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Enzymatic synthesis of structured lipids

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Abstract

Lipases are powerful tools for the syntheses of structured lipids (SLs) which are triacylglycerols (TGs) having particular fatty acid (FA) residues at specific positions. With respect to the number of FA species and their distribution in glycerol molecule, TGs are classified into several types; AAA, ABA, AAB, and ABC types. Among them, AAA-type TGs can be synthesized from FA and glycerol either chemically or enzymatically. Even at stoichiometric mixture of the substrates, almost complete reaction is possible. Syntheses of the other types of TGs require positionally specific reactions, for which the use of regiospecifc lipases are effective. ABA-type SLs are synthesized by (1) *sn*-1,3-position-specific lipase-catalyzed interesterification of two different TGs, (2) *sn*-1,3-position-specific lipase-catalyzed acyl exchange of TG with FA or with FA ethylester (FAEt), (3) *sn*-1,3-position-specific lipase-catalyzed acylation of glycerol with FA giving symmetric 1,3-diacyl-*sn*-glycerol, followed by chemical acylation at *sn*-2 position. ABB-type SL is prepared by lipase-catalyzed monosubstitution at either *sn*-1 or -3 position of TG with FA or with FAEt, avoiding formation of disubstituted by-product. Stereopreference to *sn*-1 position over *sn*-3 position of a certain kind of lipase enables the syntheses of chiral ABB- and ABC-type TGs.

Keywords: Lipase; Triacylglycerols; Esterification; Transesterification; Acidolysis

1. Introduction

Structured lipids (SLs) are defined as triacylglycerols (TGs) which are modified chemically or enzymatically to change the fatty acid (FA) composition and/or positional distribution in the glycerol backbone. In a stricter definition, SLs are referred particular molecular species of TGs with defined molecular structure (i.e. specific FA residues in specific positions).

Molecular structure of TGs influences their metabolic fate in organisms (i.e. digestion and absorption) as well as their physical characteristics (e.g. melting points). Consequently, when designing SLs with particular chemical structure, it is possible to control the behavior of TGs, thereby improving the nutritional and pharmaceutical properties of TGs.

Based on this perspective, much attention has been directed to the syntheses of SLs. The syntheses of SLs of particular structure require

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specific modifications at the desired positions in the glycerol backbone. Although chemical interesterification catalyzed by metal alkoxides is simple and inexpensive, it is not capable of modifying specific positions due to the random nature of the reactions. In contrast, the reactions catalyzed by *sn*-1,3-specific lipases are more promising for positionally specific modification of lipids.

In this article, the recent advances in lipase-catalyzed syntheses of SLs are summarized. TGs with defined structure are focused, although SLs might include any mixture of lipids rearranged, in a broader sense. Here, SLs are classified into several groups based on their structures, and synthetic strategies for each group are described showing several examples.

2. Synthesis of MLM-type SLs

2.1. Importance of MLM-type SLs

Among reports of lipase-catalyzed syntheses of SLs, most of them deal with MLM-type SLs, in which medium-chain FAs (MCFA, having 6–10 carbons) are attached to *sn*-1,3 positions and long-chain FAs (LCFA, having more than 12 carbons) at *sn*-2 position. The interest in MLM-type SLs might be related to so-called medium-chain TG (MCT) comprising MCFA, which is useful for clinical purpose as rapid energy source for patients suffering from malabsorption of lipids [1–3].

Since MCT itself does not contain essential FAs (EFAs), supplementary use of EFAs to MCT is necessary. A simple mixture of MCT and long-chain TG (LCT) containing EFAs, however, does not provide enough absorbable EFAs. Mammalian pancreatic lipase hydrolyzes the ester linkages at the *sn*-1 and 3-positions with a preference for MCFAs over LCFAs [4,5]. The resulting *sn*-2-monoacylglycerols (2-MGs) are better absorbable forms of FAs through the intestinal mucosa [6]. Therefore, LCFAs located at *sn*-2-position of MLM type SLs are expected to be well absorbed, bringing the idea to ''build-in EFAs into MCT molecule'' [6–8].

The idea was extended to an alternative concept to use MLM-type SLs as effective carriers of LCFAs, especially bio-active FAs such as polyunsaturated FAs (PUFAs). This concept might be related to drug delivery technology including so-called ''DG prodrugs'', which are 1,3-diacyl-*sn*-glycerols with various drugs attached to glycerol's *sn*-2 position [9–12].

Two possible methods for the production of the MLM-type SLs are shown in Fig. 1. A key point for both strategies is the use of *sn*-1,3-position-specific lipase especially of fungal origin such as *Rhizomucor miehei* and *Rhizopus delemar*.

2.2. Interesterification of MCT and LCT

In this strategy, a mixture of MCT and LCT is reacted with 1,3-specific lipase (Fig. 1A). In the reaction, all the substances in the reaction mixture (i.e. the interesterified products and unreacted substrates) are TGs theoretically, if the hydrolysis of the substrates can be suppressed. Thus, the product as ''a TG fraction'' is recovered very easily just by removing the catalyst. However, the reaction always gives a nonhomogeneous mixture of many TG species (i.e. MLM, LML, MLL, LMM, MCT and LCT), each of which is very difficult to isolate by practical means. Therefore this strategy might be preferable for altering the properties of a physical mixture of MCT and LCT by a simple method rather than for preparing defined species. This type of reaction was demonstrated by Akoh's research group [13,14]. A mixture of tricapronylglycerol (C10:0) and trilinoleoylglycerol (C18:2) [13], or tricaproylglycerol (C6:0) and trioleoylglycerol (C18:1) [14] was used as the substrate, resulting in a mixture of TGs consisting of all possible combinations of capronic and linolenic acids or caproic and oleic acids (OA).

