Production of MAG of CLA in a Solvent-Free System at Low Temperature with *Candida rugosa* Lipase

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ABSTRACT: We attempted to produce MAG of CLA through lipase-catalyzed esterification of a FFA mixture containing CLA (referred to as FFA-CLA) with glycerol. Screening of lipases showed that MAG-CLA was produced efficiently at 5°C with *Penicillium camembertii, Rhizopus oryzae,* and *Candida rugosa* lipases. Among them, *C. rugosa* lipase was selected because the lipase is widely used as a catalyst for oils and fats processing. The reaction was conducted with agitation of a 300-g mixture of FFA-CLA/glycerol (1:5, mol/mol), a 200-U/g mixture of *C. rugosa* lipase, and 2% water. When the reaction was conducted at 30°C, the esterification scarcely proceeded, owing to inhibition of the reaction by glycerol. But the reaction at 5°C eliminated the inhibition and produced MAG efficiently: The degree of esterification reached 93.8% after 58 h, and MAG content in the reaction mixture was 88.4 wt%. To reduce the reaction time, the reactor was connected with a vacuum pump after 24 h, and the reaction was continued with dehydration at 5 mm Hg. The degree of esterification reached 94.7% after 24 h of dehydration (48 h in total), and MAG content increased to 93.0 wt%. *Candida rugosa* lipase acted a little more strongly on *cis*-9,*trans*-11 CLA than on *trans*-10,*cis*-12 CLA, but the contents of the two isomers in MAG obtained from a 48-h reaction were the same as the contents in FFA-CLA.

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KEY WORDS: *Candida rugosa,* CLA, esterification, lipase, low-temperature reaction, MAG.

CLA is a group of C_{18} FA containing a pair of conjugated double bonds in either *cis* or *trans* configuration. A commercially available product contains almost equal amounts of *cis*-9,*trans*-11 (*c*9,*t*11)-CLA and *trans*-10,*cis*-12 (*t*10,*c*12)-CLA. The mixture of the two isomers has various physiological activities, such as reduction of the incidence of cancer (1–3), decrease in body fat content (4–6), beneficial effects on atherosclerosis (7,8), and improvement of immune function (9).

Considerable attention has been focused on the useful physiological activities of CLA, and CLA has been used as a functional food. If MAG of CLA were able to be produced efficiently, the new product could be used as a functional emulsifier for various kinds of foods and could also be added to beverages. Hence, development of a process for producing MAG-CLA has been strongly desired. MAG are produced industrially by chemical glycerolysis of oils and fats at high temperatures of 210–240°C (10,11), but the process cannot be applied to synthesize MAG of unstable FA including CLA. We thus attempted to synthesize MAG-CLA *via* a lipase-catalyzed reaction.

Organic solvent-free systems are attractive from the viewpoint of industrial production of MAG, and several systems have been reported. One of them is lipase-catalyzed glycerolysis of TAG, developed by McNeill *et al.* (12,13), in which MAG was produced efficiently by a stepwise decrease in the reaction temperature. On the other hand, CLA is produced industrially by alkali conjugation of oils containing high concentrations of linoleic acid. Because the first product is a FFA mixture, an esterification reaction with glycerol is advantageous. Production of MAG through lipase-catalyzed esterification has been reported, but the processes required organic solvents (14–16). Yamaguchi and Mase (17) reported that MAG of oleic acid was produced in an organic solvent-free system using *Penicillium camembertii* MAG and DAG lipase (referred to as *P. camembertii* lipase), but the product contained almost equal amounts of MAG and DAG. We also reported that MAG-CLA was produced efficiently in a two-step *in situ* reaction system with *P. camembertii* lipase that comprised esterification at 30°C and glycerolysis at 5°C (18). However, this system included the drawback that glycerolysis at a low temperature required a long period (2 wk). In this paper, we show that esterification at a low temperature is very effective for reducing the reaction time.

