Selection of Surfactant-Modified Lipases for Interesterification of Triglyceride and Fatty Acid

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ABSTRACT: Interesterification of tripalmitin and stearic acid in *n*-hexane was investigated with surfactant-modified lipases. Various kinds of lipases and surfactants were screened for high interesterification activity of the modified lipases. The modified-lipase activity was influenced by both the lipases and the hydrophile–lipophile balance value and fatty acid group of the surfactants. The modified lipase obtained from *Rhizopus japonicus* with sorbitan monostearate as surfactant had the highest activity in the *n*-hexane system. The interesterification activity of the selected modified lipase in molten substrates at 75°C without solvents was the same as that in the *n*-hexane system at 40°C.
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KEY WORDS: Fatty acid, interesterification, modified lipase, surfactant, triglyceride.

The physical and nutritional properties of oils and fats are related to the fatty acid composition of the triglycerides. Triglyceride quality can be improved by controlling their fatty acid composition (1–3). Interesterification is one modification method for oils and fats. Two types of interesterification have been developed (4). One is a random interesterification with a catalyst, such as sodium methylate or nonspecific lipase. By this process, the fatty acids in the triglycerides can be exchanged randomly. The other is an interesterification with 1,3-specific lipase. By means of 1,3-specific lipase, only the fatty acids in positions 1 and 3 of triglycerides can be exchanged, which makes it easier to control specified fatty acid location in the triglyceride compared to random interesterification. Interesterification has been applied to the production of cocoa butter equivalent (CBE) or cocoa butter substitute (CBS) (5–7). Recently, middle-chain triglycerides (MCT), high-value polyunsaturated fatty acids [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] and human milk fat substitutes were noted for their nutritional value and function (8,9). MCT consist mainly of octanoic and decanoic acids, which are more easily hydrolyzed by pancreatic lipase compared to long-chain triglycerides. Accordingly, MCT are commonly used in medical science for patients with pancreatic insufficiency, where MCT are provided in triglyceride form to utilize energy (9). EPA and DHA have been reported to have beneficial therapeutic (antitumor, antiinflammatory, and antiviral) and nutritional effects (10,11). Triglycerides of human milk contain palmitic acid almost exclusively in the 2-position, with 1,3-dioleoyl-2-palmitoyl glycerol being the major individual component. The saturated fatty acid in the 2-position of triglycerides is efficiently produced without soap formation. It is believed that the saturated fatty acid (palmitic acid) in the 2-position is better absorbed by infants than saturated fatty acids in positions 1 and 3 of the triglycerides (8). By interesterification, fats and oils with special properties, such as CBE, MCT, EPA, and DHA, can be produced from inexpensive, conventional materials (12–16).

Several methods for enzymatic interesterification have been investigated (17–21). Often, the reaction rate is not high, and side reaction products [diglycerides (DG) and monoglycerides (MG)] are produced due to addition of water. To improve the activity, polyethylene glycol modified lipase (22,23), covalent attachment of fatty acids (24), and immobilization on anion exchange resins (25) have been studied. Recently, a surfactant-modified enzyme, which is a complex of enzyme and surfactant, was proposed (26–28). It was assumed that the hydrophilic moiety of the surfactant attached to the enzyme surface and that the hydrophobic moieties arranged themselves on the outer side of the enzyme (26). By this modification, the enzyme becomes soluble or well dispersed in the organic solvent and can have activity in the organic solvent. In our previous publication, we showed that the lipase modified by surfactant showed much higher interesterification activity than the original crude lipase and surfactant mixture (29).

In this paper, the interesterification activities of modified lipases in organic solvent for various kinds of lipases and food-grade surfactants were investigated. Tripalmitin and stearic acid were used as model substrates, and interesterification reaction experiments were carried out in *n*-hexane. The best combination for a surfactant-modified lipase to reach the highest activity and protein recovery is proposed. Further, the effect of lipase–surfactant ratio during the modification process was studied. With the selected modified lipase, the interesterification activity was investigated in a nonsolvent system at high temperature.