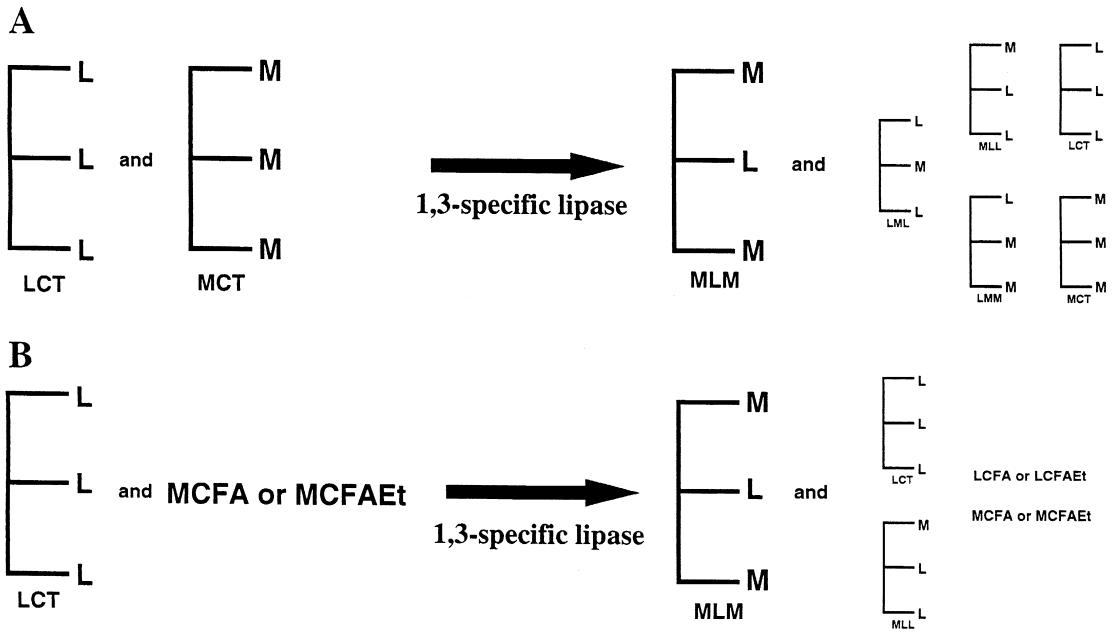


Fig. 1. Synthesis of MLM-type SLs. (A) Interesterification between LCT and MCT. (B) Acidolysis of LCT with MCFA or interesterification between LCT and MCFAEt. TGs are schematically represented. ''M'' and ''L'' indicate MCFA and LCFA residues, respectively. Possible by-products (and impurities) are shown as small schemata.

2.3. Acidolysis of TG with FA or interesterification of TG with FAEt

The second method shown in Fig. 1B is acyl exchange of oils with excess of MCFA (for acidolysis) [15–17] or its ethyester (MCFAEt, for interesterification) [18]. The strategy is to substitute the FA residues specifically at the *sn*-1- and 3-positions of the oils with desired ones by a 1,3-specific lipase, leaving the FA residues at the *sn*-2-position unchanged. Theoretically, these reactions give mixtures of TGs (containing the desired SLs) and FAs or its esters (FAEts). The FAs or FAEts can be removed after the reaction by molecular distillation for both or alkali extraction for FAs. Therefore, if all the FA residues at *sn*-1,3-positions are replaced with the desired FAs (so that neither LCT nor MML remain), eventually the TG species with specific structure can be obtained with quite high purity.

Intensive studies on acidolysis reactions were performed by Shimada et al. [15–17]. *Rh. delemar* lipase immobilized on a ceramic carrier was employed for acidolysis of various oils with caprylic acid (CA). Hydrolysis of the substrate is a side reaction which should be minimized. They found that the enzyme, which was used first in the presence of a certain amount of water for activation, after recovery and reuse, did not hydrolyze TG further in the subsequent reactions. With this ''activated enzyme'', modification of oils containing EFAs [15], γ-linoleic acid [16], arachidonic acid [16] and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [16,17] were successfully achieved without formation of partial glycerides. In addition, to enhance the incorporation of CA, the reaction was repeated for three cycles [16]. After each cycle, the TG fraction was recovered and reacted with fresh CA in the subsequent cycle. Consequently, the FA residues at *sn*-1 and 3 positions could be replaced completely, while the ones at *sn*-2 position remained unchanged. The final products were quite pure with respect to heterogeneity of constitutive molecular

species (i.e. comprising only a few TG species). Thus, depending on the choice of the oils as starting material, various kinds of MLM-type SLs can be obtained by 1,3-specific-lipase-mediated acidolysis.

