The Isolation and Recovery of Fatty Acids with Δ5 Unsaturation from Meadowfoam Oil by Lipase-Catalyzed Hydrolysis and Esterification

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This report examines the use of lipases for isolating fatty acids with Δ5 unsaturation from the seed oil of *Limnanthes alba*, or meadowfoam. Seven lipase types and three enzyme configurations (immobilized, "free" and reverse-micellar encapsulated) were examined. All lipases discriminated against Δ5 acids to varying degrees, but the degree of discrimination was independent of enzyme configuration. Lipase-catalyzed esterification of meadowfoam oil's free fatty acids was much more successful for isolating Δ5 acyl groups than was lipolysis. For example, esterification directed by *Chromobacterium viscosum* lipase yielded a free fatty acid product containing >95% of the Δ5 acyl groups at >99% purity.

KEY WORDS: Esterification, hydrolysis, *Limnanthes alba*, lipases, lipozyme, meadowfoam oil, reverse micelles, Δ5-unsaturated fatty acids.

Limnanthes alba, or meadowfoam, a winter-annual plant native to the U.S. Pacific Northwest and British Columbia, is being investigated as a potential new crop. Meadowfoam oil is unique: it contains triglycerides of long-chain fatty acids (94 wt% of the fatty acids (FAs) is 20 carbons or longer), with the majority of containing a *cis* double bond at the Δ5 position (83.5%). Moreover, the two most abundant FA groups present in the oil are 5-eicosenoic ($20{:}1^{5}$) and 5,13-docosadienoic ($22{:}2^{5,13}$) acid, present at 62.4 and 17.1%, respectively. Our research group is developing industrial applications for this oil. From its Δ5 acids, the following materials can be derived: chemical intermediates such as lactones (1) and diepoxides (2), polymer mold-release agents (3), superior-quality factises for rubber manufacture (4), dimer acids for polyamide synthesis (5) and estolides for lubrication and the chemical synthesis of hydroxy FAs (6). In addition, oxidative cleavage of the Δ5 acids produces important chemicals such as pentadecanoic, glutaric and suberic acids (7). Furthermore, industrial research has already demonstrated the potential use of sulfurized meadowfoam oil for lubrication (8).

Thus, the motivation for isolating the Δ5 FAs of meadowfoam has been shown. In this investigation, lipase-catalyzed reactions have been employed to meet this goal. Various lipases and enzyme/reactor configurations have been screened for both hydrolysis and esterification. Earlier studies have indicated that the rate of lipase catalysis is quite sensitive to the double-bond position within FA groups. In most cases, the rate is lessened when the double bonds are located near the carbonyl terminus (9–12). This type of substrate specificity has recently been utilized for reovery of eicosapentaenoic (Δ5) and docosahexaenoic (Δ4) acids from marine oils (13–15) and γ-linolenic acid (Δ6) from evening primrose oil (16).

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EXPERIMENTAL PROCEDURES

Materials. Lipases from *Rhizopus arrhizus* (Type XI), porcine pancreas (Type II), *Candida cylindracea* (Type VII-S), *Pseudomonas* sp. (Type XIII), *Chromobacterium viscosum* (Type XII) and wheat germ (Type I) were purchased from Sigma (St. Louis, MO) and used without further purification. Immobilized *Mucor miehei* lipase (Lipozyme-IM20™) was a gift from Novo-Nordisk (Danbury, CT). The surfactant Aerosol-OT (AOT, sodium *bis*-[2-ethylhexyl] sulfosuccinate) was purchased from Sigma. All other materials, including derivatizing agents and fatty acids for internal standards, were of high purity and employed without further purification. Refined meadowfoam oil was obtained from the Oregon Meadowfoam Growers Association (Salem, OR); meadowfoam free fatty acids (FFA) were obtained by processing the oil with high-pressure steam (performed by Witco Corp., Humko Chemical Division, Memphis, TN). Industrial rapeseed oil, prepressed and solvent-extracted, and crambe oil, extruded and solvent-extracted, were gifts from Dr. Kenneth D. Carlson at our facility (USDA, ARS, NCAUR, Peoria, IL). Deionized water was used throughout.

