incorporation of fatty acids into triglycerides (24). However, to study the substrate specificity of the modified lipase with respect to stearic and palmitic acids, the following two experiments were performed: (i) stearic acid (35.1 mmol/L) and PPP (12.4 mmol/L), and (ii) palmitic acid (35.1 mmol/L) and SSS (12.4 mmol/L) were each dissolved in 55 mL *n*-hexane and stirred magnetically with 30 mg modified lipase at 800 rpm while thermostated at 40°C. The results presented in Figure 2 clearly show that the modified lipase does not distinguish between stearic and palmitic acids. Also, it was noticed that the biocatalyst has no preferential specificity toward PPP and SSS when used as substrates (25).

Kinetics of the interesterification reaction. It has been reported in many studies that organic solvents used for lipase-catalyzed reactions have a significant effect on the steady-state position of the reaction systems. This effect is most likely to be attributed to the variable solubility of water in different organic solvents (26,27). Therefore, in the present study, the interesterification reaction system model of PPP and stearic acid was performed in a water-dried *n*-hexane system that contained constant concentrations of substrates,

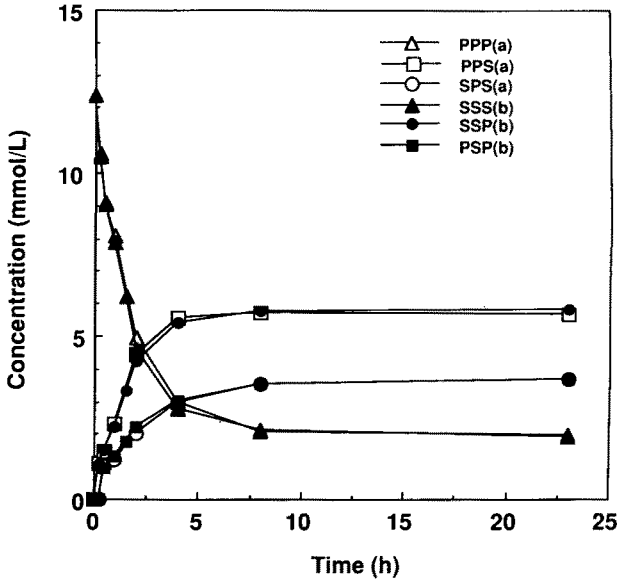

FIG. 2. Time course of the lipase-catalyzed interesterification of (a): PPP and stearic acid, and (b): the interesterification of SSS and palmitic acid. Initial reaction conditions of (a) were 35.1 mmol/L stearic acid and 12.4 mmol/L PPP, and of (b) were 35.1 mmol/L palmitic acid and 12.4 mmol/L mmol SSS. The substrates in both systems were dissolved in 55 mL *n*-hexane and stirred magnetically with 30 mg biocatalyst at 800 rpm and thermostated at 40°C. Abbreviations as in Figure 1.

whereas the concentration of the modified lipase was varied accordingly. Figure 3 shows the concentration profiles of PPP with varying concentrations of the biocatalyst. GC analysis

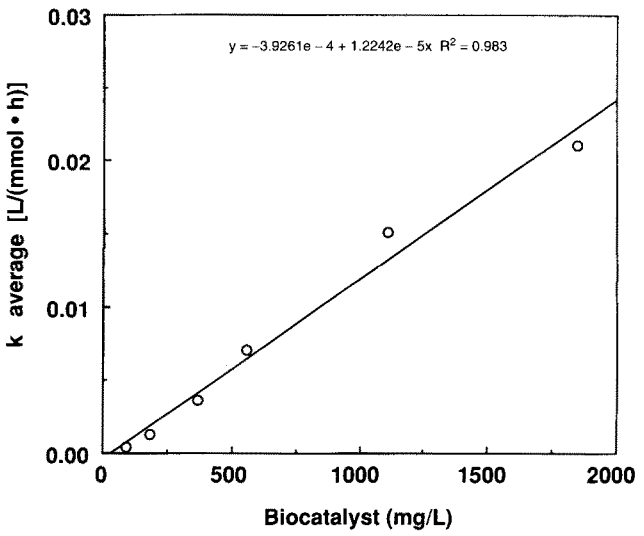

FIG. 4. The average of k values calculated according to Equation 14 for each experiment at different time intervals against the biocatalyst concentration.