MATERIALS AND METHODS

Materials. A FFA mixture containing *c*9,*t*11- and *t*10,*c*12-CLA was a commercial product (CLA-80; Rinoru Oil Mills Co. Ltd., Tokyo, Japan) obtained by alkali conjugation of safflower oil in propylene glycol. The product contained 33.9 wt% *c*9,*t*11-CLA, 35.5 wt% *t*10,*c*12-CLA, 0.8 wt% *c*9,*c*11-CLA, 1.4 wt% *c*10,*c*12-CLA, and 1.9 wt% other CLA isomers. This FFA mixture is referred to as FFA-CLA. The molar amount of FFA was calculated based on the acid value. Glycerol (water content, 0.21%) was purchased from Wako Pure Chemical Industry Co. (Osaka, Japan).

Lipases were obtained from the following companies: *C. rugosa* lipase (Lipase OF) and *Alcaligenes* sp. lipase (Lipase QLM), Meito Sangyo Co. (Aichi, Japan); *P. camembertii* lipase (Lipase G), Amano Enzyme Inc. (Aichi, Japan);

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Rhizopus oryzae lipase (Ta-lipase), Tanabe Seiyaku Co. Ltd. (Osaka, Japan). Activity of lipases except *P. camembertii* lipase was measured by titrating FA liberated from olive oil (Wako Pure Chemical Industry) with 50 mM KOH as described previously (19): Hydrolysis was conducted at 30°C in a 5-mL mixture of 0.5 mL olive oil, 50 mM Na-acetate buffer (pH 5.6), and 100 mM $CaCl_2$, with stirring for 30 min. *Penicillium camembertii* lipase is a MAG and DAG lipase. The activity was therefore measured by titrating FA liberated from monoolein (Tokyo Chemical Industry Co. Ltd., Tokyo, Japan) with 50 mM KOH (20). Hydrolysis was performed under conditions similar to those for the other lipases. One unit (U) of lipase activity was defined as the amount of enzyme that liberated 1 μmol of FA per min.

Reactions. A small-scale reaction was conducted in a 50-mL vessel with stirring at 500 rpm. The standard reaction was conducted in a mixture of 4.9 g FFA-CLA/glycerol (1:5, mol/mol), 200 U/g lipase, and 0.1 mL of water originating from the enzyme solution with stirring at 500 rpm. A large-scale reaction was performed at 5°C in a 1-L, four-necked, round-bottomed flask containing 294 g FFA-CLA/glycerol (1:5, mol/mol) and 6 mL *C. rugosa* lipase solution (10,000 U/mL) with agitating at 250 rpm. Dehydration was performed by evaporation at 5 mm Hg using a vacuum pump.

Purification of acylglycerols. The reaction mixture was separated into oil and glycerol layers by centrifugation (6,500 × *g*, 5 min). The solidified mixture was heated at 40°C before centrifugation. The resulting oil layer (15 g) was diluted with 15 mL *n*-hexane and was then applied to a silica gel column (120 g; 30 × 390 mm; Merck, Darmstadt, Germany). After washing the column with 200 mL *n*-hexane/ethyl acetate (98:2, vol/vol), DAG and FFA were eluted with a mixture of *n*-hexane/ethyl acetate (80:20, vol/vol), and MAG were eluted with a mixture of *n*-hexane/ethyl acetate (50:50, vol/vol). Organic solvents in the DAG/FFA and MAG fractions were removed with an evaporator. To the DAG/FFA fraction was added 50 mL of 0.5 N KOH (20% ethanol solution), and DAG were extracted twice with 70 mL *n*-hexane.

Analyses. About 1 g of the reaction mixture obtained from esterification of FFA-CLA was separated into oil and glycerol layers by centrifugation (6,500 × *g*, 5 min). A solid-state mixture was heated at 40°C before centrifugation. The contents of MAG, DAG, TAG, and FFA in the oil layer were measured by a TLC/FID analyzer (Iatroscan MK-5) after development with a mixture of *n*-hexane/ethyl acetate/acetic acid (90:10:1, by vol). Analysis of a mixture of TAG/FFA/DAG/MAG (1:1:1:1, by wt) showed the same areas under the peaks. Hence, the contents of acylglycerols and FFA were determined from the area percentages of their peaks.