2.4. Conversion of oils containing fungal lipase-resistant FAs

In spite of versatility of the lipase-catalysed acidolysis, DHA-containing oils such as tuna oil is exceptional [16,17]. Since fungal lipases scarcely act on DHA residues of TG [19,20], the DHA residues at the *sn*-1 and 3-positions of the starting material remained [16]. We also pointed out a similar problem in acidolysis of single cell oil rich in DHA and docosapentaenoic acid (DPA) both of which were resistant to the common fungal lipases [21]. *R. miehei* lipase (Lipozyme™, 1,3-specific but inactive in DHA and DPA residues) and a *Pseudomonas* lipase (non-regiospecific but active on DHA and DPA residues) were compared as biocatalysts for the reaction. The *Pseudomonas* lipase incorporated CA to more than 60 mol% of the total acyl groups, replacing DHA and DPA as well as the other FAs. In contrast, in the case of Lipozyme, CA content reached only 23 mol% in TGs, leaving a large amount of DHA and DPA residues unexchanged. As shown in Fig. 2, the contents of the desired SLs (1,3-dicapryloyl-2-docosahexaenoyl-*sn*-glycerol, CDhC and 1,3-dicapryloyl-2-docosapentaenoyl-*sn*-glycerol, CDpC) were higher in the case of the *Pseudomonas* lipase than in the case of Lipozyme, although it generated the positional isomers of the desired SLs (CCDh and CCDp) also. The Lipozyme-treated oil was rich in the TG species having more than two DHA or DPA residues. Thus, we concluded that the use of non-regiospecific lipase such as the *Pseudomonas* lipase might be sometimes useful for the syntheses of SLs, when the starting materials are oils with high contents of TG species having two or three FA residues which are resistant against fungal lipases.

3. Synthesis of SLs with high purity

3.1. Classification of TGs with respect to their structure

To make use of SLs as fine chemicals, it is necessary that the product is a single compound consisting of exclusively the desired molecular species with defined chemical structure.

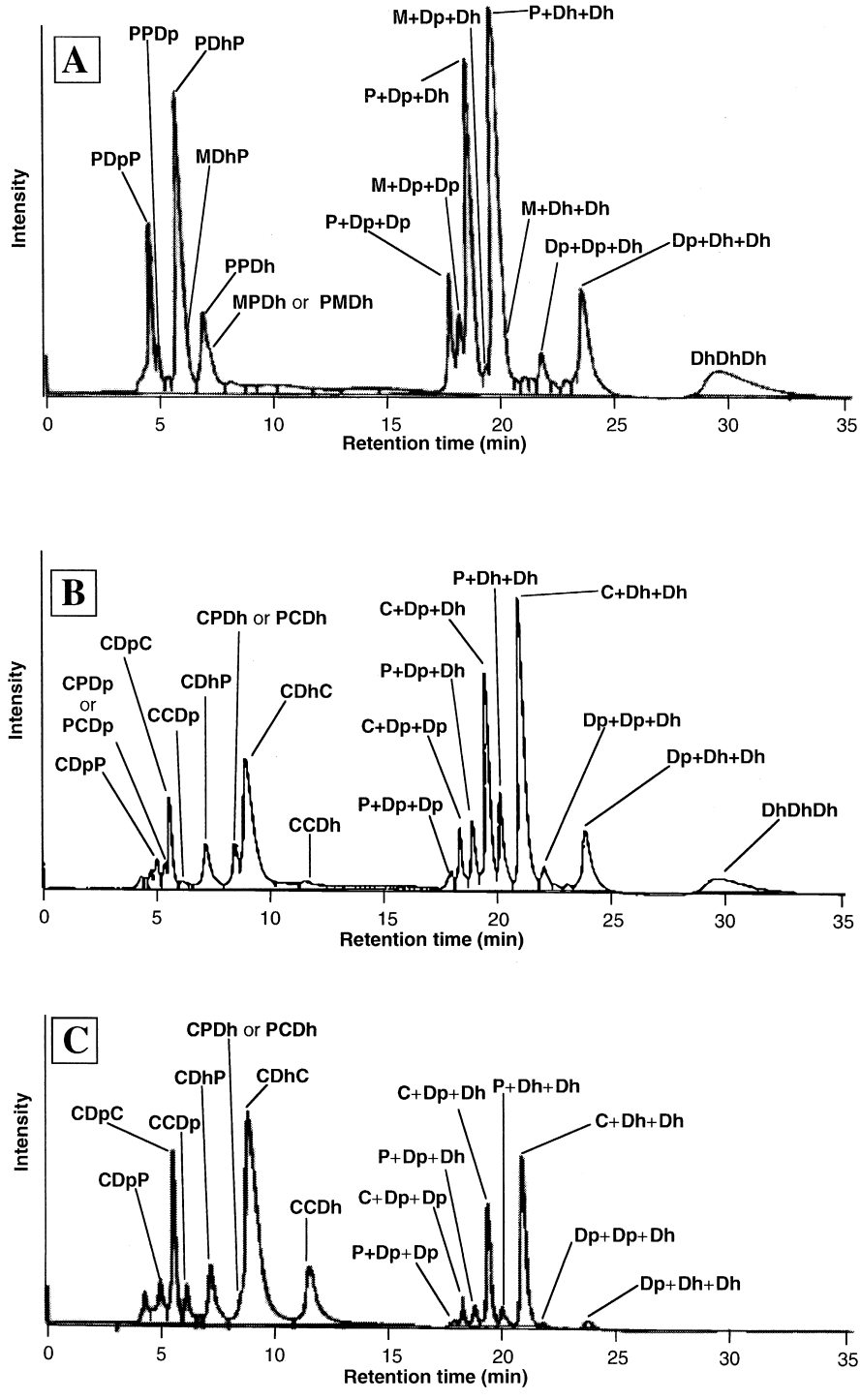
Based on the number of constitutive FA residues and their positions on the glycerol backbone, TGs are categorized into several groups, monoacid, diacid and triacid TGs (Fig. 3). Monoacid TG (AAA type) is the simplest one with only one kind of FA species. Diacid TGs include ABA and AAB types, which are positional isomers. The MLM-type SLs are included in ABA-type TGs. Since AAB type has a chiral center at *sn*-2 carbon, optical isomerism is generated. Triacid TGs (ABC type) are the most complicated ones which comprise three different kinds of FA species. Theoretically, six isomers of an ABC-type TG species are possible with respect to positional and optical isomerism.