showed that around 6% diglycerides of initial PPP concentration in all reaction systems were produced as by-products, due to an instantaneous hydrolysis reaction, which rapidly reached steady-state. Figure 3 shows that the interesterification reaction system reached steady-state after 4 h when more than 1,111 mg biocatalyst/L was used, and 8 h for those containing 370 and 556 mg biocatalyst/L. The values of k at different time intervals for each experiment were calculated ac-

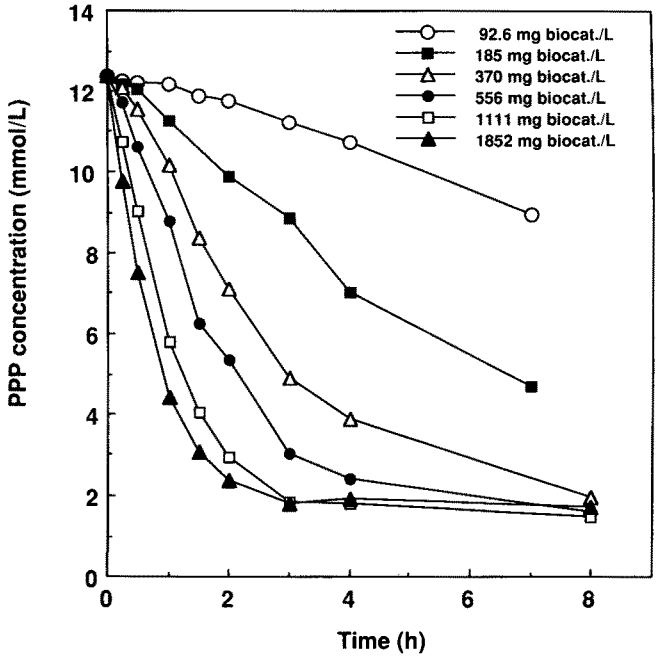

FIG. 3. The concentration of PPP as function of time in the reaction system with different concentrations of biocatalyst. Reaction conditions: 0.55 g stearic acid and 0.55 g PPP dissolved in 55 mL *n*-hexane and stirred at 800 rpm and 40°C. The concentrations of the biocatalyst (biocat.) were 92.6, 185, 370, 556, 1111, and 1852 mg/L. Abbreviation as in Figure 1.

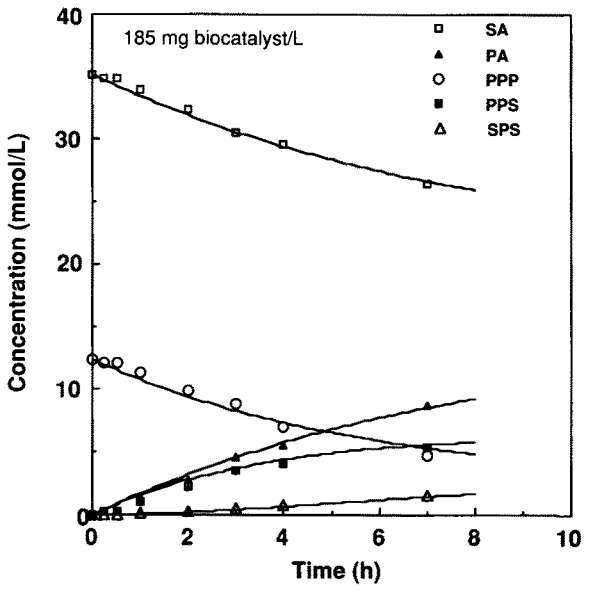

FIG. 5. Concentration profiles of fatty acids and triglycerides in the reaction system with 185 mg biocatalyst/L. Reaction conditions as in Figure 3. The points in the graph represent the experimental data, and the lines represent the analytical solutions of Equations 9–13 for each component in the reaction mixture with $k^* = 1.22 \cdot 10^{-5}$ [L^2/(mmol • mg biocatalyst • h)]. SA, stearic acid; PA, palmitic acid; PPP, tripalmitin; PPS, 1,2-palmitoyl 3-stearoyl glycerol; SPS, 1,3-stearoyl 2-palmitoyl glycerol.