Methods. All reactions were performed upon 20 vol% meadowfoam oil (for hydrolytic reactions) or 5 vol% meadowfoam fatty acids (for esterification reactions) in isooctane at ambient temperature (22 ± 1°C). In reverse micelles, hydrolysis was initiated by the addition of an aqueous solution of lipase in 50 mM phosphate buffer (PBS) at pH = 6.8 ± 0.05 to the isooctane/meadowfoam oil mixture containing 100 mM AOT. This combination produced a water-AOT molar ratio, or w_0 value, of 8.3. A brief period of mixing was required to solubilize the aqueous solution. The resulting mixture became clear and remained so throughout the course of the reaction. Agitation for "free" and immobilized lipase reactions was supplied by a Vortex/Evaporator from Haake-Buchler (Saddlebrook, NJ). To initiate free lipase reactions, a small amount of highly concentrated aqueous lipase solution was added to 3 mL 20% meadowfoam oil, yielding an initial water content of about 0.3%. The reactions stopped after 200 h. At this point, additional water and lipase were introduced (equal to the amounts present initially); however, no further reaction was detected. Optimization of conditions (*e.g.*, water content) for free lipase hydrolysis was not attempted. During the course of free lipase hydrolysis, adsorption of lipase to the (glass) container walls was observed. Before initiating the Lipozyme-catalyzed reactions, the 20% meadowfoam oil solution was first saturated with (aqueous) PBS (any water present above saturation, *i.e.*, existing as a second phase, led to poor dispersion of the immobilized biocatalyst). Twice during the course of the reaction the solvent and Lipozyme were separated, the former was resaturated with PBS (to replace water consumed during lipolysis), then returned to the biocatalyst. The esterification reactions were per-

formed in reverse micelles containing 1.8 vol% 1-butanol. The concentration of AOT was 91 mM, and the initial value of w_0 was 9.0. An esterification reaction between 1-butanol and linoleic acid (each at 100 mM) in reverse micelles ([AOT] = 100 mM; w_0 = 9.23) was used to assay enzyme activity. A Unit (U) of activity is defined as the quantity which produces 1 mM ester per hour. Table 1 displays the amount of lipase activity employed for each reaction with the exception of that for porcine pancreas lipase (PPL), which was not determined due to experimental difficulty.

Three chromatographic analyses were performed to monitor the course of reaction. Each employed an internal standard for quantitation. The first method was gas chromatography of trimethylsilane-derivitized samples on a neutrally polar 25 m × 0.25 mm methyl 65% phenyl silicone capillary column from Quadrex (New Haven, CT) as described previously (17), except slower oven temperature programming (3°C/min) was used here. The second method determined the FFAs liberated during the course of hydrolysis and provided superior separation of the FA species. The FFAs from aliquots of reaction medium were converted to methyl esters (FAMEs) by adding 10% BF_3 in methanol and heating over a steam bath. FAMEs were then recovered by adding small aliquots of saturated salt solution followed by extraction with hexane. The analysis was performed on a model 7100 gas chromatograph from Spectra-Physics (San Jose, CA) with a 25 m × 0.22 mm i.d. CP-Sil 84 polar capillary column from Chrompack (Raritan, NJ) and with the following oven temperature program and conditions: from 90 to 240°C at 3°C/min; injector and detector temperature at 250°C, and hydrogen as carrier split at a ratio of 50:1. The FA composition of meadowfoam, crambe and rapeseed oils (after saponification with methanolic KOH) was also determined by the second method. To determine relative amounts of FAs, partial glycerides and triglycerides (*i.e.*, the percent hydrolysis), supercritical fluid chromatography (SFC) was employed on a model 600 chromatograph from Dionex (Salt Lake City, UT) with a 10 m × 50 mM 5B-Methyl-100 capillary column also from Dionex. The carrier fluid was SFC/supercritical fluid extraction-grade carbon dioxide from Air Products (Tamaqua, PA). The chromatographic procedure used was the same as that described previously (17).

RESULTS AND DISCUSSION

Oil composition. The FA composition of oil from *Limnanthes alba* is depicted on a weight percentage basis in the first row of Table 2 (components present at <1 wt% are not included). These results closely match those reported previously (18,19). Note the large percentage of Δ5 FAs (83.5% with inclusion of $16{:}1^5$ and $18{:}1^5$ species); and, among the non-Δ5s, erucic acid ($22{:}1^{13}$) is the major component.