MATERIALS AND METHODS

Materials: *lipases*. Crude lipases, Newlase F (*Rhizopus niveus*), Lipase F Amano (*Rhizopus* sp.), Lipase AY 30 (*Can-*

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dida rugosa), Lipase A Amano 6 (*Aspergillus niger*), and Lipase M Amano 10 (*Mucor javanicus*) were supplied by Amano Pharmaceutical Co., Ltd. (Tokyo, Japan). Lipase A 5 (*Rh. japonicus*) Lipase Saiken 100 (*Rh. japonicus*), Lipase B 4 (*Rh. japonicus*), and Roosepase FD (*Rh. japonicus*) were supplied by Nagase Biochemicals Ltd. (Kyoto, Japan). Lipase OF (*Ca. cylindracea*), Lipase MY (*Ca. cylindracea*), and Lipase PL (*Alcaligenes* sp.) were supplied by Meito Sangyo Co., Ltd. (Tokyo, Japan). Lipolase 100 T (*As. oryzae*) and Palatase M 1000 L (*Mucor miehei*) were supplied by Novo Nordisk A/S, (Bagsvaerd, Denmark). Lipase Sankyo (*Aspergillus* sp.) was supplied from Sankyo Co., Ltd. (Tokyo, Japan). Talipase (*Rh. delemar*) was supplied by Tanabe Seiyaku Co., Ltd. (Tokyo, Japan). Lipase Asahi (*Chromobacterium viscosum*) was supplied by Asahi Chemical Industry Co., Ltd. (Tokyo, Japan). Lipase Kurita (*Pseudomonas* sp.) was supplied by Kurita Co., Ltd. (Tokyo, Japan).

Surfactants. Food-grade, sorbitan esters, and sugar esters were used.

Sorbitan esters. Emazol L-10(F) (sorbitan monolaurate), Emazol P-10(F) (sorbitan monopalmitate), Emazol S-10(F) (sorbitan monostearate), Emazol S-30(F) (sorbitan tristearate), Emazol O-10(F) (sorbitan monooleate), and Emazol O-30(F) (sorbitan trioleate) were supplied by Kao Co., Ltd. (Tokyo, Japan). Sorgen 30 (sorbitan sesquioleate) was supplied by Daiichi Kogyo Seiyaku Co., Ltd. (Tokyo, Japan). Nonion CP-08R (sorbitan monocaplirate) was supplied by Nippon Oil & Fats Co., Ltd. (Tokyo, Japan).

Sugar esters. DK-Ester F-10, F-20, F-50, F-70, F-90, F-110, F-140, F-160 (sucrose palmitate and stearate mixtures) were supplied by Daiichi Kogyo Seiyaku Co., Ltd. (Tokyo, Japan). Ryoto sugar esters ER-190, ER-290 (sucrose erurate), and O-170 (sucrose oleate) were supplied by Mitsubishi Kasei Corp. (Tokyo, Japan).

Phospholipids. Lecithin DX and Beisisu LG-10E were supplied by Nisshin Oil Co., Ltd. (Tokyo, Japan).

Other chemicals. Tri-, di-, monopalmitin, tri-, di-, monostearin, and 1-palmitoyl-3-stearoyl glycerol with more than 99% purity were purchased from Sigma Chemical Company (St. Louis, MO). 1,2-Dipalmitoyl-3-stearoyl glycerol (PPS) and 1,3-distearol-2-palmitoyl glycerol (SPS) with more than 98% purity were supplied from Unilever Research Colworth Laboratory (Sharnbrook, Bedford, United Kingdom). Palmitic acid, stearic acid, *n*-hexane, ethanol, pyridine, molecular sieves (4A 1/16), and *N,O-bis* (trimethylsilyl) trifluoroacetamide (BSTFA) of analytical grade were purchased from Wako Pure Chemical Ind. (Tokyo, Japan). Trimethyl chlorosilane (TMS) was purchased from GL Science Co., Ltd. (Tokyo, Japan).

Lipase modification procedure. Crude lipase (3 g) was dissolved in 1 L ion-exchanged water. Surfactant (0.75 g), dispersed in 20 mL ethanol (40°C), was added to the lipase solution. The mixture was sonicated at 600 W for 15 min and stirred at 600 rpm and 5°C for 2 h. The precipitate was obtained by centrifugation at 8000 rpm (7000 × *g*) and 5°C for 10 min, frozen, and freeze-dried. The modified lipase was obtained as a powder.

Interesterification experiment in n-*hexane system.* The substrates of tripalmitin (0.5 g) and stearic acid (0.5 g) were dissolved in 50 mL *n*-hexane, and the water concentration was decreased by molecular sieves. The modified lipase (30 mg) was added to the reaction mixture, and the interesterification reaction was carried out at 500 rpm stirring and 40°C for 3 h. Samples were taken from the reaction system at prescribed time intervals and were filtered (LCR13-LH, pore size 0.5 μm; Millipore Co., Milford, MA) and then analyzed by gas chromatography (GC).