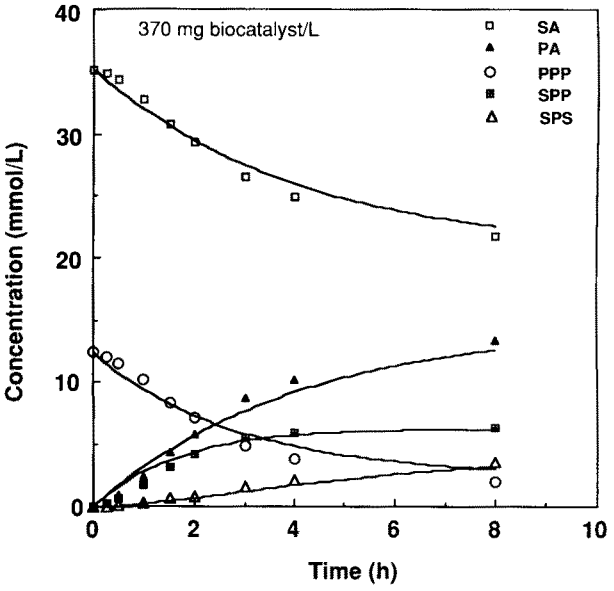

FIG. 6. Concentration profiles of fatty acids and triglycerides. Reaction conditions as in Figure 5 but with 370 mg biocatalyst/L. Abbreviations as in Figure 5.

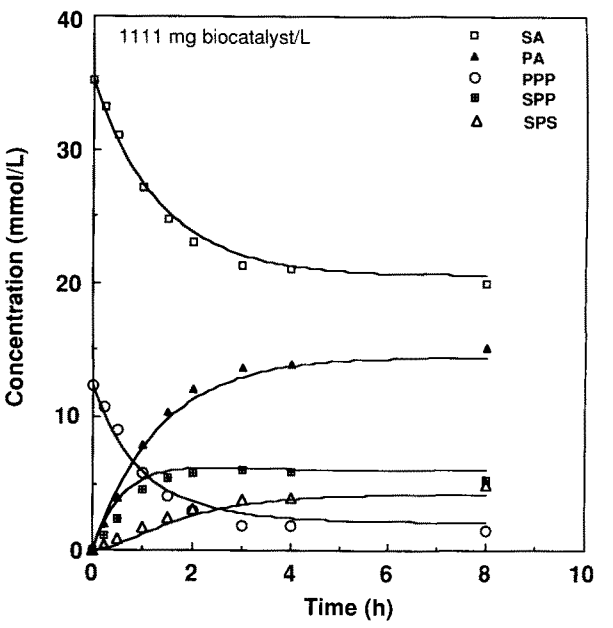

FIG. 8. Concentration profiles of fatty acids and triglycerides. Reaction conditions as in Figure 5 but with 1111 mg biocatalyst/L. Abbreviations as in Figure 5.

cording to Equations 14 and 15. The average k values of each experiment, when the concentrations of PPP and stearic acid are not limiting factors, were plotted against the biocatalyst concentration (Fig. 4). The plot is close to a straight line with slope k^* equal to $1.22 \cdot 10^{-5}$ [L^2/(mmol · mg biocatalyst · h)]. The obtained k^* value was substituted into Equations 9–13 to calculate the changes of all components in the interesterification reaction system with time. It is apparent that the obtained k^* value gave a satisfactory fit with all of the experimental data at constant substrate concentrations of PPP and stearic acid and varied modified lipase concentrations (Figs. 5–9). Therefore, this method demonstrates a simple model to describe the interesterification reaction of triglycerides and fatty acids at constant concentrations of substrates and water, so that the steady-state of the interesterification reaction is not shifted. The proposed model has the advantage that it has only one parameter that can be determined simply and allows a comparison of different lipases and different substrates, as well as different reactions with a minimum of experimenta-