Recovery of Δ5 FAs via hydrolysis. Seven lipase types (listed in the previous section) and three different enzyme configurations were employed for hydrolysis of meadowfoam oil. The objective is to isolate the Δ5 acyl groups either in the free acids released or in the remaining partial glycerides and triglycerides. The FFAs can be separated from the monoglycerides (MG), diglycerides (DG) and triglycerides (TG) through contact with a strong basic aqueous extractant. However, for the first enzyme configuration (Configuration 1), lipases encapsulated in water/AOT/isooctane reverse micelles, the presence of surfactant complicates this procedure by promoting emulsification. (Reverse micellar enzymology is reviewed in Reference 20). The separation problems that accompany Configuration 1 provide one reason for examining the two other configurations. In both Configurations 2 and 3, lipases are suspended in lipophilic medium lacking surfactant or emulsifier. Configuration 2 is *Mucor miehei* lipase immobilized onto an exchange resin (Lipozyme™). Configuration 3 is the suspension of lyophilized lipase powders, or "free" lipases.

The results for the hydrolytic screening are contained in Tables 2 and 3 and Figure 1. Of the various lipase types examined, three—from *R. arrhizus*, porcine pancreas and *M. miehei* (Lipozyme)—possess 1,3-positional specificity. These will be discussed first. An earlier report by Phillips *et al.* (21) described the positional location of fatty acids in the triglycerides of *Limnanthes douglasii* oil, which is similar in composition to *Limnanthes alba* oil. The authors found that the majority of the Δ5 species are located at the primary positions, while most of the other unsaturates, such as linoleic and erucic acid, are at the 2-position. The second row of Table 2 estimates the FFA composition resulting from 1,3-specific lipolysis of *L. alba* oil based on the data of Phillips *et al.* (21). The table suggests 1,3-specific lipolysis could isolate Δ5 acids at 91% purity. Results for hydrolysis by *M. miehei* and *R. arrhizus* lipase (with all three enzyme configurations being represented) are shown in Tables 2 and 3. Here, the percentages of the Δ5 components in FFAs do not match the expected values based on ideal, 1,3-specific lipolysis and are not greatly different from those present in the oil before hydrolysis. Any of three reasons can explain the difference. First, our assumption of the similarity of TG structure/composition for *L. alba* and *L. douglasii* oils may not be valid. Second, the reaction may not have reached completion; moreover, these lipases release Δ5 acids quite

TABLE 1

The Overall Enzyme Concentrations (in Units/mL) Employed for Each Reaction

Reaction type	*Rhizopus arrhizus*	*Mucor miehei*	*Candida cylindracea*	*Pseudomonas* sp.	*Chromobacterium viscosum*
Hydrolysis[a]	2.02		4.52	5.38	36.8
Hydrolysis	30.8[b]	36.3[c]	3.98[b]		
Esterification[a]	3.87		15.1	10.7	22.8

[a]Reverse-micellar encapsulated lipase.
[b]Free lipase.
[c]Immobilized lipase (Lipozyme).

TABLE 2

The Composition (in wt%) of the Free Fatty Acids Released *via* Lipolysis at Various Times During the Reaction[a]

Lipase	Time (h)	%C[b]	$18:1^{9}$	$18:2^{9,12}$	$20:1^{5}$	$20:1^{11}$	$22:1^{5}$	$22:1^{13}$	$22:2^{5,13}$
Overall		(100)	1.53	2.28	62.4	1.34	3.02	9.31	17.1
1,3-positions[c]		(66.7)	0.99	1.25	68.7	1.08	3.28	4.33	18.1
Rhizopus arrhizus[d]	2.9	9.0	5.64	11.5	48.3	1.66	2.38	11.0	14.5
R. arrhizus[d]	816	42.5	2.10	3.80	59.5	1.54	2.65	10.5	17.1
R. arrhizus[e]	200	42.2	1.94	3.13	59.7	1.21	3.49	8.46	18.2
Mucor miehei[f]	350	40.2	1.69	2.96	64.0	0.83	2.61	7.17	17.1
PPL[d]	640	5.4	10.1	24.0	31.5	0.98	2.54	1.35	11.1
Candida cylindracea[d]	43	4.0	12.5	22.9	37.7	1.00	1.86	6.85	11.0
C. cylindracea[d]	816	10.0	20.2	32.5	17.1	3.87	0.00	6.45	5.35
C. cylindracea[e]	200	7.1	16.7	28.2	24.3	5.67	0.84	0.56	6.31
Pseudomonas[d]	640	3.5	15.7	36.7	24.5	1.01	0.87	3.42	5.41
Chromobacterium viscosum[d]	7.1	8.5	4.40	7.16	48.2	3.78	2.28	20.8	7.68
C. viscosum[d]	34.7	26.8	2.75	4.34	55.4	2.43	2.11	21.8	7.47