3.2. Critical factors in pure SL syntheses

Four requirements should be addressed in designing synthetic methods for pure SLs. The

Fig. 2. Analyses of DHA-rich single cell oil before and after acidolysis with Lipozyme™ or PSL. The single cell oil (A) and the oils acidolyzed by Lipozyme™ (B) and *Pseudomonas* lipase (C) are analyzed by silver ion HPLC. The peaks were identified as indicated. Acyl groups are abbreviated as C, M, P, Dp and Dh for capryloyl, myristoyl, palmitoyl, docosapentaenoyl and docosahexaenoyl groups, respectively. TG species are represented using triplets of the abbreviated forms of the corresponding acyl groups with or without ''+''. Directly connected (without ''+'') triplets indicate the species of which the positional isomerism is taken into consideration. The FA residues at the sn-2 position of such species are indicated at the middle of the triplets. Triplets connected with ''+'' indicate the species of which the positional isomerism was not referred. These species may comprise two or more different positional isomers. (Source, Ref. [21]).

first requirement is the use of the homogeneous reactants in order to assure the intended FA composition of the product. Natural oils and fats are not suitable as the substrates, because of their heterogeneity. The second is the choice of highly regiospecific reactions to obtain the de-



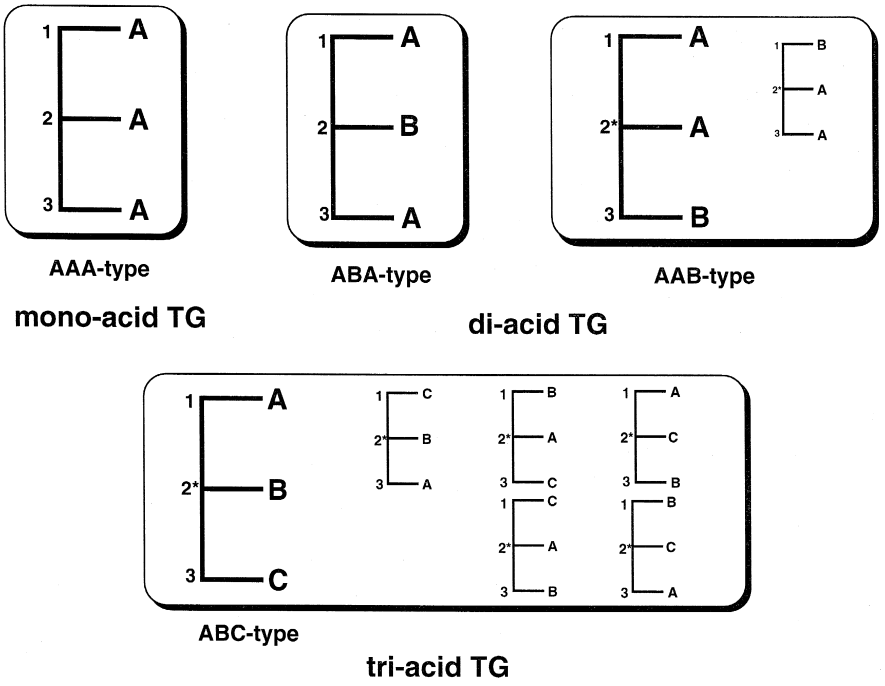


Fig. 3. Classification of TGs with respect to their structure. Structure of TGs are schematically represented. ''A'', ''B'' and ''C'' denote any acyl groups, but they are not identical. TGs are classified into mono-, di- and triacid TGs. Diacid TGs are further categorized into ABA and AAB types. AAB- and ABC-type TGs have chiral centers at the sn-2 carbons as indicated with asterisks. Possible isomers (optical isomer for AAB-type and positional/optical isomers for ABC-type) are shown as small schemata. ABA- and AAB-type TGs are the positional isomers of each other.

sired isomeric configuration of the product. This is solved by the use of regiospecific lipases. Another important requirement is minimizing acyl migration during the reaction, which affects isomeric purity of the final product. The last requirement is easy recovery and/or purification of the product from the reaction mixture. The ease of TG fraction isolation from the other lipid fraction should be considered when choosing the reactants and the type of reaction (e.g. reactions between different lipid classes: TG and free FA, TG and FAEt, partial glyceride and free FA should be compared). The most difficult part in product purification is the isolation of the aimed SL from a mixture of TGs. To simplify this step, it is recommended that reactions proceed completely so that neither unreacted TG nor incompletely modified TG is left in the final reaction mixture.