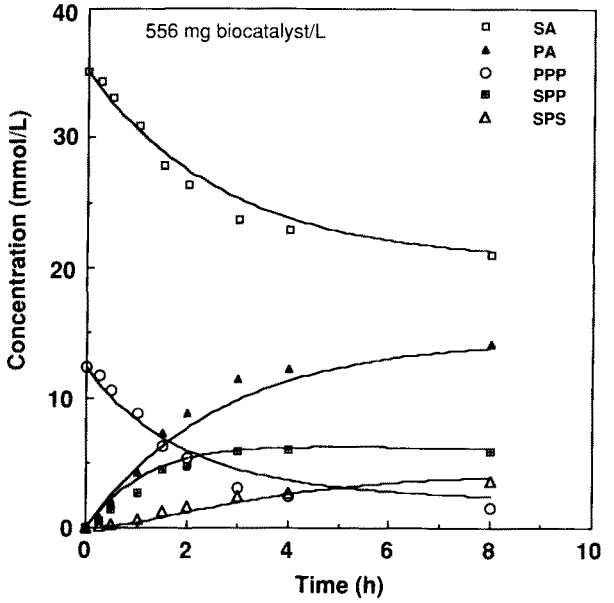

FIG. 7. Concentration profiles of fatty acids and triglycerides. Reaction conditions as in Figure 5 but with 556 mg biocatalyst/L. Abbreviations as in Figure 5.

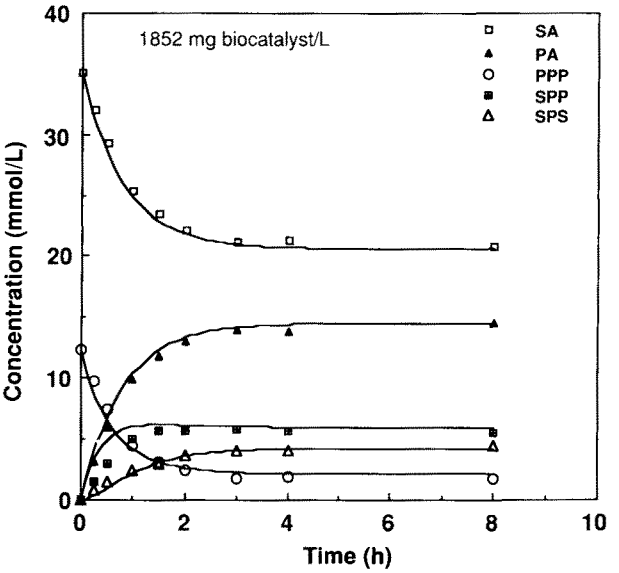

FIG. 9. Concentration profiles of fatty acids and triglycerides. Reaction conditions as in Figure 5 but with 1852 mg biocatalyst/L. Abbreviations as in Figure 5.

tion. This approach has proven to be effective in screening of different lipase and surfactant combinations for interesterification activity, while the concentrations of substrates are kept constant in micro-aqueous media (15).

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APPENDIX

This appendix contains the derivation of the kinetic model employed to describe the interesterification reaction of triglycerides and fatty acids in the presence of a modified lipase.

For simplicity, A = [PPP], B = [PPS], C = [SPS], P = [P], and S = [S]. The rate equation for each component described in Equations 1–3 is as follows:

$$\frac{dS}{dt} = k[-2AS + BP - BS + 2CP] \quad [1a]$$

$$\frac{dP}{dt} = k[2AS - BP + BS - 2CP] \quad [2a]$$

$$\frac{dA}{dt} = k\left[-2AS + BP + \left(\frac{k^0}{k}\right)\left(B^2 - 4AC\right)\right] \quad [3a]$$

$$\frac{dB}{dt = k}\left[2AS - BP - BS + 2CP + \left(\frac{k^0}{k}\right)\left(4AC - B^2\right)\right] \quad [4a]$$

$$\frac{dC}{dt} = k\left[-2CP + BS + \left(\frac{k^0}{k}\right)\left(B^2 - 4AC\right)\right] \quad [5a]$$

Initial conditions: $A = T$, $B = 0$, $C = 0$, $S = S_0$, $P = 0$. Mass balances on the triglycerides (T) and fatty acids give:

$$A + B + C = T \quad [6a]$$

$$S + P = S_0 \quad [7a]$$

A mass balance on stearic acid yields:

$$S + B + 2C = S_0 \quad [8a]$$

where the subscript zero denotes the initial value. Symbols without subscripts denote concentrations at any reaction time, t.