[a](100) = Assuming 100%, hydrolysis. (66.7) = Assuming complete 1,3-specific lipolysis.
[b]Degree of hydrolysis (%).
[c]Based on Reference 21 distribution data (see text).
[d]Reverse micellar encapsulated lipase; PPL, porcine pancreas lipase.
[e]"Free" lipase.
[f]Immobilized lipase (Lipozyme).

TABLE 3

Mole Percent of the Δ5 Fatty Acid Species Among the Free Fatty Acids (FFAs) and the Partial Glycerides/Triglycerides (TG) at Various Times During the Course of Lipolysis

Lipase type	Time (h)	%C[a]	mol% Δ5:FFA	mol% Δ5:MG/DG/TG[b]
Rhizopus arrhizus[c]	2.87	9.0	65.2	85.8
R. arrhizus[c]	42.9	19.0	76.8	86.7
R. arrhizus[c]	816	42.5	78.2	87.2
R. arrhizus[d]	200	42.2	82.4	84.4
Mucor miehei[e]	350	40.2	84.8	82.7
PPL[c]	640	5.4	39.2	87.3
Candida cylindracea[c]	42.9	4.0	50.6	84.7
C. cylindracea[c]	816	10.0	25.3	88.4
C. cylindracea[d]	200	7.1	25.6	88.9
Pseudomonas[c]	640	3.5	29.3	86.8
Chromobacterium viscosum[c]	7.1	8.5	59.4	86.0
C. viscosum[c]	34.7	26.8	66.5	90.3
Overall		(0.0)[f]		83.5

[a]Degree of hydrolysis (%).
[b]MG, monoglyceride; DG, diglyceride.
[c]Reverse micellar encapsulated lipase. PPL, porcine pancreas lipase.
[d]"Free" lipase.
[e]Immobilized lipase (Lipozyme).
[f]Among meadowfoam oil TG at 0% hydrolysis.

slowly, as will be discussed. Third, acyl migration may have permitted acids originally in the 2-position to be released, as demonstrated previously for lesquerella oil lipolysis (17).

Tables 2 and 3 demonstrate that *R. arrhizus* lipase releases Δ5 acids more slowly than any of the other acids. As will be demonstrated throughout the rest of this report, all enzymes examined, regardless of reaction scheme, discriminated against Δ5 acids to varying degrees. The discrimination applied to hydrolysis ideally would promote retention and concentration of the Δ5 acids in the MG/DG/TG by release of the non-Δ5 acids.

Figure 1A illustrates that both *R. arrhizus* and *M. miehei* lipase-catalyzed hydrolyses released Δ5 and non-Δ5 acids from TG at similar rates. Moreover, Table 3 indicates the percentage of Δ5 acids among FFA and MG/DG/TG is not significantly different from the overall percentage in the starting material (83.5%) for these two lipolyses. Employment of the third positionally specific lipase, PPL, led to better isolation of the Δ5 acids. Moreover, nearly 100% of the Δ5 acids remained in the TG/DG/MG, while 30% of the non-Δ5 acids were released (Fig. 1). Thus, it appears PPL isolated Δ5 acids more effectively through discrimination based on double-bond position. Similarly, PPL was found to discriminate against unsaturated FAs with double-bond position between Δ3 and Δ8, and the Δ5 acids led to the strongest degree of discrimination (9–11,21). But to contrast, PPL was also shown to be a poor catalyst for erucic acid release during rapeseed hydrolysis (data not shown). This suggests that PPL does not effectively catalyze reactions of long-chain acids. The low extent of conversion noted in Tables 2 and 3 may be due in part to enzyme inactivation because conditions of the aqueous pseudophase were not optimized.