Interesterification experiment in nonsolvent system. Substrates of tripalmitin (5 g) and stearic acid (5 g) were melted at 75°C in a flask with a lid. The modified lipase (300 mg) was added to the reaction mixture, and interesterification was carried out at 500 rpm and 75°C for 3 h. Samples were taken from the reaction system at prescribed time intervals, dissolved in *n*-hexane, and filtered prior to GC injection.

Interesterification activity. Interesterification activity of the modified lipase was described in terms of the specific reaction rate constant, k^* [m^6/(mol • g • s)], which was based on a two-substrate, two-product reversible reaction system, assuming second-order reaction kinetics (27). The value of k^* reflects the conversion rate of tripalmitin to PPS and SPS per gram protein of the modified lipase.

First screening of modified lipase. Nineteen different lipases were screened to obtain the modified lipase that have high interesterification activity in the *n*-hexane system and high protein recovery. Emazol S-10(F), sorbitan monostearate, was used as surfactant in each modification process.

Second screening of modified lipase. The modified lipases that were obtained in different solutions of McIlvaine buffer, ranging from pH 4 to 8, were analyzed for their interesterification activities and protein recoveries. Lipases, selected earlier by the first lipase screening, were used for the investigation. The ratio of lipase protein weight and surfactant weight during the modification was the same for each modified-lipase preparation.

Surfactant screening for the modified lipase. Twenty-two kinds of surfactants were screened to use for modified-lipase formation. Lipase used for the screening was the lipase selected by the second lipase screening.

Investigation of lipase–surfactant ratio. To obtain the modified lipase efficiently, the weight ratio of lipase and surfactant used in the modification process was investigated. Lipase Saiken 100 as lipase and Emazol S-10(F) as surfactant were used. The lipase–surfactant ratio was described as the *R* value, which is the weight ratio of lipase protein amount and surfactant amount during the modification (lipase protein/surfactant). The modified lipases were obtained from the combination of 0.5–5.0 g lipase and 0.4–5.0 g. The interesterification activity and protein yield were investigated for different *R* values.

Analysis method. For the analysis of triglycerides and fatty acids that existed in the reaction system, the sample was injected in the GC with an internal standard (*n*-hexadecane). For partial glycerides, the sample silylated as follows (30). The reaction solvent, *n*-hexane, was evaporated from 0.5 mL

of the sample at 60°C with nitrogen. The dried sample was mixed with 1 mL of internal standard solution (n-hexadecane in pyridine), 0.2 mL BSTFA, and 0.1 mL TMS. The mixture was kept at 70°C for 30 min to allow silylation of the carboxyl and hydroxyl groups. After cooling down to room temperature, the mixture was injected into the GC.

GC analysis was performed on a GC-14AH gas chromatograph (Shimadzu Co., Kyoto, Japan), equipped with a capillary column (ULBON HR-TGC1, 0.25-mm i.d., 25-m column length, 0.1-μm film thickness; Shinwa Chemical Industries, Ltd., Tokyo, Japan) and a flame-ionization detector. The operating temperature was from 80 to 360°C, programmed at the rate of 10°C/min, with a final hold of 5 min. The injector and detector temperatures were set up at 370 and 400°C, respectively. Helium was used as the carrier gas with 5 mL/min flow rate and 1:10 split ratio. Air (0.5 kg/cm^2) and hydrogen (0.6 kg/cm^2) were also used.

Water concentration analysis. Water concentration of the reaction system was analyzed by Karl Fischer determination, with a 684 KF Coulometer (Metrohm, Herisau, Switzerland). The sample for water analysis from the reaction system was injected into the coulometer without filtration. The water concentration of n-hexane, in which water content was decreased by molecular sieve, was below 10 mg/L.

Protein content analysis. The protein contents of crude and modified lipases were analyzed by Automatic Nitrogen Analyzer, FP-428 (Leco Co., St. Joseph, MI).