In the following, some examples for syntheses of each type of SLs are described.

3.3. AAA type

This type of TG is the simplest one among the classes described above. Most of this type of TGs can be prepared by chemical methods from glycerol and FAs. Lipase-catalyzed reactions are also possible. Since PUFAs such as EPA and DHA are very unstable, enzymatic synthesis of PUFA-rich TGs under mild conditions is worthwhile. Non-regiospecific lipases are favorable rather than 1,3-specific ones because all three hydroxyl groups should be esterified.

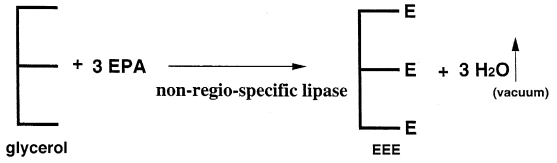


Fig. 4. Enzymatic synthesis of an AAA-type TG, EEE. EPA and glycerol are condensed in the presence of a non-regiospecific lipase under reduced pressure, yielding EEE.

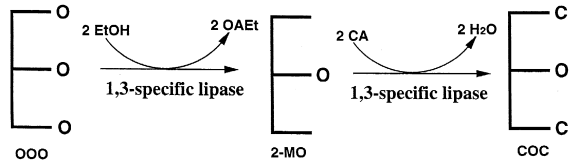


Fig. 5. Synthetic route for pure COC via 2-MO. OOO is alcoholyzed with ethanol, resulting in 2-MO. After recrystalization, 2-MO is esterified with CA, yielding COC in high purity.

The authors' group synthesized trieicosapentaenoylglycerol (EEE) from EPA and glycerol with an immobilized non-regio specific lipase from *Candida antarctica* (Novozyme™) (Fig. 4) [22]. By removing water under reduced pressure, a yield of more than 98% was achieved even at stoichiometric ratio of glycerol and EPA (i.e. glycerol/EPA = 1:3).

3.4. ABA type

3.4.1. Route via 2-MGs

Soumanou et al. [23,24] employed a two-step reaction for the synthesis of pure 1,3-dicapryloyl-2-oleoylglycerol (COC) (Fig. 5). The first step was preparation of 2-monooleoylglycerol (2-MO). Pure trioleoylglycerol (OOO) was deacylated by a 1,3-specific lipase to yield 2-MO. The resultant 2-MO was isolated by recrystallization at cold temperature. In the second step, 2-MO was subjected to enzymatic esterification (condensation) with CA, resulting in COC formation. A key step in this method is suppressing acyl migration, which isomerizes 2-MO into 1(3)-monooleoylglycerol (1-MO) during both the first and the second steps. Performing deacylation of OOO by alcoholysis (not hydrolysis) in methyl-*tert*-butylether (ether solvents suppress acyl migration) with fungal lipases immobilized on Celite (immobilization supports greatly influenced the isomeric purity of 2-MO) successfully suppressed the acyl migration, giving 2-MO without 1-MO formation in 71.8% yield (after recrystallization). The isomeric purity of 2-MO was confirmed by ^{13}C NMR. Esterification of the purified 2-MO with CA gave the desired COC with more than 91% purity. In a similar way, they synthesized 1,3-dioleoyl-2-palmitoylglycerol (OPO), the main component of Betapol™ used for infant formulations [25].

A drawback of this method is that it requires recrystallization of 2-MGs at low temperature, which can not be applied for 2-MGs with very low melting points, such as PUFA containing 2-MGs [25].

3.4.2. Pure ABA-type SL containing EPA

In the authors' laboratory, 1,3-dicapryloyl-2-eicosapentaenoylglycerol (CEC) was synthe-

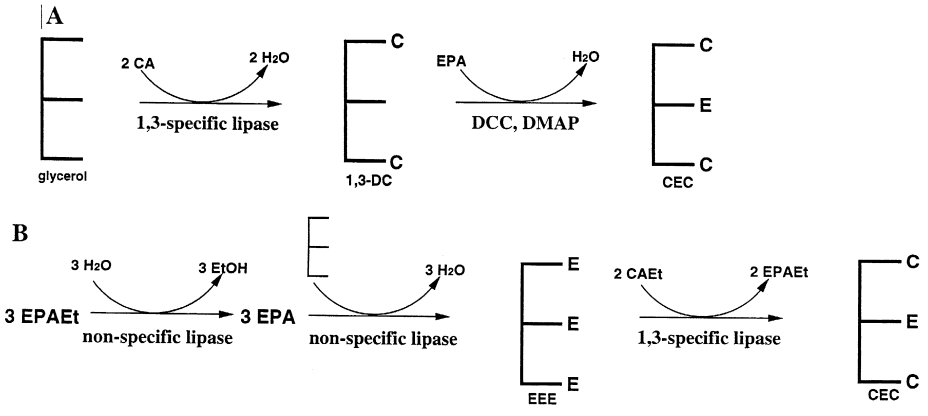


Fig. 6. Two different synthetic routes for pure CEC. (A) 1,3-DC is prepared by 1,3-specific lipase-mediated esterification of glycerol with CA. Then 1,3-DC is chemically esterified with EPA. (B) EEE is prepared by non-regiospecific lipase-catalyzed esterification of glycerol with EPA. Afterwards, EEE is transesterified with CAEt by 1,3-specific lipase.

sized from glycerol, CA or its ethylester (CAEt) and EPA or its ethylester (EPAEt).