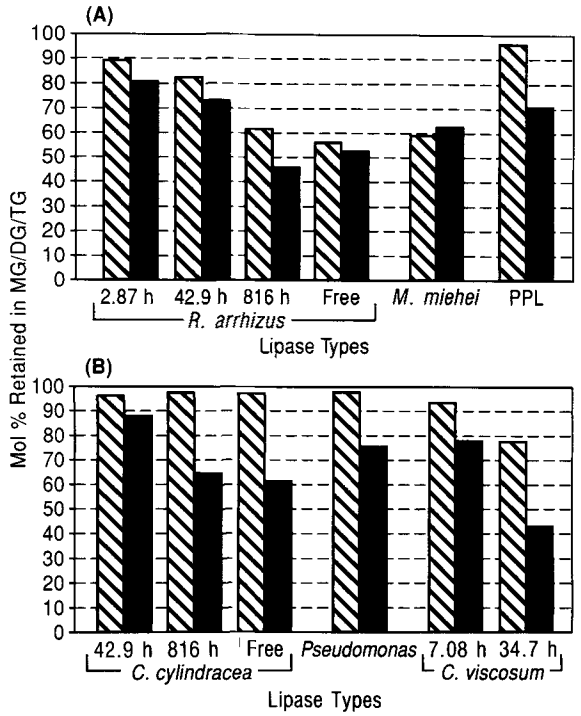

FIG. 1. Mole percent of Δ5 (▨) and non-Δ5 (■) fatty acid species retained in the combined triglycerides (TG) and partial glycerides for lipolysis by (A), 1,3-specific lipases and (B) random lipases. All results are for reactions in reverse micelles and at "final" reaction conversions (listed in Tables 2 and 3) unless otherwise noted. Reaction conditions are listed in the text; enzyme concentrations in Table 1. Abbreviations: MG, monoglycerides; DG, diglycerides; PPL, porcine pancreas lipase.

Moreover, it is well known in aqueous media that PPL requires calcium cations, and in our case, phosphate buffer was employed. In addition, the crude lyophilized PPL employed demonstrated poor solubility in aqueous solution as well as in reverse micelles. In conclusion, the isolation of Δ5 acids by 1,3-specific lipolysis did not occur to a desirable level.

The other lipases examined are of the random type meaning they possess no positional specificity. Of these, *C. cylindracea* and *Pseudomonas* sp. lipases yielded similar results. As Figure 1B indicates, little of the Δ5 acids was released from TG by either lipase, even after long times. This demonstrates that both lipase possess strong discrimination against Δ5 FA. To agree with this finding, *C. cylindracea* lipase was also found to strongly discriminate against Δ4 acids (15) and meadowfoam Δ5 acids during esterification (see below). *Pseudomonas* sp. lipase also discriminated against Δ5 acids during esterification, but to a lesser extent.

Hydrolysis directed by *C. cylindracea* lipase over an extended period of time yielded a TG product retaining over 95% of the Δ5 acids (Fig. 1B). However, over 60% of the non-Δ5 acids were also retained. Moreover, Δ5 acid purity in TG/DG/MG increased only slightly with time (Table 3). Although over 90% of shorter-chain acids (≤C18) were released during *C. cylindracea* lipase-catalyzed hydrolysis, less than 5% of erucic acid, the most abundant non-Δ5 component, was liberated. Likewise, less than 40% of gondoic ($20:1^{11}$) acid was released. Thus, it appears that *C. cylindracea* lipase also discriminates against long-chain acids, which agrees with the findings of Sugiura and Isobe (22). We found the release of erucic acid from industrial rapeseed and crambe oils (which contain 42 and 56% erucic acid, respectively, and lack acids with Δ5 unsaturation) by *C. cylindracea* lipolysis also to be quite slow. But in these cases, the amount of free erucic acid produced was significant. In addition, we found *C. cylindracea* lipase readily catalyzes esterification of free erucic acid to nearly 100% conversion, although at a slow rate. Thus, the inability of *C. cylindracea* lipase to release erucic acid from meadowfoam oil appears to be an oddity. However, we believe the solution to this mystery is related to the TG structure of meadowfoam oil. As discovered by Nilova-Damyanova *et al.* (18) and supported by our SFC TG analysis, the majority of erucic acid is contained in TG possessing two Δ5 acid groups, which, based on the data of Table 2, are located at the primary positions. But the majority of shorter-chain non-Δ5 acids belong to TG lacking Δ5 acids. Hence, we suggest that *C. cylindracea* lipase cannot act upon the former TG type. Analogous results were reported by Österberg *et al.* (23) for isolation of γ-linolenic ($18:3^{6,9,12}$) acid. The authors speculated that the inability of lipase to act upon such TGs was due to the restriction of chain rotation near the ester linkages imposed by the Δ6 double bonds.