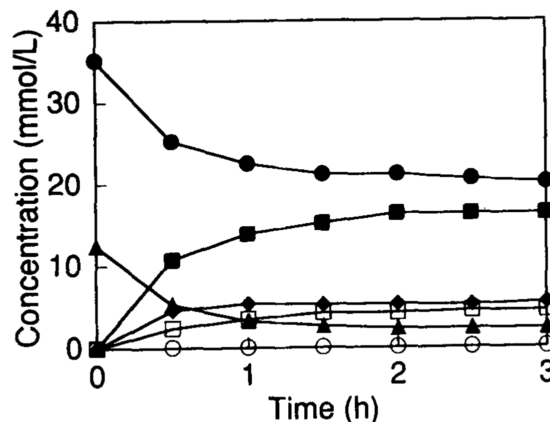

FIG. 1. Interesterification reaction time course for the modified lipase obtained from Lipase Saiken 100 (*Rhizopus japonicus*; Nagase Biochemicals, Kyoto, Japan) and Emazol S-10(F) (sorbitan monostearate; Kao Corp., Tokyo, Japan). Substrates were 0.5 g of tripalmitin and 0.5 g of stearic acid. The amount of modified lipase was 30 mg. The reaction was carried out at 500 rpm stirring and 40°C in n-hexane system. Palmitic acid, ■; stearic acid, ●; tripalmitin, ▲; 1,2-dipalmitoyl-3-stearoyl glycerol (PPS), ◆; 1,3-distearol-2-palmitoyl glycerol (SPS), □; tristearin (SSS), O.

RESULTS AND DISCUSSION

Figure 1 shows the typical time course for the interesterification of tripalmitin and stearic acid with the modified lipase obtained from Lipase Saiken 100 and Emazol S-10(F). The concentrations of tripalmitin and stearic acid decreased, and palmitic acid, PPS, and SPS were produced with time. The reaction system seemed to reach steady state after 2 h reaction. Tristearin (SSS) was not produced at all, which implied that the modified lipase had 1,3-specificity. MG were not produced at all. The amount of DG produced was less than 6% of the triglycerides. In this system, the initial water concentration was below 30 mg/L. The modified lipase had interesterification activity, even at such low water concentrations.

To obtain modified lipase with high interesterification activity, the modified lipases that were obtained from various lipases and surfactants were analyzed for activity and protein yield.

First lipase screening. Table 1 shows the interesterification activity and protein yield for the modified lipases obtained from various kinds of lipases that were classified by their 1,3-specificity. Sorbitan monostearate was used as surfactant, and all crude lipases produced precipitates. Protein concentrations of the modified lipases varied from 1.15 to 18.56%. The k^* values of the modified lipases with 1,3-specificity were 3 to 32 × $10^{-9}m^6/(mol \cdot g \cdot s)$. On the other hand, the k^* values of modified lipases having no 1,3-specificity were almost zero. Total activity of modified lipases was evaluated by the relative activity yield, per unit of crude lipase protein, which was obtained from k^* multiplied by the protein recovery. Based on total activity evaluation, Lipase Kurita was determined to have the highest activity yield. From the first screening, the lipases with high total activity, Lipase Kurita from *Pseudomonas* sp., Lipase Saiken 100 from *Rh. japonicus*, Talipase from *Rh. delemar*, and Lipase Asahi from *Ch. viscosum* were selected for further investigation. Because it has been reported that the lipase from *M. miehei* has 1,3-specific interesterification activity in the immobilized form in organic solvent, (31–33) *M. miehei* was selected as a standard.

Second lipase screening. The modified lipases that were prepared from Lipase Kurita, Talipase, Lipase Saiken, Lipase Asahi, and Palatase M 1000 L, and surfactant Emazol S-10 (F) in McIlvaine buffer solution, pH 4 to 8, were investigated for the effect of pH during the modification process. Table 2 shows the effect of pH on protein recovery and interesterification activity. Total activity of the modified lipase can be evaluated from relative activity yield. Except for Platase M 1000 L, the pH condition during modification affected the modified-lipase amount, protein concentration, protein recovery, and interesterification activity. The amount of modified Lipase Kurita obtained at pH 5 (2.153 g) was almost three times that obtained at pH 6–8. The protein recovery (14.9%) and activity of modified Lipase Kurita [80 × $10^{-9}m^6/(mol \cdot g \cdot s)$] at pH 5 were high, giving the highest relative activity yield of all modified lipases. For Lipase Saiken 100, the amount of the modified lipase obtained at pH 5 and 6 was higher than at pH 7 and 8. Modified Lipase Saiken obtained at pH 5 had high protein recovery (13.7%) and activity [87 × $10^{-9}m^6/(mol \cdot g \cdot s)$], and the relative activity yield was the highest of all modified lipases (same value as the modified lipase obtained from Lipase Kurita at pH 5). For Lipase