One method included preparation of 1,3-dicaprylolylglycerol (1,3-DC) from glycerol and CA by Lipozyme-catalyzed esterification, followed by acylation with EPA at *sn*-2-position (Fig. 6A) [26]. Since the synthesis of the 1,3-DC is a condensation reaction, it is important to remove water generated during the reaction course. The reaction underwent successfully by removing the water under vacuum or nitrogen blowing (Fig. 7). The targeted 1,3-DC could be obtained with more than 80% purity even from the stoichiometric mixture of the substrates (CA: glycerol = 2:1). After purification by a silica gel column, the 1,3-DC was chemically reacted with EPA in the presence of dicyclohexylcarbodiimide as a condensation agent and dimethyaminopyridine as a catalyst, yielding the desired CEC with 90% purity. In fact, we tried to introduce EPA to 1,3-DC by lipase-catalyzed reactions also, but the results were unsatisfactory, due to the formation of various by-products.

Alternatively, we tried a three-step method which did not require isolation of intermediates (Fig. 6B) [22]. The first step was hydrolysis of EPAEt to free EPA by Novozyme™ under controlled vacuum in order to remove the formed ethanol and promote faster hydrolysis. The residual water was removed completely at the end of the reaction. In the second step, EPA was esterified with the stoichiometric amount of glycerol added directly to the reaction vessel. High yield of EEE (over 90%) was obtained by removal of water under vacuum. After the reaction, the non-regiospecific enzyme (i.e. Novozyme™) was removed by filtration. The final step was transesterification of EEE with excess amount of CAEt by 1,3-specific Lipozyme, resulting in the desired CEC. The excess of CAEt and the by-products (CA, EPAEt and EPA) can be removed fractionally by molecular distillation from the glyceride fraction which contained more than 90% CEC (Fig. 8). EPAEt and EPA can be reused in the first step and CAEt in the third step. The main advantages of this procedure are: (1) no organic solvent is employed, (2) isolation and purification of intermediates is not necessary, and (3) the reuse of the excess of reactants (CAEt) and main by-products (EPAEt and EPA) is feasible.

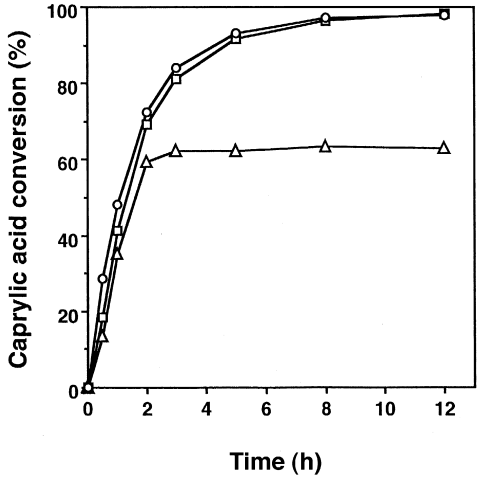


Fig. 7. Effect of water removal on conversion of CA for 1,3-DC synthesis. Glycerol (12.5 mmol) and CA (25 mmol) were condensed in the presence of Lipozyme. The reaction was performed without water removal at normal pressure (-△-), with water removal either by evaporation under reduced pressure (-○-) or by evaporation under nitrogen stream (-□-). (Source, Ref. [26]).

3.5. AAB type

3.5.1. AAB-type SL containing EPA

AAB-type SLs are not as well-studied as ABA-type from the view points of syntheses and biological function. However, it is known that natural oils and fats have asymmetrical distribution of FA residues, suggesting that *sn*-1 and -3 positions are not biologically equivalent. In addition, some lipases in animals (e.g. gastric lipase) digest TGs at different rates towards *sn*-1 and -3 positions [27,28]. From these points arises the necessity to establish synthetic methods for the AAB-type SLs.

In the authors' laboratory, 1,2(2,3)-dicaprylolyl-3(1)-eicosapentaenoyl-*sn*-glycerol (CCE) was synthesized from tricapryloylglyc-

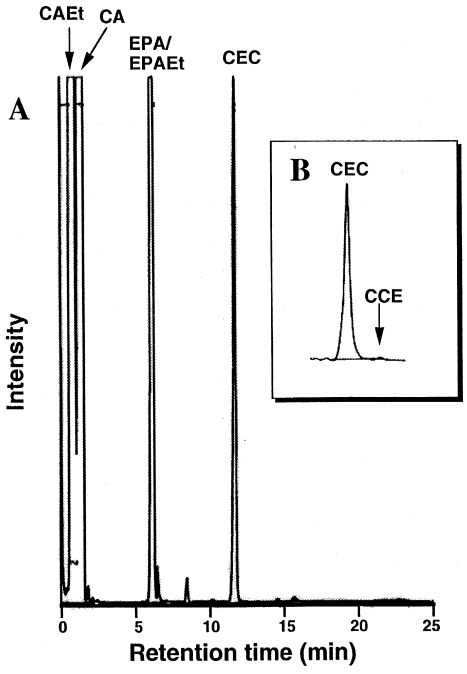


Fig. 8. Confirmation of the purity of CEC synthesized by 3-step enzymatic reaction. (A) High-temperature gas chromatography showing the purity of the product in the ''glyceride fraction''. Note that main impurities are FAs or FAEts, but not glycerides other than the desired product. (B, insert): Silver ion HPLC proving the absence of the positional isomer (CCE). (Source, Ref. [22]).