The degree of esterification was expressed as a ratio (mol%) of the amount of FA esterified to total FA in the reaction mixture. The M.W. used were 280, 354, 616, and 878 for FFA, MAG, DAG, and TAG, respectively.

FA composition was determined by GC of FAME. The constituent FA in acylglycerols were converted to their methyl esters in 3 mL methanol containing 1% Na-methylate by heating at 70°C for 15 min, and FFA were methylated in 3 mL of 5% HCl-methanol by heating at 70°C for 10 min. The resulting FAME were analyzed with a Hewlett-Packard 5890 gas chromatograph connected to a DB-23 capillary column (0.25 mm × 30 m; J & W Scientific, Folsom, CA) under the conditions described previously (21).

All analyses were performed three to five times under the same conditions, and the average values were presented.

RESULTS AND DISCUSSION

Synthesis of MAG-CLA with several lipases. FFA-CLA was esterified at 5 or 30°C with 5 molar equivalents of glycerol using four lipases. Table 1 shows the composition of FFA, MAG, DAG, and TAG in the oil layer separated from the reaction mixture. When *P. camembertii* and *R. oryzae* lipases were used as catalysts, the reaction velocity (shown by the degree of esterification after 7 h) at 30°C was faster than that at 5°C. Reactions at 30°C with the two lipases produced not only MAG but also DAG after 48 h, although TAG was not synthesized. Meanwhile, reactions at 5°C attained *ca*. 90% esterification after 48 h, and the main product was MAG. Although the reaction with *R. oryzae* accumulated 22.4 wt% DAG in the reaction mixture after 24 h, the DAG content reduced to 7.2 wt% after 48 h. This result showed that glycerolysis of DAG proceeded in the late stage of the reaction.

The reaction with *Alcaligenes* lipase proceeded faster at 30°C than at 5°C: esterification at 30°C after 7 h, 30.5%; esterification at 5°C after 7 h, 5.5%. The degree of esterification at 5 and 30°C reached *ca*. 80% after 48 h, and the MAG content was higher at 5°C than at 30°C, showing that esterification of MAG and DAG with FFA-CLA proceeded poorly at a low temperature.

When the esterification with *C. rugosa* lipase was conducted at 30°C, the degree of esterification reached only 14.0% even after 48 h. However, the reaction proceeded efficiently at 5°C, and the MAG content reached 85.4 wt% after 48 h at 93.5% esterification. In this reaction, the DAG content decreased from 11.9 to 7.7 wt% between 24 and 48 h, indicating that glycerolysis of DAG occurred weakly in the late stage of the reaction.

The screening test revealed that MAG-CLA was produced successfully by esterification of FFA-CLA with glycerol at a low temperature using *C. rugosa*, *R. oryzae*, and *P. camembertii* lipases. Although the optimal temperature of *C. rugosa* lipase was reported to be 40–50°C (22,23), the enzyme catalyzed the esterification at 5°C more efficiently than at 30°C. For further study of this phenomenon in addition to the efficient production of MAG-CLA, we selected *C. rugosa* lipase as a catalyst.