TABLE 1
Modified-Lipase Activities Obtained from Various Kinds of Lipases During First Lipase Screening

Lipase[a] (%)	Source	1,3-Specificity[i] of crude lipase	Modified lipase amount (g/L)	Modified lipase protein (%)	Protein recovery (%)	Activity $k^* \times 10^9$ [m^6/(mol • g • s)]	Relative activity yield (%)
Newlase F[a]	*Rhizopus niveus*	Y	0.303	2.61	3.2	11	17
Lipase F Amano[a]	*Rhizopus* sp.	Y	0.366	6.64	5.1	19	46
Lipolase 100 T[b]	*Aspergillus oryzae*	Y	0.477	3.26	33.6	4	64
Palatase M 1000 L[b]	*Mucor miehei*	Y	1.864	1.15	2.9	3	4
Lipase A Amano 6[a]	*As. niger*	Y	0.329	4.05	6.2	3	9
Lipase M Amano 10[a]	*Mucor javanicus*	Y	0.502	12.68	22.2	4	42
Talipase[c]	*Rh. delemar*	Y	1.868	2.08	5.1	32	77
Lipase Saiken 100[d]	*Rh. japonicus*	—	0.364	7.15	3.5	44	73
Lipase A 5[d]	*Rh. japonicus*	—	0.392	2.84	7.0	17	56
Lipase B 4[d]	*Rh. japonicus*	—	0.593	15.04	35.5	0	0
Roosepase FD[d]	*Rh. japonicus*	—	0.869	15.01	45.6	0	0
Lipase PL[e]	*Alcaligenes* sp.	—	0.449	14.57	19.3	3	27
Lipase Asahi[f]	*Chromobacterium viscosum*	—	0.419	4.49	2.5	60	71
Lipase Kurita[g]	*Pseudomonas* sp.	—	0.333	13.10	5.7	37	100
PPL[j]	*Porcine pancreas*	—	0.538	18.56	12.4	0	0
Lipase AY 30[a]	*Candida rugosa*	N	0.348	3.11	20.1	0	0
Lipase of[e]	*Ca. cylindracea*	N	0.170	11.60	7.9	1	4
Lipase MY[e]	*Ca. cylindracea*	N	0.362	4.39	12.6	0	0
Lipase Sankyo[h]	*Aspergillus* sp.	N	0.320	2.64	5.9	0	0

[a]From Amano Pharmaceutical (Tokyo, Japan).
[b]From Novo Nordisk A/S (Bagsvaerd, Denmark).
[c]From Tanabe Seiyaku (Tokyo, Japan).
[d]From Nagase Biochemicals (Kyoto, Japan).
[e]From Meito Sangyo (Tokyo, Japan).
[f]From Asahi Chemical Industry (Tokyo, Japan).
[g]From Kurita (Tokyo, Japan).
[h]From Sankyo (Tokyo, Japan).
[i]Y, yes; N, no; —, unknown.
[j]From Biocatalyst (Mid Glamorgan, United Kingdom).

TABLE 2
Effect of pH During Modification on the Interesterification Activities of Selected Modified Lipases During the Second Lipase Screening[a]

Lipase[a]	Source	pH	Modified lipase amount (g/L)	Modified lipase protein (%)	Protein recovery (%)	Activity $k^* \times 10^9$ [m^6/(mol • g • s)]	Relative activity yield (%)
Lipase Kurita	*Pseudomonas* sp.	5.0	2.153	5.30	14.9	80	100
		6.0	0.713	11.10	10.4	42	37
		7.0	0.333	13.10	5.7	37	18
		8.0	0.527	10.40	7.2	44	27
Talipase	*Rhizopus delemar*	4.0	2.335	0.83	2.6	4	0.9
		5.0	2.367	1.34	4.2	20	7
		6.0	1.042	1.22	1.7	30	4
		7.0	1.868	2.08	5.1	32	14
Lipase Saiken 100	*Rh. japonicus*	5.0	0.866	11.90	13.7	87	100
		6.0	0.491	10.30	6.7	59	33
		7.0	0.364	7.15	3.5	44	13
		8.0	0.159	7.95	1.7	49	7
Lipase Asahi	*Chromobacterium viscosum*	5.0	1.092	8.07	11.8	93	92
		6.0	1.248	7.09	11.8	100	99
		7.0	0.419	4.49	2.5	60	13
		8.0	0.694	3.11	2.9	99	24
Palatase M 1000 L	*Mucor miehei*	5.0	1.784	0.97	2.3	5	1
		6.0	1.547	1.28	2.6	3	0.7
		7.0	1.864	1.15	2.9	3	0.7
		8.0	1.574	1.36	2.9	0	0

[a]Company locations as in Table 1.