erol (CCC) and EPAEt by Lipozyme-catalyzed transesterification (Fig. 9) [29,30]. An important aspect of this synthesis was to substitute only one CA residue of CCC molecule with an EPA residue, avoiding the formation of 1,3-dieicosapentaenoyl-2-capryloyl-*sn*-glycerol (ECE). This was achieved by the use of CCC in molar excess (CCC: EPAEt = 3:1, the stoichiometric ratio is 1:1). Since CCC has low molecular weight, the remaining CCC after the reaction is expected to be separated from the product by molecular distillation. Another point was to shift the equilibrium towards CCE formation by removing CAEt (which is generated during the reaction) under vacuum, keeping the water content constant by periodical addition of water necessary for maintaining the enzyme activity.

Under the optimized conditions, 95% of EPAEt was incorporated to glyceride fractions. Among the EPA-containing glycerides obtained, 91.5% was CCE (the desired product), 3.1% was ECE and 5.4% was diacylglycerol containing EPA and CA [31]. HPLC analysis using a silver ion column confirmed the absence of the positional isomer (CEC) in the reaction product (Fig. 10) [29–31]. Optical purity of the product will be published elsewhere soon.

3.5.2. Chiral AAB-type SLS

For practical synthesis of chiral AAB-type SLs, a very interesting study was reported by Chandler et al. [32], who investigated stereospecificity of lipases in acidolysis of tripalmitoylglycerol (PPP) with oleic acid (OA) in organic solvent (Fig. 11A). They found that *R. miehei* lipase showed a preference towards *sn*-1 position over *sn*-3 position to several fold, yielding chiral 1-oleoyl-2,3-dipalmitoyl-*sn*-glycerol (OPP). The stereospecificity depended on water activity, chain length of the TG and type of the solvent.

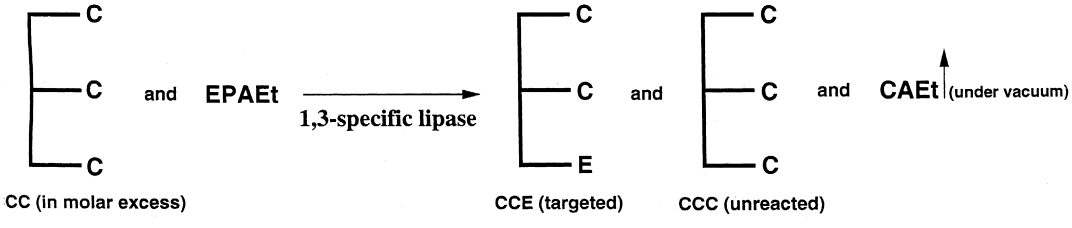


Fig. 9. Synthetic strategy for CCE. EPAEt is interesterified with an excess amount of CCC so that only one CA residue of CCC is substituted for EPA residue. CAEt formed during the reaction is removed by evaporation under reduced pressure to shift the equilibrium towards CCE formation.

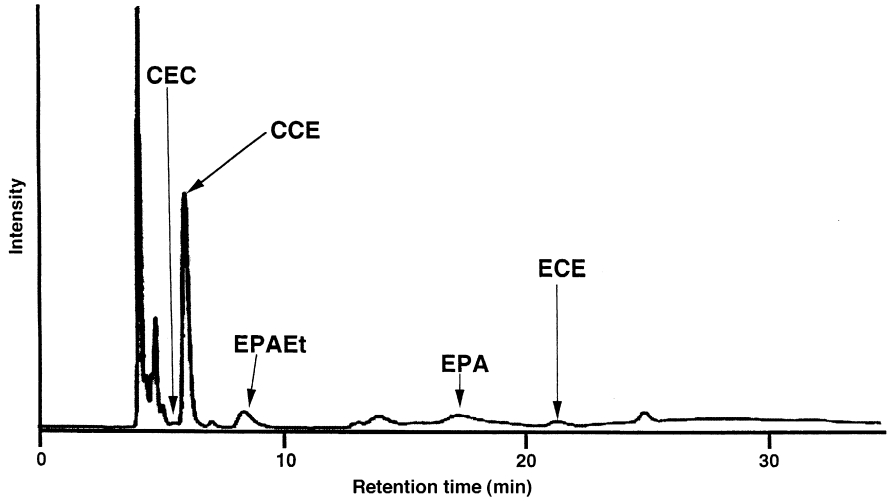


Fig. 10. Silver ion HPLC analysis of CCE synthesized by enzymatic reaction. The chromatogram proves abundance of the desired product (CCE) and the absence of the positional isomer (CEC) as well as suppression of the disubstituted by-product (ECE) formation. (Source, Ref. [29]).