From Equations 3a–6a:

$$\left(\frac{1}{k}\right)\frac{d(A + B + C)}{dt} = \left(\frac{k^0}{k}\right)\left(B^2 - 4AC\right) = 0 \quad [9a]$$

Substituting Equations 6a–8a in Equations 1a–5a and 9a produces:

$$\left(\frac{1}{k}\right)\frac{dS}{dt} = -(S_0 + 2T)S + {S_0}^2 \quad [10a]$$

$$\left(\frac{1}{k}\right)\frac{dP}{dt} = -(S_0 + 2T)P + TS_0 \quad [11a]$$

$$\left(\frac{1}{k}\right)\frac{dA}{dt} = -2S_0A + 2TS_0 - 2TS - P^2 \quad [12a]$$

$$\left(\frac{1}{k}\right)\frac{dB}{dt} = -2S_0B + 2TS + P^2 - SP \quad [13a]$$

$$\left(\frac{1}{k}\right)\frac{dC}{dt} = -2S_0C + SP \quad [14a]$$

Solving Equations 10a and 11a for the initial conditions of the interesterification reaction at t = 0 gives the concentrations of S and P measured as a function with time:

$$S = \frac{S_0}{S^*}\left\{S_0 + 2T\exp(-kS^*t)\right\} \quad [15a]$$

$$P = \frac{2TS_0}{S^*}\left\{1 - \exp(-kS^*t)\right\} \quad [16a]$$

where $S^* = S_0 + 2T$.

Substituting Equations 15a and 16a in Equations 12a–14a gives:

$$\left(\frac{1}{kS_0}\right)\frac{dA}{dt} = -2\left(A - \frac{4T^3}{S^{*2}}\right) - \left\{\frac{4T^2(2T - S_0)}{S^{*2}}\right\}\exp(-kS^*t) - \left\{\frac{4T^2S_0}{S^{*2}}\right\}\exp(-2kS^*t) \quad [17a]$$

$$\left(\frac{1}{kS_0}\right)\frac{dB}{dt} = -2\left(B - \frac{4T^2S_0}{S^{*2}}\right) + \left\{\frac{2T(2T - S_0)^2}{S^{*2}}\right\}\exp(-kS^*t) + \left\{\frac{8T^2S_0}{S^{*2}}\right\}\exp(-2kS^*t) \quad [18a]$$

$$\left(\frac{1}{kS_0}\right)\frac{dC}{dt} = -2\left(C - \frac{TS_0^2}{S^{*2}}\right) + \left\{\frac{2TS_0(2T - S_0)}{S^{*2}}\right\}\exp(-kS^*t) - \left\{\frac{4T^2S_0}{S^{*2}}\right\}\exp(-2kS^*t) \quad [19a]$$

Equations 17a–19a can be solved to give:

$$\frac{A}{T} = \frac{4T^2}{S^{*2}} + A_1\exp(-2kS_0t) + \frac{4TS_0}{S^{*2}}\exp(-kS^*t) + \frac{S_0^2}{S^{*2}}\exp(-2kS^*t) \quad [20a]$$

$$\frac{B}{T} = \frac{4TS_0}{S^*} + B_1\exp(-2kS_0t) + \frac{2S_0(S_0 - 2T)}{S^{*2}}\exp(-2kS^*t) - \frac{2S_0^2}{S^{*2}}\exp(-2kS^*t) \quad [21a]$$

$$\frac{C}{T} = \frac{S_0^2}{S^{*2}} + C_1\exp(-2kS_0t) - \frac{2S_0^2}{S^{*2}}\exp(-kS^*t) + \frac{S_0^2}{S^{*2}}\exp(-2kS^*t) \quad [22a]$$

Substituting the initial concentrations $A = T$, $B = 0$, $C = 0$ at t = 0 in Equations 20a–22a gives the change of triglycerides concentrations with time:

$$A = \frac{T}{S^{*2}}\left\{4T^2 + 4TS_0\exp(-kS^*t) + S_0^2\exp(-2S^*t)\right\} \quad [23a]$$

$$B = \frac{T}{S^{*2}}\left\{4TS_0 + (2S_0^2 - 4TS_0)\exp(-kS^*t) - 2S_0^2\exp(-2kS^*t)\right\} \quad [24a]$$

$$C = \frac{TS_0^2}{S^{*2}}\left\{1 - 2\exp(-kS^*t) + \exp(-2kS^*t)\right\} \quad [25a]$$

The reaction rate constant k as a function of either stearic or palmitic acid concentration with time can be obtained by rearranging Equation 15a or 16a as:

$$k = \frac{-1}{S^*t}\ln\left\{\frac{\left(\frac{S^*S}{S_0} - S_0\right)}{2T}\right\} \quad [26a]$$

or

$$k = \frac{-1}{S^*t}\ln\left\{1 - \left(\frac{S^*P}{2TS_0}\right)\right\} \quad [27a]$$