Asahi, the amount of modified lipases obtained at pH 5 and 6 was almost twice of those at pH 7 and 8. Modified Lipase Asahi at pH 6 showed the highest activity [$100 \times 10^{-9} m^6/(mol \cdot g \cdot s)$] among all modified lipases, and the relative activity yield reached 99%. For Talipase, higher amounts of modified lipases were collected than for other kinds of lipases. However, protein recovery and activity were low, and, the relative activity yields were 0.9 to 14%. For Palatase M 1000 L, relatively high amounts of modified lipases were collected, but, activities were low.

Table 3 shows DG and MG production of modified lipases after a 3-h reaction. Modified Lipase Kurita at pH 5 produced 17.5 wt% DG and 2.76 wt% MG after 3 h. However, modified Lipase Saiken at pH 5 produced 3.58 wt% DG and no MG at all. DG produced by modified Lipase Asahi at pH 5 and 6 was 5.58 and 5.33 wt%, respectively, and no MG was produced by either modified lipases. All modified lipases were freeze-dried and all free water was assumed to be removed during the process. The initial water concentrations in all reaction mixtures ranged from 20 to 80 mg/L. The produced amounts of DG and MG were dependent on lipases and modification conditions.

On the basis of the total activity yield, modified Lipase Kurita at pH 5, modified Lipase Saiken 100 at pH 5, and modified Lipase Asahi at pH 6 were estimated to be acceptable. However, because of the considerable amount of DG and MG production, Lipase Kurita was not selected. Lipase Asahi was not selected because of its high cost for diagnostic use in medical areas.

Because of interesterification activity, DG and MG production and cost performance, Lipase Saiken 100, from *Rh. japonicus* was selected as the most suitable lipase for modified lipase formation.

Surfactant screening. Surfactant screening was carried out with Lipase Saiken 100. Table 4 shows the surfactants used for modification and the obtained modified-lipase activities. Three solid types and six liquid types of sorbitan ester were used. Eight solid types and three liquid types of sugar ester and two types of phospholipids were also used. Sorbitan esters and sugar esters were classified by hydrophile–lipophile balance (HLB) as proposed by Griffin (34). HLB describes the ratio of the hydrophilic and hydrophobic moieties in a surfactant, and it varies from 0 to 20 (0 corresponds to highly hydrophobic, 20 to highly hydrophilic compounds) (35).

For sorbitan esters of HLB below 4.3, almost no precipitates were observed, or the obtained precipitates (modified lipases) showed no activity. With sorbitan monostearate of HLB 4.7, the highest k^* and activity yield were obtained. With the other sorbitan esters of HLB 6.7–9.5, the activity yields were 12–68% of the sorbitan monostearate. For sugar esters of HLB below 2, almost no precipitates were observed, or the obtained precipitates (modified lipases) showed no activity. With sugar esters of HLB in the range 6.0–13.0, the k^* value, protein recovery, and relative activity yield did not depend on HLB. At HLB 15, the highest protein recovery (5.9%) and the highest relative activity yield (27%) were observed among the modified lipases with sugar esters. No precipitates were obtained with phospholipids, and cloudy solutions were obtained after centrifugation. Among the nine liquid surfactants (six sorbitan esters and three sugar esters), six did not give any precipitates but stable cloudy solutions. The other three gave precipitates with considerable protein recoveries. However, the observed k^* values were not high, which is probably due to differences in fatty acid groups. From these results, Emaol S-10(F), sorbitan monostearate, HLB 4.7, was selected as the most suitable surfactant for modified lipase

TABLE 3
Monoglycerides (MG) and Diglycerides (DG) Produced by Modified Lipases After Three Hours