Interestingly, other lipases tested, such as *Rh. delemar* lipase, showed no stereospecificity, despite the lipases from *Rh. delemar* [33] and *R. miehei* [34] being closely related in their molecular structure level. This implies that a slight difference in the structure of enzymes' catalytic sites might bring a significant difference in the stereospecificity, and that stereospe-

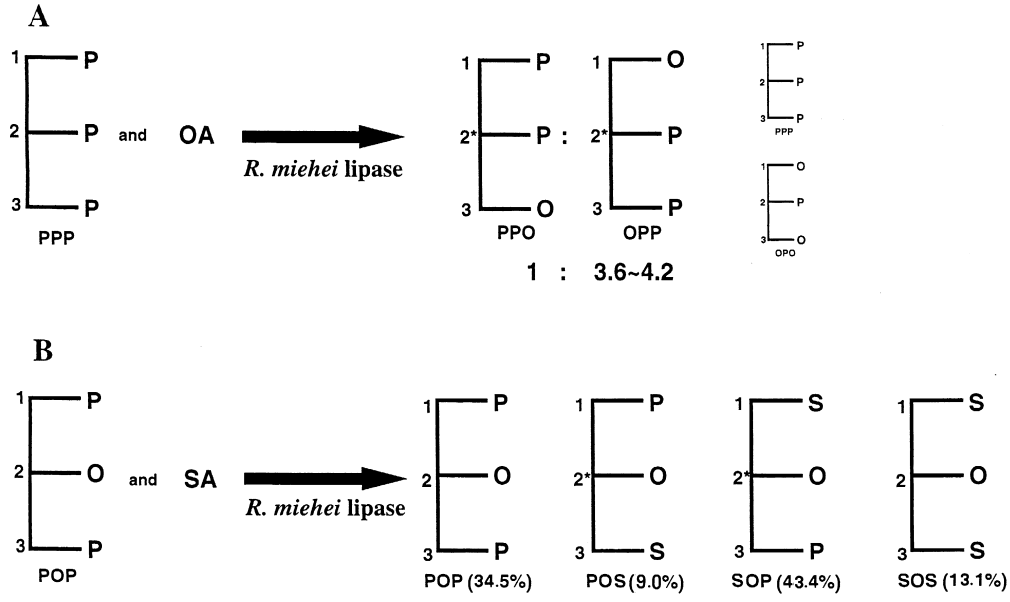


Fig. 11. Synthesis of chiral TGs. (A) Synthesis of chiral OPP. Acidolysis of PPP with OA by *R. miehei* lipase yielded chiral OPP with 3.6–4.2-fold excess over its enantiomer, PPO. (B) Synthesis of chiral SOP. Acidolysis of POP with stearic acid (SA) gave a TG mixture, in which SOP was predominant over its enantiomer, POS.

cific lipases might be found by careful screening and/or mutagenesis techniques.

These findings are very helpful for further investigations, although the product obtained was a nonhomogenous mixture (contained disubstituted OPO and unreacted PPP as impurities).

3.6. ABC type

Chandler et al. [32] also demonstrated the synthesis of chiral ABC-type SLs in a similar way to the chiral AAB-type SL synthesis. The strategy employed was partial stereospecific acidolysis of ABA-type TG with a FA, resulting in the ABC-type SL. By the reaction of 1,3-dipalmitoyl-2-oleoyl-*sn*-glycerol (POP) and stearic acid with *R. miehei* lipase (Fig. 11B), they obtained a mixture of 34.5% of POP (unreacted substrate), 9.0% of 1-palmitoyl-2-oleoyl-3-stearoyl-*sn*-glycerol (POS), 43.4% of 1-stearoyl-2-oleoyl-3-palmitoyl-*sn*-glycerol (SOP) and 13.1% of 1,3-distearoyl-2-oleoyl-*sn*-glycerol (SOS). The amount of SOP was 4.8-fold larger than that of POS.

4. Future perspectives

Since the realization that oils and fats are not just energy sources, researchers have become focused on the function of each FA. However, the quality of oils and fats are still evaluated based only on the average FA composition or contents of particular FAs. In contrast, the concept involved in the studies of SLs is to elucidate oils' and fats' functions based on the molecular structure. The studies of SLs include understanding the structure–function relationships of TG species as basic science and design and utilization of more functional products for application purposes. Enzymologists can contribute to both by providing effective synthetic methods of SLs which can be used as research reagents or as industrial products marketed.

Some of the synthetic methods described in this article might be improved further with respect to yield, purity and cost. The cost of enzymes is usually most predominant among the factors which affect the production costs. Immobilized enzyme would facilitate continuous operation or repeated use of the biocatalysts, thereby lowering the costs. Effective production of enzymes themselves by recombinant DNA techniques is also promising. In addition, obtaining novel enzymes with enhanced stability by screening or by mutagenesis techniques is worthwhile. The mutagenesis techniques are also promising for creating novel lipases with altered characteristics, with which an alternative synthetic route might be found. For example, an *sn*-2-specific lipase would facilitate the production of ABA-type SLs.

Besides the synthetic procedures, analytical methods are also very important in order to confirm that the product is surely the one targeted. For example, at present, there is no method by which optical isomers of TGs are quantified without laborious derivatization. Simple and accurate analytical methods will help the development of synthetic procedures.

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