Effect of the amount of glycerol. A mixture of FFA-CLA, different amounts of glycerol, a 200-U/g mixture of *C. rugosa* lipase, and 2% water was stirred at 5 or 30°C (Fig. 1). In the reaction at 5°C (Fig. 1A), an increase in the amount of glycerol enhanced the esterification of FFA. Addition of <1 molar equivalent of glycerol for FFA resulted in a decrease in MAG and an increase in DAG and TAG, owing to the acceleration

TABLE 1
Production of MAG-CLA with Several Lipases[a]

Lipase	Reaction temperature (°C)	Reaction time (h)	Esterification[b] (%)	Composition (wt%)[c] FFA	MAG	DAG	TAG
Penicillium camembertii	5	7	27.6	67.2	31.9	0.9	ND[d]
		24	84.4	12.6	83.9	3.5	ND
		48	88.9	8.7	84.0	7.3	ND
	30	7	71.2	23.4	68.2	8.4	ND
		24	78.4	15.3	50.4	34.3	ND
		48	79.6	13.9	44.5	41.6	ND
Rhizopus oryzae	5	7	16.6	76.9	14.2	8.9	ND
		24	65.4	26.7	50.9	22.4	ND
		48	92.8	5.6	87.2	7.2	ND
	30	7	42.4	46.7	30.1	23.2	ND
		24	72.7	19.6	46.3	34.1	ND
		48	76.1	15.3	30.4	54.3	ND
Alcaligenes sp.	5	7	5.5	91.3	4.0	4.7	ND
		24	29.5	58.2	16.9	22.1	2.8
		48	81.8	11.8	44.7	28.5	15.0
	30	7	30.5	55.1	14.3	22.7	7.9
		24	80.9	11.0	27.2	38.9	22.9
		48	81.7	9.6	16.4	46.3	27.7
Candida rugosa	5	7	47.5	45.2	47.7	7.1	ND
		24	78.3	16.9	69.9	11.9	1.3
		48	93.5	5.0	85.4	7.7	1.9
	30	7	10.1	86.8	11.1	2.1	ND
		24	13.6	81.1	12.7	5.7	0.5
		48	14.0	80.3	12.6	5.9	1.2

[a]A mixture of 4.9 g FFA-CLA/glycerol (1:5, mol/mol) and 0.1 mL of lipase solution was incubated at 5 or 30°C with stirring at 500 rpm. *Penicillium camembertii, Alcaligenes* sp., and *C. rugosa* lipases were used at the amount of 200 U/g mixture, and *R. oryzae* lipase was used at the amount of 50 U/g because of the strong activity. MAG-CLA, MAG of CLA; FFA-CLA, FFA mixture containing CLA.
[b]The degree of esterification was expressed as the molar ratio of FA in acylglycerols to total FA in the reaction mixture.
[c]Content in the oil layer separated from the reaction mixture.
[d]Not detected (<0.3 wt%).

of esterification of partial acylglycerols with FFA. On the other hand, larger amounts of glycerol inhibited the esterification of MAG and accumulated MAG efficiently in the reaction mixture. When 5 molar equivalents of glycerol was used as a substrate, the degree of esterification reached 78.8% after 24 h and the MAG content increased to 70.8 wt%. When the amount of glycerol was increased to 10 molar equivalents, the degree of esterification and the MAG content were improved only a little; the MAG content after 24 h was 74.9 wt% at 82.0% esterification.

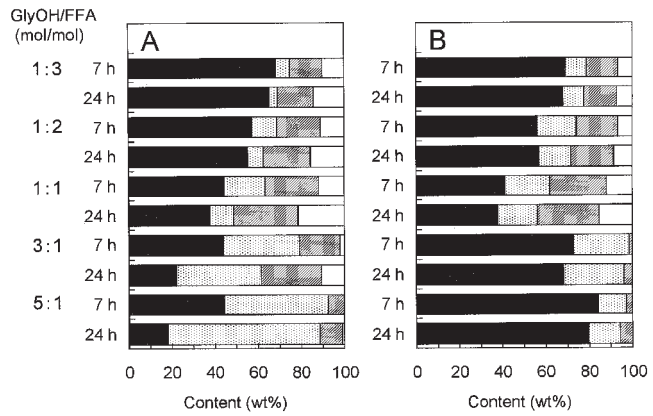

FIG. 1. Effect of amount of glycerol (GlyOH) on esterification of a FFA mixture containing CLA (FFA-CLA) with *Candida rugosa* lipase. A 5-g mixture of FFA-CLA, different amounts of GlyOH, and 0.1 mL lipase solution (10,000 U/mL) was incubated with stirring at 500 rpm. The reaction was conducted at (A) 5°C and (B) at 30°C. Black bars, FFA content in the oil layer separated from the reaction mixture; dotted bars, MAG; crross-hatched bars, DAG; white bars, TAG.