Lipase[a]	Source	pH	MG (wt%)	1,3-DG (wt%)	1,2-DG (wt%)	DG total (wt%)
Lipase Kurita	*Pseudomonas* sp.	5.0	2.76	13.0	4.50	17.50
		6.0	0.00	5.24	2.02	7.26
		7.0	0.00	5.08	1.55	6.63
		8.0	0.00	5.11	1.62	6.73
Talipase	*Rhizopus delemar*	4.0	1.22	1.63	3.03	4.66
		5.0	0.94	1.14	4.14	5.28
		6.0	0.68	1.05	2.67	3.72
		7.0	0.75	2.45	3.02	5.47
Lipase Saiken 100	*Rh. japonicus*	5.0	0.00	0.00	3.58	3.58
		6.0	0.00	0.00	4.28	4.28
		7.0	0.00	0.00	5.99	5.99
		8.0	0.00	0.00	7.48	7.48
Lipase Asahi	*Chromobacterium viscosum*	5.0	0.00	4.38	1.20	5.58
		6.0	0.00	4.29	1.04	5.33
		7.0	0.76	4.82	1.97	6.79
		8.0	0.75	5.22	1.27	6.49
Palatase M 1000 L	*Mucor miehei*	5.0	0.80	1.06	4.05	5.11
		6.0	0.86	1.19	3.50	4.69
		7.0	0.00	1.78	4.59	6.37
		8.0	0.63	1.35	4.90	6.25

[a]Company sources as in Table 1.

TABLE 4
Modified Lipase Activity Obtained from Various Kinds of Surfactants

Surfactant		HLB	Modified lipase amount (g/L)	Modified lipase protein (%)	Protein recovery (%)	Activity $k^* \times 10^9$ [m^6/(mol • g • s)]	Relative activity yield (%)
Sorbitan ester							
Emazol O-30(F)	Sorbitan trioleate[a]	1.8	NP[b]	—	—	—	—
Emazol S-30(F)	Sorbitan tristearate	2.1	0.238	3.80	1.9	0	0
Emazol O-10(F)	Sorbitan monooleate[a]	4.3	NP	—	—	—	—
Emazol S-10(F)	Sorbitan monostearate	4.7	0.364	7.15	3.5	44	100
Emazol P-10(F)	Sorbitan monopalmitate	6.7	0.236	6.08	3.0	32	62
Emazol L-10(F)	Sorbitan monolaurate[a]	8.6	0.133	7.51	2.1	8	11
Sorgen 30	Sorbitan sesquioleate[a]	3.7	NP	—	—	—	—
Nonion OP-80R	Sorbitan monooleate[a]	4.3	1.190	9.75	31.5	0	0
Nonion CP-08R	Sorbitan monocaplirate[a]	9.5	0.303	17.94	14.7	4	38
Sugar ester							
DK-Ester F-10	Sucrose palmitate/stearate	1.0	0.250	7.78	1.6	0	0
DK-Ester F-20	Sucrose palmitate/stearate	2.0	0.216	6.86	1.2	0	0
DK-Ester F-50	Sucrose palmitate/stearate	6.0	0.210	8.81	1.5	13	13
DK-Ester F-70	Sucrose palmitate/stearate	8.0	0.206	7.44	1.3	6	5
DK-Ester F-90	Sucrose palmitate/stearate	9.5	0.410	7.16	2.5	8	13
DK-Ester F-110	Sucrose palmitate/stearate	11.0	0.323	6.98	1.8	5	6
DK-Ester F-140	Sucrose palmitate/stearate	13.0	0.693	4.89	2.8	9	16
DK-Ester F-160	Sucrose palmitate/stearate	15.0	1.508	4.76	5.9	7	27
Ryoto sugar ester							
DK-Ester ER-190	Sucrose erurate[a]	1.0	NP	—	—	—	—
DK-Ester ER-290	Sucrose erurate[a]	2.0	NP	—	—	—	—
DK-Ester O-170	Sucrose oleate[a]	1.0	NP	—	—	—	—
Phospholipid							
Lecithin DX[a]		—	NP	—	—	—	—
Beisisu LG-10E[a]		—	NP	—	—	—	—

[a]Liquid, the others are solid.
[b]NP, no precipitate was observed.

formation. Goto *et al.* (36) reported that the esterification activity of surfactant-modified lipase in organic solvent was influenced by the hydrophobic groups of the surfactant. They suggested that a surfactant with a large number of hydrophobic groups is better for lipase modification because of its solubility in organic solvents. Our results showed that, both HLB and fatty acid group in surfactants significantly affected the activities and yields.