The other random lipase examined, from *C. viscosum*, also released Δ5 acid ester bonds relatively slowly. But unlike *C. cylindracea* and *Pseudomonas* sp. lipases, it was able to liberate large amounts of free Δ5 acids (and erucic acid). Moreover, the extent of hydrolysis was much greater (see Tables 2 and 3). The production of free Δ5 and non-Δ5 acids by this biocatalyst is plotted in Figure 2. The figure indicates after a few minutes the difference in production between free non-Δ5 and Δ5 acids is greatest, but only after *ca.* 30 h is the release of non-Δ5 acids nearly completed. Based on this rationale, the reaction times of 7.1 and 34.7 h were selected for further examination. At 7.1 h, only about 7% of the Δ5 acids have been liberated, while 23% of the non-Δ5 acids have been released. At 34.7 h, the separation between the Δ5 acids in MG/DG/TG and the non-Δ5 components in the FFAs is greater (Fig. 1B); also, the percentage of Δ5 components in MG/DG/TG is over 90% (Table 2). However, the separation is not greater than that obtained by *C. cylindracea* lipase, and the recovery of Δ5 acids is less (Fig. 1B). In conclusion, the best isolation, separation and recovery of Δ5 acids through lipolysis is obtained with *C. cylindracea* lipase. However, the degree of separation here is much less than desirable. This lack of success motivated us to examine isolation *via* enzymatic esterification. However, if the goal was to isolate the long-chain acids from meadowfoam oil, hydrolysis catalyzed by lipase from *C. cylindracea*, *Pseudomonas* sp. or porcine pancreas could readily perform the task. Wheat germ lipase was determined to be ineffective toward hydrolysis.

Recovery of Δ5 FAs by esterification. As a second means of isolating the Δ5 acids from meadowfoam, its FFAs (formed by high pressure steam) were esterified with 1-butanol. This method takes advantage of the discrimination lipases possess against Δ5 acids; thus, esters of non-Δ5 acids should form more quickly. After the reaction is

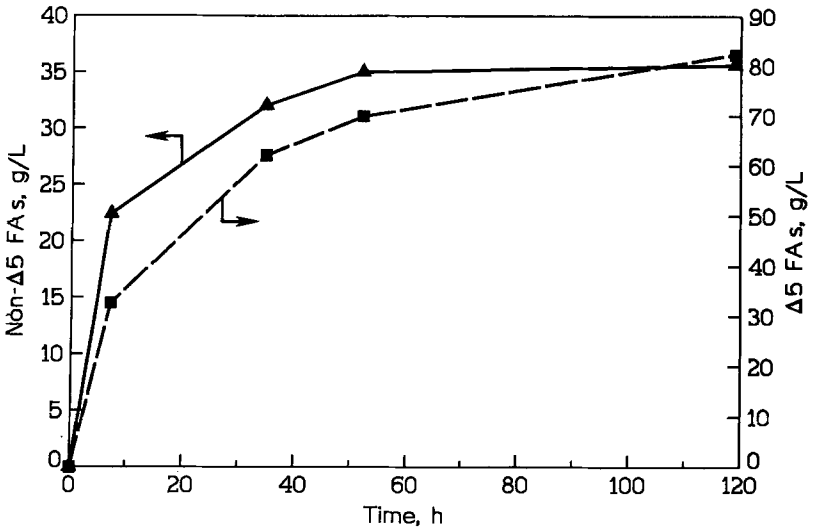

FIG. 2. The amount of Δ5-containing and non-Δ5-containing free fatty acid (FA) formed during hydrolysis by lipase from *C. viscosum*. Reaction conditions are listed in the text; enzyme concentration in Table 1.