At 30°C with <1 molar equivalent of glycerol (Fig. 1B), esterification was enhanced with an increase in the amount of glycerol. But MAG was not produced efficiently because esterification of partial acylglycerols with FFA was also accelerated. In addition, 3 and 5 molar equivalents of glycerol significantly inhibited the esterification. These results show that MAG-CLA cannot be produced efficiently at 30°C even though the amount of glycerol is controlled.

Comparison of the esterification at 30°C with that at 5°C indicated that esterification at 30°C was significantly inhibited by 3 and 5 molar equivalents of glycerol. In addition, the esterification efficiency at 30°C was almost the same as that at 5°C in the presence of <1 molar equivalent of glycerol, and the temperature dependency of the reaction was not observed. It was therefore found that even <1 molar equivalent of glycerol inhibited esterification at 30°C.

Inhibition of esterification at 30°C by glycerol. A high concentration of glycerol inhibited esterification of FFA-CLA at 30°C in the presence of 2% water, but not at 5°C (Fig. 1). Because water is easily attracted to glycerol at high temperatures, the effect of water on the esterification at 30°C with a high concentration of glycerol was studied.

To a mixture of FFA-CLA and 3 or 5 molar equivalents of glycerol was added 10% water, and the reaction was conducted at 30°C with 200 U/g mixture of *C. rugosa* lipase (Fig. 2). In the esterification with 3 molar equivalents of glycerol, the degree of esterification after 24 h was only 24.8% in the presence of 2% water. However, addition of 10% water increased the degree of esterification to 65.2%, and not only MAG but also DAG and TAG were synthesized. This relatively low degree of esterification can be explained by the simultaneous hydrolysis of acylglycerols attributable to the presence of large amounts of water. Similar results were observed in the esterification with 5 molar equivalents of glycerol. Addition of 10% water increased the degree of esterification after 24 h to 68.0%, and MAG, DAG, and TAG were synthesized. These results indicated that one reason for the inhibition of esterification by glycerol was the decrease in water participating in the reaction because the high concentration of glycerol attracts more water to glycerol at high temperatures.

Effect of temperature. A mixture of FFA-CLA/glycerol (1:5, mol/mol), a 200-U/g mixture of *C. rugosa* lipase, and 2% water was stirred at a range of temperatures from 5 to 40°C (Fig. 3). The esterification proceeded efficiently with a decrease in temperature, showing that the inhibition of the esterification by glycerol was eliminated at lower temperatures. The contents of MAG after 24 h were *ca*. 70 wt% at 5 and 10°C, and 59.1 wt% at 15°C. The reaction temperature should therefore be set at <10°C for efficient production of MAG-CLA.