Investigation of lipase–surfactant ratio. Figure 2 shows the relationship of relative interesterification activity and protein yield against the *R* value (lipase protein/surfactant). As the *R* value increased between 0 and 0.5, protein yield became lower. However, the interesterification activity became higher. Interesterification activity and protein yield were not changed at *R* values above 0.5. Approximately one molecule of modified lipase was found to have 400–4000 molecules of surfactant at *R* values of 2.5–0.25, respectively. In our previous paper, the modified lipase obtained at the *R* value of 1 could keep the original activity for more than 70 h in continuous operation (37). An *R* value of 1 was selected as the most suitable for the modification process.

Summarizing the conditions of modification, Lipase Saiken 100 from *Rh. japonicas* as lipase, Emazol S-10 (F), sorbitan monostearate with HLB 4.7 as surfactant, and an *R* value of 1 were chosen.

Interesterification in nonsolvent system. The interesterification experiment of tripalmitin and stearic acid without solvent was carried out at 75°C with the modified lipase that was obtained from the selected modification conditions. Figure 3 shows the comparison of interesterification activity in *n*-hexane and in a solvent-free system. The interesterification activity is described as the conversion of stearic acid incorpo-

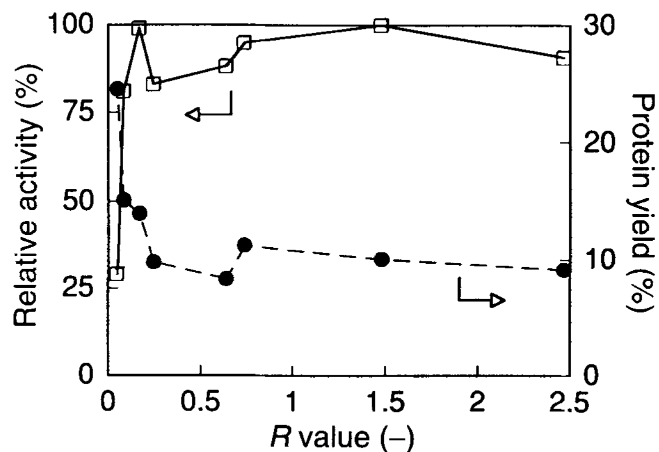

FIG. 2. Relative interesterification activity and protein yield of modified lipase obtained with different *R* values (lipase protein/surfactant); □, relative activity; ●, protein yield.

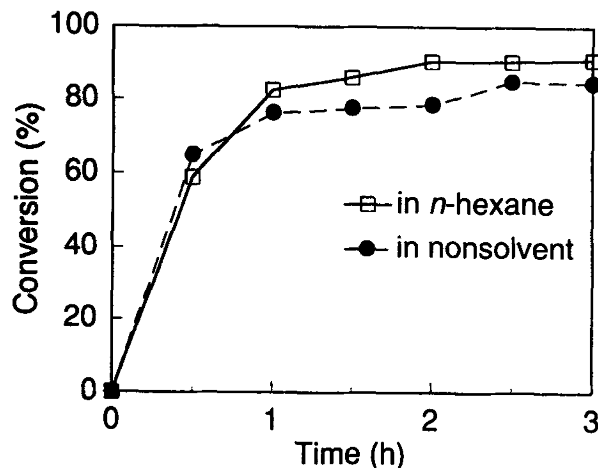

FIG. 3. Comparison of interesterification activities in *n*-hexane and without solvent in the reaction system. The modified lipase obtained from Lipase Saiken 100 and Emazol S-10(F) was used. In *n*-hexane, the reaction was carried out in 50 mL *n*-hexane with 0.5 g tripalmitin, 0.5 g stearic acid, and 30 mg of modified lipase at 40°C. In the nonsolvent system, the reaction was carried out with 5 g tripalmitin, 5 g stearic acid, and 300 mg of modified lipase at 75°C. Company sources as in Figure 1.

rated into tripalmitin. The reaction temperatures in the nonsolvent and the *n*-hexane systems were 75 and 40°C, respectively. Substrates were tripalmitin and stearic acid. The amount of modified lipase was 3% of the amount of substrates in both systems. Changes in conversion from tripalmitin to PPS and SPS were almost the same in both systems. Both systems almost reached steady-state after 2–3 h. The activity of modified lipase at 75°C in the nonsolvent system was almost the same as that at 40°C in *n*-hexane.

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