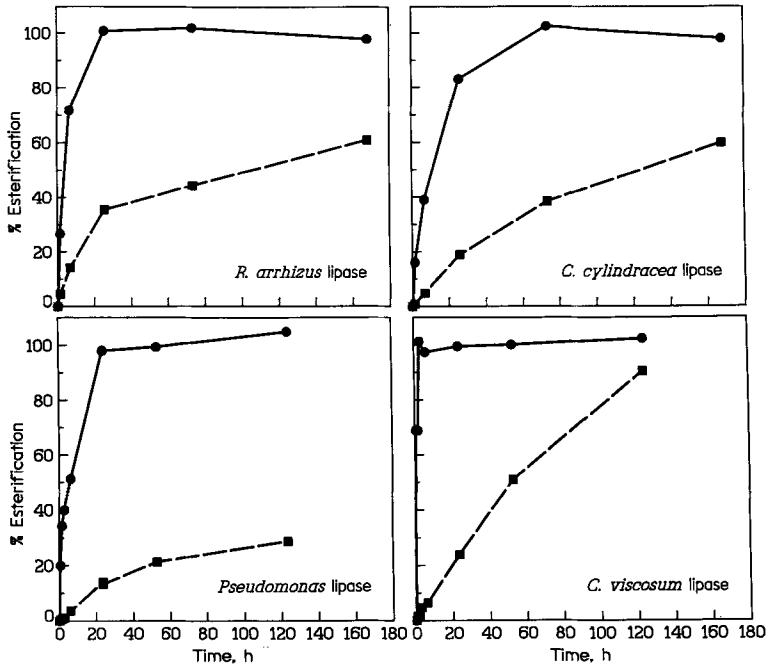

FIG. 3. Change in percent of esterification of Δ5 (---■---) and non-Δ5 (— ● —) acyl groups during the course of reaction. Conditions are noted in the text and enzyme concentrations are listed in Table 1. Lipase types are noted in the figure.

stopped—either through thermodynamic equilibrium or by denaturing or removing the biocatalyst—the unesterified acids, of which the majority should be Δ5 acids, can be recovered by extraction with a strongly alkaline aqueous solution. Reverse micelles were employed for hosting this reaction because of the experimental simplicity and degree of success (95–100% conversion) exhibited previously for esterification in this medium (24). Four lipases were examined for esterification: *R. arrhizus, C. cylindracea, Pseudomonas* sp. and *C. viscosum*. The production of non-Δ5 and Δ5 FA butyl esters for each reaction is plotted in Figure 3. One can see for all four reactions that esterification for non-Δ5 acids was much more rapid than for Δ5 acids.

Ideally, the reaction sould be stopped at the moment where >99% of the non-Δ5 acids have been esterified. The FFA product remaining would then contain Δ5 acids at nearly 100% purity. This instance or "critical" time, given the symbol t_{cr}, is estimated for the four lipase-catalyzed esterifications in Table 4. Part of the differences in t_{cr} are

TABLE 4

Results from Lipase-Catalyzed Esterification of Meadowfoam Free Fatty Acids (FFA)

Lipase type	t_{cr} (h)	t_{cr} (h) (at 10 U lipase/mL)[a]	% Esterification of Δ5 FFA (at $t = t_{cr}$)
Candida cylindracea	73.6	111	38.3
Chromobacterium viscosum	2.9	6.6	4.8
Pseudomonas	23.9	25.5	13.9
Rhizopus arrhizus	25.9	13.6	35.4

[a]Estimated by relationship t_{cr} [E] = a constant.

due to the different enzyme concentrations used (Table 1). Both the Michaelis-Menten and Ping-Pong Bi-Bi kinetic models, which have been used to describe lipase-catalyzed reactions (25), predict a hyperbolic relationship between t_{cr} and enzyme concentration for a given set of initial conditions, substrate concentrations and enzyme. Experimental data published for Lipozyme-catalyzed reactions also reflect this trend (26). Based on this relationship, estimates of t_{cr} for a common enzyme concentration (10 U/mL) were derived and are reported in Table 4. These results show that the reaction catalyzed by *C. cylindracea* lipase is almost an order of magnitude slower than that for the other three reactions. This probably reflects *C. cylindracea* lipases's discrimination toward erucic acid.

Table 4 also contains information on the recovery of Δ5 acids at t_{cr}. At the critical time, only *ca.* 4.8% of the Δ5 acids have been esterified for esterification by *C. viscosum* lipase. Moreover, for this reaction, <95% of Δ5 acids can be recovered at *ca.* 100% purity. The recovery for *Pseudomonas* lipase-catalyzed esterification is also high at >85%. However, recoveries obtainable for esterification catalyzed by *C. cylindracea* and *R. arrhizus* lipases are much lower. The results obtained for *C. viscosum*-catalyzed esterification are quite impressive and merit further investigation. The superiority of esterification over hydrolysis for isolation of acyl groups containing positions of unsaturation near the carboxyl group has also been encountered for the isolation and recovery of γ-linolenic acid from evening primrose oil (27).

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