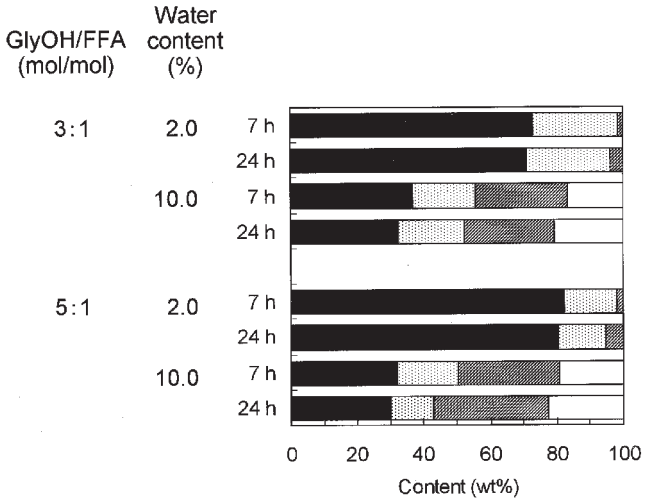

FIG. 2. Effect of the amount of water on esterification at 30°C with high amounts of glycerol. A 5-g mixture of FFA-CLA/GlyOH (1:3 or 1:5, mol/mol), 2 or 10% water, and a 200-U/g mixture of *C. rugosa* lipase was incubated at 30°C with stirring at 500 rpm. Black bars, FFA in the oil layer separated from the reaction mixture; dotted bars, MAG; cross-hatched bars, DAG; white bars, TAG. See Figure 1 for abbreviations.

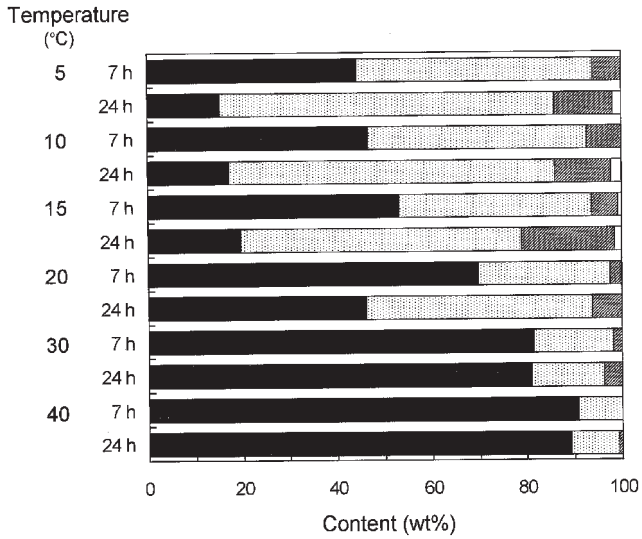

FIG. 3. Effect of temperature on esterification of FFA-CLA with *C. rugosa* lipase. A mixture of 4.9 g FFA-CLA/GlyOH (1:5, mol/mol) and 0.1 mL lipase solution (10,000 U/mL) was incubated at 5 to 40°C with stirring at 500 rpm. Black bars, FFA in the oil layer separated from the reaction mixture; dotted bars, MAG; cross-hatched bars, DAG; white bars, TAG. See Figure 1 for abbreviations.

Effect of the amount of lipase. A mixture of FFA-CLA/glycerol (1:5, mol/mol), 20 to 400 U/g mixture of *C. rugosa* lipase, and 2% water was stirred at 5°C. The velocity of esterification was enhanced by an increase in the lipase amount, and the degree of esterification after 24 h reached a constant value (80–82%) with >200 U/g mixture of the lipase. The MAG contents after 24 h were 70.7 wt% with 200 U/g of lipase and 74.6 wt% with 400 U/g of lipase.

Effect of the amount of water. A mixture of FFA-CLA/glycerol (1:5, mol/mol), a 200-U/g mixture of *C. rugosa* lipase, and different amounts of water was stirred at 5°C (Fig. 4). The reaction scarcely proceeded with the addition of

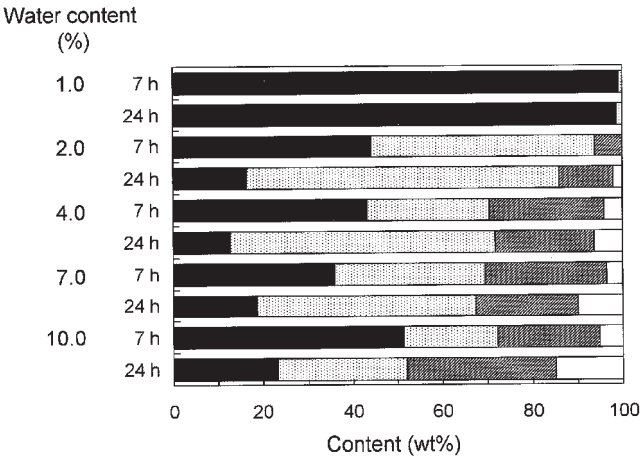

FIG. 4. Effect of the amount of water on esterification of FFA-CLA with *C. rugosa* lipase. Water was added to a mixture of 4.95 g FFA-CLA/GlyOH (1:5, mol/mol) and 50 µL lipase solution (20,000 U/mL) at the concentrations of 1, 2, 4, 7, and 10%. The mixture was incubated at 5°C with stirring at 500 rpm. Black bars, FFA in the oil layer separated from the reaction mixture; dotted bars, MAG; cross-hatched bars, DAG; white bars, TAG. See Figure 1 for abbreviations.

1% water (water content in the reaction mixture, 1.16%). With the addition of 2–7% water, the velocity (shown by the degree of esterification of 7 h) increased slightly with an increasing amount of water. These results showed that some water is necessary for expression of the lipase activity. The degree of esterification after 24 h showed a maximal value with the addition of 4% water, suggesting that simultaneous hydrolysis occurs in the presence of larger amounts of water.

In the reactions with 2–10% water, MAG content decreased with increasing water content after 24 h, whereas TAG content increased. These results indicate that the larger amount of water accelerated esterification of partial acylglycerols with FFA, converting MAG to TAG *via* DAG. The reaction with an addition of 2% water produced 69.6 wt% MAG-CLA after 24 h at 79.0% esterification.

Effect of dehydration on the reaction after steady state. Addition of 2% water produced MAG most efficiently, and larger amounts of water accelerated esterification of MAG with FFA (Fig. 4). Esterification generates 2% water at 80% esterification. Hence, the effect of dehydration on esterification was studied.

Esterification was conducted at 5°C by agitating a 300-g mixture of FFA-CLA/glycerol (1:5, mol/mol), 200 U/g mixture of *C. rugosa* lipase, and 2% water. Figure 5A shows a typical time course of the reaction without dehydration. The reaction mixture changed from liquid to creamy after 2 h and its hardness increased gradually. Esterification gradually proceeded even after 24 h, and the degree of esterification reached 93.8% after 58 h. During the first 24 h, MAG accumulated efficiently in the reaction mixture, and DAG was also synthesized by esterification of MAG with FFA. The DAG content reached 11.3 wt% at 24 h, and then decreased to 5.4 wt% after 58 h. This result showed that glycerolysis of DAG occurred after 24 h in preference to esterification of MAG with FFA.

To see the effect of dehydration, esterification of FFA-CLA with glycerol was conducted under the same conditions as stated above, and dehydration was started after 24 h by evaporation at 5 mm Hg using a vacuum pump (Fig. 5B). The degree of esterification increased concomitantly with dehydration and reached 94.7% after 24 h of dehydration (48 h in total). Glycerolysis of DAG was also accelerated by dehydration, and MAG content after 48 h in total reached 93.0 wt%. Without dehydration, MAG content was 88.4 wt% after 58 h, although the degree of esterification was 93.8% (Fig. 5A). From these results, we concluded that dehydration after reaching the steady state is effective for a reduction in reaction time and for an increase in MAG yield.

We previously reported a two-step *in situ* reaction system with *P. camembertii* lipase for production of MAG-CLA, which was composed of esterification of FFA-CLA with glycerol at 30°C and glycerolysis of DAG at 5°C (18). This reaction system required a long period of 2 wk for glycerolysis. In this study, we clarified that esterification of MAG with FFA was inhibited at low temperatures. This reaction may be explained by the following hypothesis. The m.p. of MAG-CLA is lower than FFA-CLA; thus, MAG-CLA solidifies at the reaction temperature (5°C). Since the solid-state MAG-CLA no longer participates in the reaction, the equilibrium of the reaction shifts to the accumulation of MAG-CLA.

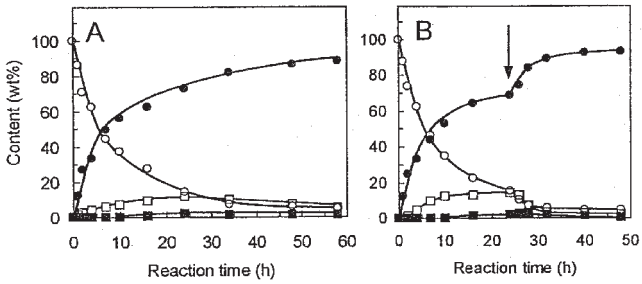

FIG. 5. Effect of dehydration on esterification of FFA-CLA with GlyOH using *C. rugosa* lipase. A mixture of 294 g FFA-CLA (1:5, mol/mol) and 6 mL *C. rugosa* lipase (10,000 U/mL) was agitated at 30°C and 250 rpm. The reaction was conducted (A) without dehydration and (B) with dehydration: The reactor was connected to a vacuum pump at 24 h (indicated with arrow), and the reaction was continued with dehydration at 5 mm Hg. ○, FFA content in the oil layer separated from the reaction mixture; ●, MAG; □, DAG; ■, TAG. See Figure 1 for abbreviations.

Specificity of C. rugosa *lipase toward CLA isomers*. *Candida rugosa* lipase acted more strongly on *c*9,*t*11-CLA than on *t*10,*c*12-CLA in the esterification with lauryl alcohol (21). To study the selectivity of the lipase toward CLA isomers in the esterification with glycerol, FA compositions synthesized in MAG and DAG (Fig. 5B) were analyzed (Table 2). In the early stage of the reaction (<4 h), the content of *c*9,*t*11-CLA in MAG was higher than the content of *t*10,*c*12-CLA. When the degree of esterification reached 31.4% at 4 h, the ratio of *c*9,*t*11-CLA content in MAG to total content of *c*9,*t*11-CLA and *t*10,*c*12-CLA was 64.9%. Meanwhile, in the esterification with lauryl alcohol, the ratio of *c*9,*t*11-CLA in the lauryl esters to the total content of the two CLA isomers was >80% even at *ca*. 60% esterification (21). These results indicate that selectivity of the FA is low in the esterification with glycerol compared to that in the esterification with lauryl alcohol. This difference may result from the substrate used (lauryl alcohol or glycerol) and/or the reaction temperature (30°C or 5°C).

When the esterification with glycerol was conducted for 48 h, the degree of esterification reached 94.7% and the two isomers were esterified efficiently; the contents of the two CLA isomers in MAG were the same as those in FFA-CLA. Hence, the reaction system described in this paper may be suitable for the production of MAG esterified with each CLA isomer as well as MAG with the two isomers.

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TABLE 2
FA Composition in Acylglycerols Obtained by Esterification of FFA-CLA with Glycerol[a]

Reaction time (h)	Esterification (%)	Fraction	FA composition (wt%)					
						CLA[b]		
			16:0	18:0	18:1	*c*9,*t*11	*t*10,*c*12	Others
0	0	FFA[c]	6.4	2.6	15.7	33.9	35.5	4.1
2	21.6	MAG	4.8	1.4	17.8	48.5	25.1	2.3
4	31.4	MAG	5.1	1.7	16.9	47.6	25.8	2.7
10	57.5	MAG	5.5	2.1	16.5	44.2	28.2	2.8
		DAG	5.6	2.0	16.4	45.1	25.9	4.2
24	80.4	MAG	6.2	2.4	16.3	32.4	36.8	4.1
		DAG	4.7	1.8	16.4	42.2	29.4	3.9
48	94.7	MAG	6.3	2.5	15.9	34.2	35.7	4.2

[a]MAG and DAG were purified by silica gel column chromatography of the reaction mixture obtained from the reaction stated in Figure 5B.
[b]*c, cis*; *t, trans*.
[c]FA composition in FFA-CLA. For other abbreviation see Table 1.

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